MEGESTROL ACETATE

In the Treatment of Metastatic Breast Cancer

(Megestrolacetaat in de behandeling van gemetastaseerd mammacarcinoom)

Cover: S. Botticelli: Birth of Venus (ca. 1486)

MEGESTROL ACETATE

In the Treatment of Metastatic Breast Cancer

(Megestrolacetaat in de behandeling van gemetastaseerd mammacarcinoom)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN
DOCTOR IN DE GENEESKUNDE
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. M.W. VAN HOF
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 29 FEBRUARI 1984
TE 14.00 UUR

DOOR

JANA ALEXIEVA-FIGUSCH
geboren te Bratislava (Tsjechoslowakije)

1984

grafische verzorging:
ROCCADAVIDS
ALBLASSERDAM

PROMOTOR: PROF. DR. S.W.J. LAMBERTS

To my family

CONTENTS

		Page
INTRODUCTION		9
ABBREVIATIONS		10
CHAPTER I	Hormonal influences on breast cancer, progesterone and progestins.	11
	1. Historical overview of hormonal therapy	11
	2. The physiological action of progesterone and the pharmacological effects of progestins, metabolism and excretion, plasma levels	15
	3. Mechanism of action of hormones on breast cancer	22
	4. Progestin induced tumor regression	26
	5. Effects of progestins on carbohydrate metabolism and insulin, lipid-, protein-, water- and salt metabolism	30
	6. Relation of progesterone and progestins to prolactin	33
	7. Adverse effects	36
	8. Evaluation of clinical results	38
CHAPTER II	Progestin Therapy in Advanced Breast Cancer Megestrol Acetate - An Evaluation of 160 Treated Cases.	55
CHAPTER III	Estrogen, Androgen, Glucocorticoid and Progesterone Receptors in Progestin-Induced Regression of Human Breast Cancer.	59
CHAPTER IV	Steroid Receptors in Megestrol Acetate Therapy.	65
CHAPTER V	Treatment of Metastatic Breast Cancer Patients with Different Dosages of Megestrol Acetate: Dose relations, metabolic and endocrine effects.	71
CHAPTER VI	Sequential Treatment of Metastatic Breast Cancer with Tamoxifen after Megestrol Acetate - Vice Versa.	81
CHAPTER VII	Endocrine Effects of the Combination of Megestrol Acetate and Tamoxifen in the Treatment of Metastatic Breast Cancer.	97
GENERAL DISCUSSION, SUMMARY AND CONCLUSIONS		109
SUMMARY IN DUTCH (SAMENVATTING)		115
ACKNOWLEDGEMENTS		119
CURRICULUM VITAE		120

INTRODUCTION

There are many non-elucidated questions concerning cancer, especially of the breast, in which hormones are involved. The scope of this particular study is to bring more clarity on the role of the progestin megestrol acetate in the hormonal treatment of breast cancer.

It should be kept in mind that this is a clinical study. Biochemical details mentioned in Chapter I serve primarily as a background for a better understanding of clinical effects.

The study was done from 1974-1983 and 5 questions have provided the frame for this thesis:

1. How effective is megestrol acetate in the treatment of metastatic breast cancer? (Chapter II)
2. What is the role of steroid receptors in the mechanism of action of megestrol acetate in inducing tumor regression? (Chapter III + IV)
3. Has the effect of megestrol acetate on some endocrine parameters any relation to tumor regression? What is the optimal dose? (Chapter V)
4. In what sequence of hormonal treatment should megestrol acetate be used? Is there a difference with regard to the age and/or menopausal state of the patient? (Chapter VI)
5. Does the combination of megestrol acetate and tamoxifen have any additional effect on endocrine events; especially can megestrol acetate-induced hyperprolactinaemia be suppressed by the administration of tamoxifen? (Chapter VII)

These questions are considered in the general discussion and summary.

ABBREVIATIONS

AR	androgen receptor
7,12-DMBA	7,12-dimethyl-benzanthracene
EORTC	European Organisation on Research and Treatment of Cancer
ER	estrogen receptor
GR	glucocorticoid receptor
MA	megestrol acetate
MCF-7	human breast cancer cell line
MPA	medroxyprogesterone acetate
PgR	progesterone receptor
RIA	radioimmunoassay
SHBG	sex-hormone-binding globulin
TAM	tamoxifen
TRH	thyreotropin-releasing-hormone
UICC	Union Internationale Contre le Cancer

CHAPTER I

HORMONAL INFLUENCES ON BREAST CANCER, PROGESTERONE AND PROGESTINS

1. HISTORICAL OVERVIEW OF HORMONAL THERAPY

The first indication that regression of advanced breast cancer can be induced by endocrine manipulation was the beneficial effect of ovariectomy on cancer of the breast of two women. This empirical observation of Beatson[1] in 1896 was remarkable since it was made before the concept of hormones had been developed. Why did he ever consider ovariectomy in the management of inoperable breast cancer? Many years before, while writing his M.D. thesis on the subject of lactation, he learned from Scottish farmers "that it is the custom in certain countries to remove the ovaries of the cow after calving if it is wished to keep up the supply of milk". This fact interested him greatly "for it pointed to one organ holding the control over the secretion of another and separate organ". Furthermore, he believed that there was a similarity between lactation and mammary cancer, since common to both conditions was the "proliferation of generations of epithelial cells which block the duct and fill the acini of the gland. In lactation the cells undergo fatty degeneration and form milk, while in carcinoma they penetrate and invade the surrounding tissues".

At the same time Schinzinger[2] (1889) in Germany suggested ovariectomy in premenopausal advanced breast cancer, which would cause mammary gland atrophy with the opportunity that the tumor would get encapsulated in the shrinking tissue of the gland.

Only decades later, ovariectomy became widely used. In large series of 99 patients Clarke[3] (1905) described remissions in about 30% of the patients. The beneficial action of ovariectomy was not understood until steroid hormones had been isolated forty years later. Since the availability of

estrogens, androgens, progesterone, corticosteroids and ACTH after the 1940's, endocrine therapy has played an important role to control the growth of metastatic breast cancer and to prolong survival of patients.[3-18]

Experimental animal models appeared to be necessary to study tumor growth and its behaviour. Most of the early laboratory investigations of cancer of the breast were carried out in mouse strains with spontaneous mammary tumors and metastatic spread.

Early experiments by Lacassagne[19] (1932) showed that mammary tumors in susceptible mice strains could be induced with large doses of estrogen. Foulds[20] (1949) described spontaneous pregnancy-dependent mammary tumors in mice, which - when they first appeared - tended to regress in pregnancies. Also the influence of pituitary hormones in the etiology was considered (Mühlbock 1956).[21]

There are serious disadvantages in the mouse tumor, they arise slowly and in most strains the mammary cancer of mice are hormone-independent when the tumors reach a palpable size.[21] Studies of the rat altered the course of research on breast cancer. This species has a remarkable propensity to develop mammary carcinoma after exposure to potent carcinogens and a hormone-dependent mammary adeno-carcinoma develops selectively, rapidly and invariably after exposure to 3-methylcholanthrene (3-MC) [22] and 7,12-dimethyl-benzanthracene (7,12-DMBA).[23] This was a very important display and soon almost absolute control over mammary cancer of the rat, its induction, prevention and cure was achieved. Rats, mice, rabbits and dogs are the only experimental animals in which spontaneous mammary tumors have been found. They have never been observed in hamsters, guinea pigs or monkeys. However, spontaneous mammary carcinoma is extremely rare in rats while induced mammary tumors are growing locally without metastatic spread and thereby differ from the human tumors.

Tumor cell studies seemed a more direct approach. It is only within the past years that an estrogen-responsive in vitro cell system has been described. Following the initial description of the MCF-7 human breast cancer cell line[24], Brooks et al.[25] (1973) demonstrated that these cells showed a variety of growth responses to physiologically relevant concentrations of

estrogen. The studying of hormonal dependency of human breast cancer in tissue culture has been started.

As mentioned, several observations suggest that estrogen plays a role in maintaining the growth of established breast cancer: a significant regression may occur after ovariectomy in premenopausal women and an exacerbation of growth occurred when physiological doses of estrogen were given to women whose tumor underwent regression following castration.[8,26,27]

The concept of estrogen dependency, although not strictly proven, offers a working hypothesis that the diminished circulating levels of estrogens as a result of ovariectomy, adrenalectomy and hypophysectomy, as well as by pharmacological dosages of androgens and progestins, could induce a regression of tumors.

On the other hand, this is unable to explain satisfactorily the tumor regression, which follows administration of estrogen to postmenopausal women.[8,28]

Two different types of endocrine therapy have been applied:

1) Ablation of endocrine glands by surgery or by irradiation.
2) Addition of pharmacological dosages of hormones: androgens, estrogens, progestins, corticosteroids.

Grossly, 30% of all human mammary cancers showed a significant regression following anyone of these treatments.

With the introduction of ovariectomy for premenopausal, adrenalectomy or hypophysectomy for the treatment of postmenopausal patients, hormone deprivation through endocrine ablation became a common therapy for advanced breast cancer. Since only one-third of human breast cancers are hormone-dependent and responsive to endocrine manipulation, there was a need to distinguish those hormone-dependent cancer patients before great ablative surgery was to be performed.

Observations, that tissues of estrogen responsive reproductive organs of laboratory animals contain characteristic estrogen-binding components, indicated by their striking uptake and retention of tritiated hexestrol or estradiol given in vivo, led to the rationale of estrogen receptor determination as a tool for estrogen sensitivity.[29,30] After techniques

were devised for the study of estrogen-receptor interaction in vitro[31], it became possible also to examine tumors and to demonstrate specific estrogen-binding by hormone-dependent rat mammary tumors. For clinical use a new area of important research on steroid receptors and their significance in human hormone-dependent cancer, particularly in relation to prognosis and treatment, was opened.

While the additive therapy with androgens and estrogens have several disadvantages, mainly due to unwanted side-effects, more attention was given during the last two decades to the apparently less disturbing anti-estrogens[47] and progestins.[32-44]

Our own interest was directed to the progestins. Although progesterone was discovered (under the term progestin) a long time ago[4,48] its clinical use was limited by short bioavailability. This problem has been overcome by the production of synthetic steroids, which are not only much more active than progesterone but which, unlike progesterone, are also active after oral administration.

Since Huggins[32] (1962) reported that DMBA-induced rat mammary tumors could be "extinguished" by a combination of progestin and estradiol, various progestins have been used in the treatment of advanced breast cancer.[33] After the synthesis of medroxyprogesterone acetate (MPA) in 1958 by Babcock et al.[34] and Sala et al.[35] independently, it has been widely used in the treatment of metastatic breast cancer.[36-41] Megestrol acetate (MA), a drug which is very close to MPA, was first described by Pagani[42] (1961), and is also widely used.[43-46]

In summary

Many endocrine manipulations, ablative and additive, have proven to beneficially influence breast cancer in a palliative way and have been therefore used in metastatic breast cancer. Progestins provide an endocrine modality which is little harmful to the patient and is easily applicable.

2. THE PHYSIOLOGICAL ACTION OF PROGESTERONE AND THE PHARMACOLOGICAL EFFECTS OF PROGESTINS, METABOLISM AND EXCRETION, PLASMA LEVELS

In the early thirties, Corner and Allen[48] (1929) isolated a crystalline substance from corpora lutea which had two important properties: it produced progestational proliferation in the rabbits' uterus and it could maintain pregnancy in castrated female rabbits. They named this extract progestin. A few years later Butenandt[49] (1934) determined the structural formula and synthesized the compound. It was a steroid with a prominent ketone group and was therefore named progesterone.

Since then many similar compounds were synthesized. The Advisory Committee on the Nomenclature of Endocrine Principles of the Council on Pharmacy and Chemistry of the American Medical Association has named the hormone progesterone[50], and to its related compounds with a similar action the generic name progestins was given.

Progesterone

Progesterone is produced and secreted by the ovary, the placenta in female and by the adrenal cortex in both sexes.[51-55] In spite of the large amounts of progesterone produced, the concentration of progesterone detected in blood is low. The rapid disappearance of progesterone from the plasma appears to be due to a rapid diffusion. It is taken up by peripheral tissues, especially those with high lipid content, and it is transformed by them into numerous metabolites; but the most important site of degradation is in the liver. Some progesterone and its metabolites are excreted into the bile and later reabsorbed (entero-hepatic circulation). The plasma level in premenopausal women varies during the menstrual cycle; it is low in the follicular (0,44 - 4,8 nmol/l) and high in the luteal phase of the cycle (16-62 nmol/l). In postmenopausal women and men the level is very low (0,50 - 1,25 nmol/l).

The major degradation products are pregnanediol, pregnanedione and pregnanolone. After the administration of radioactive progesterone to humans, 40-70% of the dose may be recovered from urine and 13-20% from faeces within the first four days, although smaller amounts of radioactivity

continue to be excreted for a longer period of time.

Progesterone is metabolised along several routes. Along one of them progesterone serves as a precursor for corticosteroids and androgens, and via androgens for estrogens (Table 1).

Most of the physiological actions of progesterone require estrogen priming. Estrogens have mainly growth stimulating effects, while the action of progesterone is more directed towards modification and differentiation. However, some stimulation of growth must be present before progesterone can be effective (vagina, uterus, mammary gland). Exceptions include influences on water and electrolyte metabolism involving interaction with aldosterone receptors, some effects on protein metabolism and possibly its action on the hypothalamus affecting body temperature and appetite (Table 2).

Practically all processes of female reproduction are regulated by progesterone and estrogens acting together. It is important to know that progesterone estrogen synergism occurs only when the ratio of estrogen to progesterone and the time-sequence of their interaction is optimal.

Progestins

It is difficult to define synthetic progestins concisely in terms of their respective physiological and pharmacological effects, since virtually none of the effects is exclusive to progesterone.[55,56] The synthetic progestins are derived from 17 α-hydroxy-progesterone, testosterone, or 19-nor-testosterone (Table 3 and Table 4). The 19-nor-testosterone derivates are distinguished by an alkyl group at carbon atom 17. This alkyl group prevents rapid metabolism in the liver and is essential for strong oral activity. The alkyl or halogen substitution of carbon atom 6 have a similar function in the case of hydroxyprogesterone derivates.

The term progestin is in fact no more than a convenient partial description, since there are some progestins which, at least on the basis of their properties and effects in many animal species, could as easily be classified as androgens or estrogens as well.

The alkylated and halogenated acetoxyprogesterone derivates are distinguished by the absence of androgenic and estrogenic effect; they have anti-androgenic and certain glucocorticoid-like effects.[57,58]

Table 1. Some metabolic pathways of the most important steroid hormones.

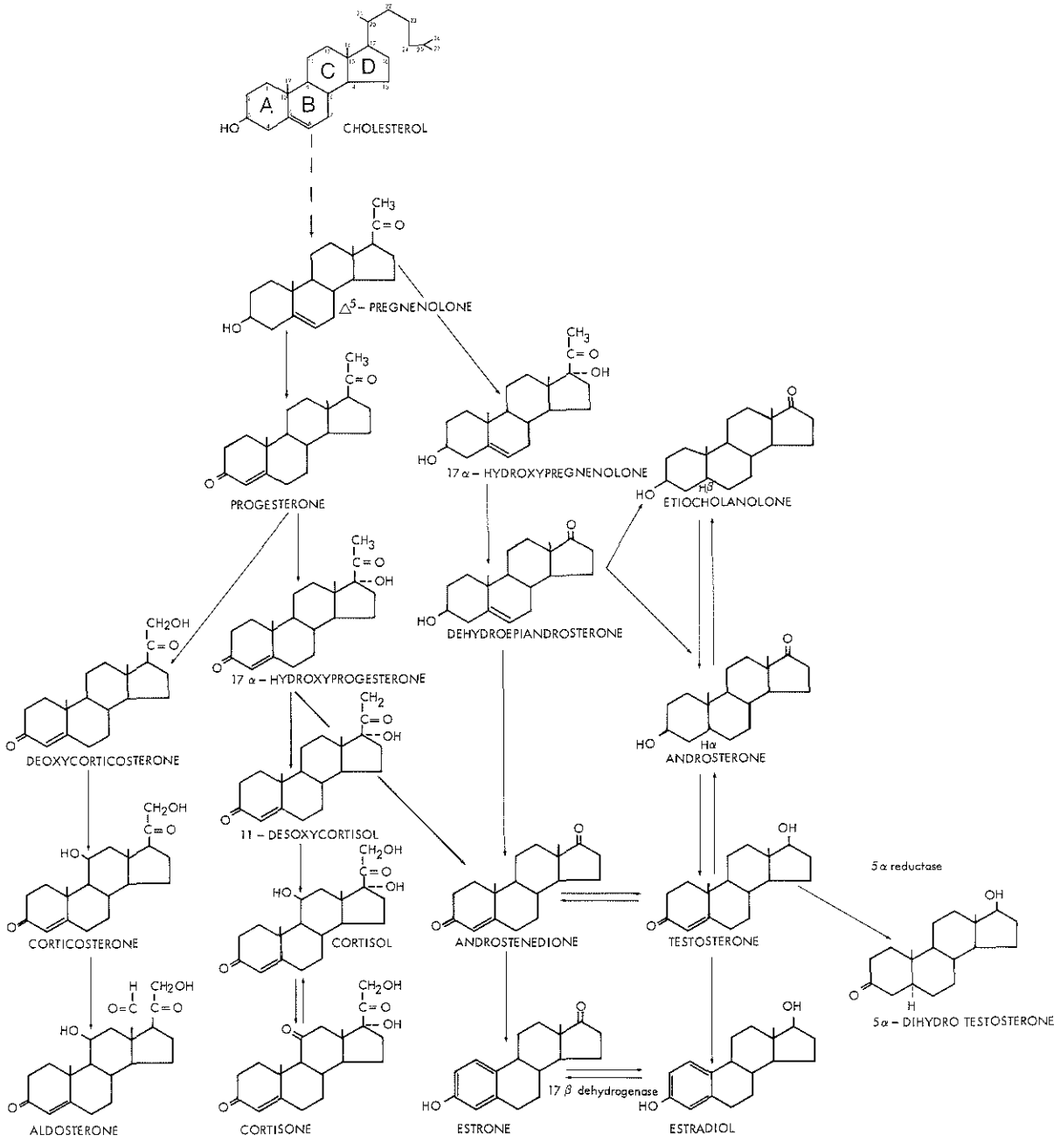


Table 2 SOME SPECIFIC ACTIVITIES OF PROGESTERONE

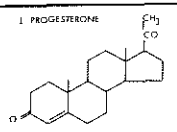
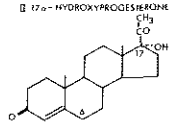
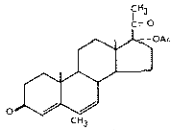
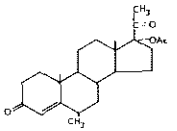
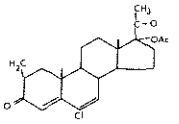
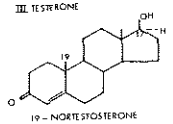
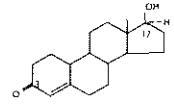
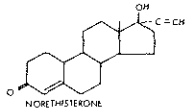
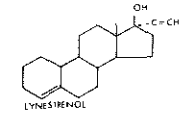
O r g a n	Influence of progesterone
Endometrium	Transformation, growth of the uterine glands, induction of decidual reaction under certain conditions
Myometrium	Reduced contractility
Cervical mucus	Increase in consistence, amount reduced
Vagina	Lowering of the karyopyknotic index
Pregnancy	Maintenance of pregnancy
Mammary gland	Stimulation of tubulo-alveolar growth in most mammals (synergism with estrogens and prolactin)
Pituitary/hypothalamic system	Inhibition of gonadotropin secretion
Sperm capacitation	Inhibition
Metabolism of sperm in the uterus	Lowering effect
Egg transport	Inconsistent effect (species differences)
Tubular secretion	Decrease
Libido	Decrease in most species
Body temperature	Increase
Appetite	Increase
Aldosterone	Competition for aldosterone receptors, natriuresis
Behaviour	Maintenance of maternal behaviour in mammals through interaction with other hormones

Table 3 SOME ACTIVITIES OF PROGESTINS

Progestational	Transformation of the endometrium
Estrogenic	Positive Allen-Doissy assay (cornification of vaginal smear
Androgenic	Stimulation of prostate and seminal vesicle growth in castrate rodents (virilisation of female fetuses)
Anti-androgenic	Inhibition of androgen-induced growth of prostate and seminal vesicle in castrate rodents (feminisation of male fetuses)
Anti-gonadotropic	Inhibition of ovulation
Glucocorticoid-like activity	Inhibition of ACTH secretion and adrenal atrophy

(Adapted from Neumann[55])

TABLE 4. ACTIVITY SPECTRUM OF PROGESTERONE AND SOME PROGESTINS

		Endometrial transformation	Estrogenic	Androgenic	Anti-estrogenic	Anti-androgenic	Anti-gonado-trophic	Glucocorti-coid-like aktivity	Virilisation	Feminisation
I PROGESTERONE 		+	–	–	+	+	+	+		
II 17α-HYDROXYPROGESTERONE 	 MEGESTROL ACETATE	+	–	–	+	+	+	+	–	+
	 MEDROXYPROGESTERONE ACETATE	+	–	–	+	±	+	+	+	±
	 CYPROTERONE ACETATE	+	–	–	+	+	+	+	–	+
III TESTERONE  19-NORTESTOSTERONE 	 NORETHISTERONE  LYNESTRENOL	+	+	+	+	–	+	–	+	–
		+	+	+	+	–	+	–	+	–

Metabolism and excretion of megestrol acetate

Introduction of a 17 α-acetoxy group, a 6 α-methyl group and a double bond at C-6 in the progesterone molecule progressively reduced the rate of metabolism of the steroid in vitro by rat and rabbit liver preparations.[59] When megestrol acetate was given orally to rabbits[60] as (1,2-$^{3}H_{2}$)megestrol acetate the recovery of tritium in urine and faeces during the first seven days was 40.9%; administered as (6-methyl-^{14}C)megestrol acetate, 70.4% of the administered isotope was recovered in the same period. In the urine 20-30% of the administered dose was in the conjugated form and 10-13% in the free form; faecal excretion accounted for 8-15% of the dose. Several urinary metabolites retained the 17 α-acetoxy-20-one and 4,6-dien-3-one structure present in the administered compound, two major metabolites, probably conjugated as glucuronide, are the 17 α-acetoxy-2 α-hydroxy-6-hydroxymethyl-pregna-4,6-diene-3,20-dione and 17 α-acetoxy-6-hydroxymethylpregna-4,6-diene-3,20-dione.

Of (6-methyl-^{14}C) megestrol acetate (4-90 mg) administered to women[61] a mean recovery of ± 70% in the urine and ± 20% in the faeces was obtained within seven days. Three major metabolites excreted as glucuronide conjugates have been found as well as free steroids. Some loss by respiration but also temporary storage in body fat may account for ± 10% not found in the excrets. The biological half-life time of doses 60-90 mg was 3½ days.

Plasma level

Several methods have been used for the estimation of plasma levels of MA, such as mass fragmentography and radioimmunoassay (RIA). For details see Adlercreutz.[62-66] These methods are not strictly comparable.

After a single oral administration of 60 mg of MA to healthy females, the plasma level reached a mean maximum of 43 ng/ml (21.7 - 87.7 ng/ml) after 1-4 hours; after 24 hours MA was still detectable (9.6 - 26 ng/ml) and after 7 days in the range of 0,7-3 ng/ml.[66]

Plasma levels are greatly dependent not only on the method used, but also on intestinal and hepatic inactivation of the drug which again may be secondary to intestinal tract motility and intestinal bacteria, or administration of antibiotics, body weight, diet, liver function.

Adlercreutz et al.[65] and Martin et al.[67] investigated the excretion of steroid hormones. They found that both endogenous and exogenous natural and synthetic estrogens and progestins are excreted to a significant degree in bile and are partly reabsorbed from the intestine. One of the most important consequences of this enterohepatic circulation is their delaying effect on the final elimination of steroids from the body. In addition, the products of intestinal metabolism may be biologically more active than those excreted via the bile.

Orally administrated progesterone has little biological effect. It has been suggested[65,67] that the 17-acetoxy group in MA and MPA hinders the removal of the side-chain by intestinal bacteria.

The double bond at C6 of MA is very resistant to reduction by intestinal bacterial enzymes. MA is relatively unaffected under incubation conditions of human mixed intestinal flora.

In summary

Progesterone was discovered in 1930, but its use in the treatment of metastatic breast cancer besides other ablative and additive hormonal treatment was limited by short bioavailability. This has been overcome by the production of synthetic progestins in sixties, which are more active than progesterone and, unlike this, are also active after oral administration.

Most of the known actions of progesterone require estrogen priming. Estrogen has mainly growth stimulating effects, while the action of progesterone is more directed towards modification and differentiation.

Synthetic progestins may, on the basis of their chemical structure, have not only progestational but also androgenic, anti-androgenic and glucocorticoid properties.

The plasma level after a single oral administration of megestrol acetate (60 mg) reached the maximum after 1-4 hours; was still detectable after 24 hours while a mean recovery of $\pm$ 70% in the urine and $\pm$ 20% in the faeces was obtained within 7 days. The biological half time of this dose was $3\frac{1}{2}$ days.

3. MECHANISM OF ACTION OF HORMONES ON BREAST CANCER

General remarks

The growth, development and function of the human mammary gland is dependent on a number of hormones, among which estrogen and prolactin exert the most promoting effects. It may be expected that some tumors arising by malignant transformation of mammary gland cells would retain at least partly the sensitivity to these hormonal controls. Striking observations in this respect are that hormone-dependent mammary tumors regress not only after the host is deprived from estradiol, but also after an excess of estradiol is given.[8,32] This suggests that an equilibrium in the turnover of the cell population can be altered by hormonal treatment and that cell destruction may prevail over cell multiplication.

McGuire[68] (1975) postulated the following hypothesis: normal mammary cells contain cytoplasmic or membrane receptor sites for each of the hormones known to influence the growth and function of the mammary gland. These receptor sites are responsible for the initial interaction between the hormone and the cell, and function to trigger the biochemical chain of events characteristic for the particular hormone. When malignant transformation occurs, the cell may retain all or only part of the normal population of receptor sites. If the cell retains the receptor sites, its growth and function are potentially influenced by changes in the hormonal environment as in a normal cell. However, if the receptors are lost from the cell as a consequence of malignant transformation, the cell is no longer recognized as a target cell by circulating hormones and endocrine control is absent.

The presence of specific receptors in mammary tumor tissue may therefore indicate hormonal dependency, thus aiding to select breast cancer patients who will actually benefit from endocrine therapy.

Although a positive estrogen receptor (ER) assay is found in 70-85% of the breast tumors, only 55-60% of these respond to endocrine therapy.[68] Not all receptors are present in each individual cell. There is now good evidence that ER assay offers also a valuable indicator of prognosis. Especially in those patients found to have axillary node metastases at operation, it has

been found that ER+ tumors recur more slowly than ER- tumors[69,70] while these patients have a longer survival.[71] This finding may be associated with the observation that ER+ tumors tend to be highly differentiated[72,73] with slower growth[74] and a lower thymidine labelling index[75] and thus being less aggressive than ER- tumors.

± Thirty per cent of ER+ tumors fail to show a significant degree of regression after endocrine manipulation while ± 10% ER- do respond. Factors responsible for this discrepancy may be variations in assay technique, different cut-off levels used to distinguish between ER+ and ER-, and the degree of cellularity of the tumor specimen.[76]

The cytoplasmic ER assay is not necessarily a measure of functional activity of estrogen receptor in the cell. The estrogen molecule combines with the receptor protein in the cytoplasma but the complex needs to be translocated to the nucleus before it can stimulate RNA synthesis. Possibly the ER complex could be defective and fail to translocate to the nucleus or to stimulate RNA and protein synthesis.

The progesterone receptor (PgR) appears to be dependent on estrogen stimulation. Its presence has even been suggested as a marker of a functional estrogen receptor. PgR has been reported in 83% of breast cancer specimens containing more than 100 fmol ER/mg cytosol protein.[77]

Based on present knowledge a generalised theory of steroid hormone action has been developed. In tissues, responsive to both estrogens and progesterone, estradiol associates with a specific estrogen receptor which, after a temperature-dependent modification, enters the nucleus, associates with the nuclear chromatin, and induces production of RNA, further production of estrogen receptor and formation of specific progesterone receptor. The latter, in turn, facilitates the action of progesterone on the nuclear chromatin.

As both androgens and glucocorticoids have therapeutic effects in breast cancer, receptors for these steroids have been sought and also found. Estrogen, progesterone, androgen (AR) and glucocorticoid (GR) receptors were found in the human breast cancer line MCF-7, [78] and in DMBA-induced rat mammary tumors.[31,79,83] Allegra et al.[84] studied the occurrence of ER, PgR,

AR and GR in a large series of patients with primary and metastatic breast cancer. The presence of ER, but not that of PgR, AR and GR, showed a relation to menopausal status. In premenopausal patients, the mean ER quantity of the tumors is lower than in postmenopausal patients; to explain this it has been suggested that in the former group free ER sites are occupied by endogenous estrogen and are therefore unavailable to the assay. Another explanation is that of Saez et al.[85] suggesting that it is the cyclic progesterone secretion which inhibits estrogen stimulation of new ER synthesis in premenopausal women.

Although the role of prolactin is still uncertain, it has become apparent that some transplantable mammary cancers of the rat[86,87] and man[88-90] also bind polypeptide hormones, in particular prolactin. Pearson et al.[91] found prolactin receptors in 51% of human breast cancers in both estrogen positive and negative tumors.

Hormone-induced tumor regression

may result in changes in one or more of cell kinetic functions such as prolongation of the cell cycle, decrease in the fraction of cells in cycle or increase in the rate of cell loss.

If a tumor is of a well-differentiated type, only a small proportion of the cells are clonogenic and if they leave the cycle by giving rise to mature cells, the tumor will regress. In the case of an initially multiclonal tumor, loss of hormonal control could occur by clonal selection.[92] The resistant clones will proliferate when the sensitive ones have been eradicated by the endocrine therapy. Endocrine therapy kills only a proportion of cells in most late cancers so that the tumor does not disappear, but merely reduces in size. It temporarily stabilizes at a size, where the number of replicating cells is more or less equal to the number of cells dying. At some stage during tumor progression, part of the malignant cell population will overcome this block imposed on their activity by the hormonal environment.[93]

 Hormonal control of breast cancer probably involves interrelationships

between the actions of a number of hormones on the same cell. Some human breast cancers are able to synthesize hormones, either steroid or protein in type. It has been reported that one and the same mammary carcinoma can synthesize progesterone, androgen or estrogen from cholesterol and if this were so, it would have a profound bearing on the approach to therapy.[94-97]

In summary

The growth, development and function of the human mammary gland is sensitive to a number of hormones. It could be expected that after malignant transformation some tumors would retain these hormone dependencies. A local hormonal action can be exerted only if specific receptors are present in the tumor cell. The presence of an estrogen receptor seems a valuable indicator for the prognosis as to response to hormonal treatment. In addition, the presence of a progesterone receptor, which appears dependent on estrogen stimulation, has been suggested as marker of a functional estrogen receptor. The presence of other hormone receptors in breast cancer cells such as for androgen, glucocorticoid and prolactin, has been established.

Hormone-induced tumor regression may be due to changes in the cell cycle leading to its prolongation, to a decrease in the number of cells in cycle or an increase in the rate of cell loss.

must be considered in relation to the complete endocrinological milieu on the breast cancer cell and should be correlated with effective interference with estrogen-regulated growth processes. Hormonal manipulation in hormone dependent tumors may be directed towards:

1. Direct interference with steroid-receptor proteins

There is very little evidence for an interaction of progestins with estrogen receptors in the estrogen target tissue.

Estrogen acts through estrogen receptors to affect transcription and protein synthesis of a new estrogen receptor. Progestins at physiological concentrations act through the progesterone receptor to produce diminution of new estrogen receptor synthesis. This diminution of the cytoplasmic ER protein results in a reduced ability of estrogen to stimulate uterine growth.[98] Di Carlo et al.[99] observed a similar effect on rat uteri using an oral dose of 8 mg/kg of medroxyprogesterone acetate.

Using pharmacological concentrations of different progestins (up to 50.000 fold in excess) an effective competition with estradiol binding has been reported in rats.[100]

The presence of a progesterone receptor suggests that the action of progestins in breast tumor cell homeostasis involves a receptor protein. In fact the evidence for interaction of progestins with estrogen receptors is sparse, which is not the case for androgen receptors.

Detailed studies on various androgen-target organs in mice, especially the kidney, have shown that progestins can exert a variety of effects dependent on steroid structure, dose, target organ and end-point studies.[101,102] These parameters together determine whether the effects of the progestins are androgenic, synandrogenic or anti-androgenic. This can be explained by the interaction of the progestin with the androgen receptor. There has been established a relation of steroidal structure to the capacity for modulation of androgen action. A 6 α-methyl substitution correlated with androgenic activity, which was enhanced by a 17 α-acetoxy substitution.[58]

In addition, introduction of a 17 α-acetoxy group produces significant anti-glucocorticoid activity. The further presence of a 6 α-methyl group enhanced this activity of synthetic progestins[57] and the similar interactions of the progestins with the glucocorticoid receptor was reported[103], if progestins were used in pharmacological (200-fold higher) concentration.

2. Direct cytotoxic effect

The morphological changes following therapy with progestins were observed on endometrial carcinoma clinically and experimentally, both in vitro and in vivo using methods such as histology, histochemistry, electron microscopy, liquid scintillation radioautography and historadio-autography.[104]

These findings suggest that the mechanism of action of progestins is also directly located within the cell. In vitro studies using short-term incubation systems suggest that the inhibition of DNA and RNA synthesis as effect of progesterone and progestins in endometrial carcinoma is dose dependent both in humans[105,106] and rodents.[107] Dose dependent was also the inhibition of ^{3}H-thymidine incorporation into DNA and the mitotic activity of experimental mammary tumor cell line MCF-7, incubated with MPA.[108]

High doses of progestins produce tissue necrosis, but at low concentrations tissue differentiation. Regression in endometrial carcinoma is attributed to growth suppression and degeneration. These alterations are accompanied by a decrease and eventual arrest in mitotic activity with a drop of the mitotic index, increase in shrinkage necrosis (apoptosis) and cell digestion (autophagy).[105]

3. Influence on several endocrine events

One of the most important effects of progestins is the lowering of LH/FSH secretion with subsequent decrease of circulating estrogens and androgens.[51,55,109]

Their anti-androgenic effect is the result of lowered plasma testosterone levels and in addition an increasing rate of catabolism of androgens due to increased hepatic 5 α-reductase activity[110] resulting in a further lowering of plasma testosterone levels. This also leads to a lower rate of androgen conversion to estrogens and consequently to lower target tissue uptake of testosterone. The decrease of the SHBG concentration in the plasma may be attributed to these anti-androgenic properties too.[111,112]

The relation between the steroidal structure of progestins and the capacity for modulation of androgenic action has been determined.[58] A 6 α-methyl substitution was correlated with androgenic activity and was enhanced by a 17 α-acetoxy substitution. Besides competing with estrogen for specific estrogen receptors, progestins produce their anti-estrogenic effect by blocking the secretion of gonadotropins as mentioned above and by accelerating the enzymatic transformation of estradiol into estrone[113]. Thus the length and degree of cell exposure to the most effective estrogen will be reduced.

A glucocorticoid-like effect has been found in animal experiments. Salander[114] reported adrenocortical atrophy after exposure to high doses of megestrol acetate (2 mg/rat/day). Orally administered progesterone is devoid of glucocorticoid activity, but the introduction of a 17 α-acetoxy group produces significant glucocorticoid activity. The additional presence of a 6 α-methyl group provides significant eosinopenic and hyperglycaemic properties, however, a 6 α-methyl in combination with 6-ene reduced these by about 50%. Megestrol acetate has a weak glucocorticoid activity, while medroxyprogesterone acetate has a stronger one, because of absence of this 6-ene binding. These glucocorticoid actions of progestins may be mediated by the compounds themselves, or by their metabolites.[57] In addition, competition for glucocorticoid receptor binding has been mentioned.[103]

There are only few data about the influence of insulin on tumor growth. During progestin therapy hyperinsulinaemia may be induced. The effect of insulin on tumor growth or regression should therefore be considered. Insulin is a critical hormone in the normal differentiation of the

mammary gland and is required for normal lactogenesis in rodents. Although an important regulatory role for insulin in human breast cancer has not been established, in vivo it has been reported that in tissue cultures at "physiologic" concentration insulin strongly stimulates the growth of both human[115] and DMBA-induced rat mammary cancer cell lines.[116] In long-term tissue culture of human mammary cancer cell lines specific receptors for insulin have been demonstrated and specific binding and its biological effect are well correlated.[117]

In summary

Progestin-induced tumor regression must be considered in relation to the complete endocrinological milieu on the breast cancer cell and should be correlated with effective interference with an estrogen-regulated growth process. Hormonal manipulation in hormone dependent tumors may be directed towards:

1. Interference with:
   - estrogen-receptor interaction, synthesis and turnover of estrogen receptor;
   - other receptor proteins than those for estrogen.
2. Cytotoxicity: large concentrations of progestin could have a damaging effect on cellular organelles.
3. Influence on several endocrine events such as decrease of concentration of LH,FSH,estrogens,androgens,cortisol and ACTH.

5. EFFECTS OF PROGESTINS ON CARBOHYDRATE METABOLISM AND INSULIN, LIPID-, PROTEIN-, WATER- AND SALT METABOLISM

Effect on carbohydrate metabolism and insulin

Because of the well-known adverse effects of pregnancy on the metabolic equilibrium in diabetic women, Beck[118] (1969) undertook a study on the pancreatic response to glucose in rhesus monkeys treated with i.m. progesterone. No consistent effects on either the mean fasting serum glucose or insulin concentrations were found but progesterone treatment caused a marked increase in the plasma insulin response to intravenously administered glucose, while the disappearance rate of glucose remained constant. At the same time progesterone reduced the sensitivity of these animals to exogenous insulin. Progesterone administration also induced pancreatic islet hypertrophy and exaggerated insulin secretion in vitro in response to glucose.[119]

The existence of sex steroid receptors in pancreatic islets has been reported.[120] Moreover, in vitro cultures of islets with progesterone showed an increased insulin secretion, suggesting a direct β-cytotropic action of this hormone.[121]

The liver is a major organ for steroid metabolism, so effects of progesterone on hepatic metabolic pathways could be expected. Progesterone administration to the intact female rat increases the glycogen content of the liver and suppresses hepatic gluconeogenesis.[122]

Progesterone also blunts the hyperglycemic effects of cortisol administration[118] and lowers the plasma glucose response to intravenous arginine infusion.[123]

Since all of these effects are insulin-like and since progesterone induces hyperinsulinaemia, one cannot distinguish between direct effects of progesterone on these processes and indirect actions mediated by augmented insulin secretion.[124]

On the other hand progesterone administration to rats reduces the sensitivity of adipocytes and skeletal muscle to insulin-induced glucose uptake and oxidation.[125,126]

Effect on lipid metabolism

In the female rat exposed to progesterone, hyperphagia and weight gain developed with substantial increases in carcass fat.[127]

Progestins enlarged the adipose cell size but did not affect the cell number.[128] Progesterone also induces increased lipoprotein lipase activity in the liver and mammary gland.[129] Administration of progesterone to humans and animals has little effect on plasma-free fatty acids or triglycerides.

Effect on protein metabolism

After protein-anabolic effects in animals have been reported[130], only few reports on protein metabolism in have been reported in literature.

Pregnancy as well as administration of estrogens or combined oral contraceptive agents are associated with significant alterations of plasma proteins.[131] There is little evidence, however, that progesterone has major effects on these.[131,132]

The work of Landau and Poulous[133] (1971) suggests that progesterone has a weak catabolic action in man. The basic effects are a lowering of several plasma aminoacids and an increased total urinary nitrogen excretion without associated aminoaciduria. They concluded that loss of protein from muscle may not be pronounced, since the increased urinary nitrogen was largely urea.

There are no reports on the effects of progestins.

Effect on water and salt metabolism

The natriuretic effect of progesterone in humans was reported by Landau et al.[134] (1965) and has further been confirmed in animals. It is likely that this hormone participates in the physiological control of water and salt metabolism, especially when its secretion rate is high, as in the second phase of the menstrual cycle and during pregnancy. A study of the binding of progesterone and some of its metabolites to the mineralocorticoid receptor[135] revealed that progesterone was able to displace ^{3}H-aldosterone from its binding sites.

Only a few studies have been carried out concerning the alteration of water and salt metabolism during progestin treatment.[136] The progestins were

shown to have a much lower natriuretic effect than progesterone itself.

In summary

Progesterone induces hyperinsulinaemia, promotes glycogen storage in the liver, stimulates deposition of body fat, increases natriuresis and might have a weak catabolic effect.

With progestins an additional effect on gluconeogenesis should also be considered because of glucocorticorticoid properties of some progestins, while the natriuretic effect seems to be lower.

6. RELATION OF PROGESTERONE AND PROGESTINS TO PROLACTIN

Prolactin is important for the development and growth of mammary tumors in mice and rats[137] and is also a hormone of primary importance that influences the growth of hyperplastic nodules and their conversion to tumors in mice. In contrast, once mammary tumors have developed, prolactin does not appear to be necessary for their maintenance or growth.[138]
In contrast to the murine mammary tumor model, in DMBA-induced mammary tumors in rat, the growth continues to be influenced by the hormonal environment, once tumors are induced. Increases in serum prolactin enhance tumor growth.[139] The stimulatory effect of prolactin on tumor growth in rats requires the presence of estrogen.[140]

In human breast cancer the role of prolactin is much less clear. The inability to demonstrate elevated prolactin levels in patients with breast cancer, however, does not preclude the possible importance of prolactin in the pathogenesis of breast cancer or the possible usefulness of its suppression in therapy.[141-144] However, some data seem to point to elevated serum prolactin levels in patients with malignant and benign breast lesions[145-147], relatives and daughters of patients with breast cancer during the luteal phase[148,149], although recently published data[150] concluded that the "early evening prolactin peak" cannot be used easily to identify individual risk in 1st degree relatives of breast cancer.
There is some evidence that seems to contradict a major role of prolactin in breast neoplasms. McMahon et al.[151] (1973) argumented that prolactin levels are very high during pregnancy and lactation and yet these two conditions do not increase the risk of breast cancer (unless the first pregnancy is after age 35). Possibly the interaction between steroid and polypeptide hormones during these periods is such that these high levels of prolactin lead to glandular differentiation rather than to growth promotion and the role of prolactin may enhance a mutagenic effect of estrogen rather than to be a primary mutagen itself.[152]
Binding of prolactin to specific receptors located on the membrane of the cell is the first event in the action of this and other polypeptide

hormones. Specific prolactin binding has been identified in a number of tissues of experimental animals and humans including the chorioid plexus of the brain, liver, kidney, mammary gland, adrenal, ovary, testis, prostate, seminal vesicle and uterus.[153-157]

Prolactin binding capacity in human mammary carcinoma is low compared with that found in prolactin-responsive experimental mammary carcinoma.[155]

The role of progesterone in prolactin release or inhibition has not been elucidated. Haug[158] (1979) studied the effects of progesterone, testosterone, corticosterone and TRH on estrogen-induced prolactin synthesis and cytoplasmic estrogen receptor levels in a clonal strain of rat pituitary tumor cells. His data strongly suggest that progesterone inhibits estrogen-induced prolactin synthesis by decreasing the number of available estrogen receptor sites.

Administration of MPA or MA to rats bearing DMBA-induced mammary tumors[159] or estrogen-induced prolactinomas[160] caused a decrease in the circulating prolactin levels.

On the other hand, progesterone administered i.m. induced an acute release of prolactin in estrogen-primed ovariectomized women.[161] In one subject given progesterone prior to ethinyl-estradiol priming, no effect on the circulating levels of prolactin was observed. They suggested that this effect of progesterone may be mediated through a reduction of hypothalamic dopamine, the prolactin inhibiting factor. Estrogen promotes dopamine turnover, progesterone on the other hand, may reduce dopamine activity by virtue of its ability to inhibit tyrosine hydroxylase (the rate-limiting enzyme for catecholamine synthesis) as has been demonstrated in the median eminence of ovariectomized rats after estrogen priming.[162,163]

In addition, progesterone and several of its A-ring saturated metabolites are potent inhibitors of the NADPH-dependent microsomal drug metabolizing system.[164] Tyrosine hydroxylase, appears to be likewise susceptible to inhibition by progestins. Hyperprolactinemia was reported in women receiving low dose of medroxyprogesterone acetate for contraception.[165] On the other hand during treatment of metastatic breast cancer with high dosages no significant changes were found.[166]

Prolactin could be involved in the development and growth of breast tumors, at least in a supportive role not only in animal models[167] but also in humans.[137,168]

Recently reports were published about the prophylaxis of spontaneous mammary tumorigenesis in rats by inhibition of prolactin secretion using the prolactin suppressor bromocriptine[169] and in DMBA-induced tumors in rats and mice.[170]

Welsch et al.[171] (1982) used the antiestrogen tamoxifen as prolactin suppressor and they found the same reduction of incidence of mammary cancer in DMBA-treated female rats as using bromocriptine, while a further reduction occurred with the combination of both.

Even though data about the involvement of prolactin in malignant breast tumor growth in humans are conflicting, the detection of prolactin receptors in about 35-50% of human breast cancer with apparent lack of correlation with steroid hormone receptors[91,172] indirectly supports an importance of this hormone as a growth factor in malignant neoplasia.

Recently Dogliotti et al.[173] (1983) reported that with a combination of the progestin MPA and bromocriptine in the treatment of metastatic breast cancer a higher response rate (C.R.+P.R. = 54%) of longer mean duration of response (24 months) was reached than could be expected from an unselected (to ER status) group of patients with metastatic breast cancer.

In summary

The influence of progesterone or progestins on the secretion of prolactin has not been elucidated. They seem to cause a release in humans, while in animal models (DMBA-induced mammary carcinoma or estrogen-induced prolactinomas in rats) they cause a decrease of the circulating prolactin levels.

There is no equivocal evidence that lowering serum prolactin levels in itself as a primary goal is of benefit for the treatment of metastatic breast cancer in man.

The influence of combination treatment of a steroid and a prolactin release inhibitor deserves further investigation.

7. ADVERSE EFFECTS

A major goal of cancer therapy is to find an optimal relationship between desired and undesired effects of a given treatment.

The pattern of undesired effects of megestrol acetate and other progestins is partly similar to that observed with other steroid-hormonal agents such as estrogens, glucocorticoids and androgens: weight gain (however, without fluid retention), increase in blood pressure, changes in carbohydrate metabolism, blood coagulation, thromboembolic disorders, vaginal bleeding. Hypercalcaemia has not been reported. There are no reports about adverse effects of megestrol acetate concerning deterioration of glucose tolerance[174], although this has been reported in patients using medroxyprogesterone acetate.[175,176] Weight gain is a very common observation.[177] Studies of blood coagulation in women treated with megestrol acetate implants as a contraceptive[178] showed normal values without a difference between short and long-term treatment.

Brema et al.[179] (1981) studying several hematologic parameters during treatment with a high dose of medroxyprogesterone acetate found that prothrombin time (PTT), thromboelastogram (TEG), anti-thrombin III and platelet adhesiveness underwent statistically significant changes, tending towards hypercoagulability, although, on the average, they did not exceed the upper normal ranges.

There are many data concerning the relation between thromboembolic diseases and oral contraceptives[180]. However, there seems to be a correlation with their estrogen content, and factors as age, weight, cigarette smoking, history of hypertension or diabetes mellitus multiplied the risk of cardiovascular disease.

In order to prevent and minimize possible adverse effects, careful clinical and laboratory assessment is important to identify before the start of therapy those patients, who are at high risk to develop undesired effects during progestin treatment. Patients with a history of hypertension, obesity, diabetes, myocardial infarction, thromboembolic disorders and heavy smokers should be carefully monitored during the treatment.

 With the accumulation of more knowledge on the mechanism of action of

natural and synthetic steroids, more rationale as with regard to the optimal effective dose, their combination or sequence of use, will follow.

In summary

The pattern of undesired effects of megestrol acetate and other progestins is similar to that observed with other steroid hormonal agents, but without fluid retention. Therefore, careful clinical and laboratory assessment as to weight gain, blood and urine sugar and cardiovascular control is recommended.

8. EVALUATION OF CLINICAL RESULTS

In the following studies the responses are assessed according to the generally accepted method, recommended by UICC (Union Internationale Contre le Cancer)[181]; the recording of lesions was done by "dominant sites", which is commonly used.[182-184] By this method patients are always classified into the visceral category when visceral lesions are present, the osseous category when skeletal lesions exist without visceral involvement, and into the soft tissue category when only sub- and cutaneous, mammary of superficial lymphatic structures are involved:

I. Visceral : - visceral (lung/liver) metastases
- visceral + bone metastases
- visceral + bone + soft tissue metastases

II. Osseous : - bone metastases
- bone + soft tissue metastases

III. Soft tissue : - soft tissue metastases

However, as Rozencweig[185] (1975) pointed out in an extensive survey article, the interpretation of the prognostic value of "dominant sites" categories, based the general view that the prognosis is increasingly worse in the order: soft, osseous and visceral tissue, is difficult due to their very definition of these categories and the criteria of response to treatment. Thus, soft tissue lesions are often the sole objective criterion of evaluation in patients belonging to the osseous or even visceral category. Other factors may be included in the evaluation of therapeutic trials, such as disease-free interval, number of sites[186], type of organ and degree of involvement.[184,187]

The categories of response are as follows:

Complete response. Disappearance of all known disease. In the case of lytic bone metastases: these must be shown radiologically to have calcified.

Partial response. 50% decrease in measurable lesions, and objective improvement in evaluable, but non-measurable lesions. No new lesions. It is

not necessary for every lesion to have regressed to qualify for partial response, but no lesion should have progressed.

No change. Lesions unchanged (i.e. < 50% decrease or < 25% increase in the size of measurable lesions).
If non-measurable, but evaluable lesions represent the bulk of disease and these clearly do not respond, even though measurable lesions have improved, then this is considered as "no change" and not "objective regression".

Progressive disease

1. Mixed - some lesions regress while others progress or new lesion appear.
2. Failure - progression of some or all lesions and/or appearance of new lesions. No lesions regress.

All lesions should be bidimensionally measured at each assessment and the sum of the product defined. When multiple lesions are present, only a representative number of eight may be selected for measurement.

In summary

The results of clinical trials should be considered with regard to inaccuracy of assessment. The prognosis and response may not only depend on the site of metastases, but also on the number of sites, size of metastases and degree of organ involved.

REFERENCES

1. Beatson, G.Th.: On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment; with illustrative cases. Lancet II, 104-107 and 162-165, 1896

2. Schinzinger, A.S.: Ueber Carcinoma mammae. Protokolle, Discussionen, kleinere Mittheilungen. Verh.dtsch.Gesellsch.Chir. 18:28-29, 1889

3. Clarke, W.: An analysis of 99 cases of inoperable carcinoma of the breast treated by oophorectomy. Lancet I, 227, 1905

4. Allen, W.M.: Physiology of the corpus luteum. V. The preparation and some chemical properties of progestin, a hormone of the corpus luteum which produces progestational proliferation. Amer.J.Physiol. 92:174-188, 1930

5. Loeser, A.A.: Male hormone in the treatment of cancer of the breast. Acta, Union internat. contre cancer 4:375, 1939

6. Ulrich, P.: Testostérone (hormone male) et son role possible dans le traitement de certains cancers du sein. Acta, Union internat. contre cancer 4:377, 1939

7. Biden, W.M.: Stilboestrol for breast tumour. Brit.med.J. II, 57, 1943

8. Haddow, A., Watkinson, J.M., Paterson, E. and Koller, P.: Influence of synthetic oestrogens upon advanced malignant disease. Brit.med.J. II, 393, 1944

9. Adair, F.E., Mellors, R.C., Farrow, J.H., Woodard, H.Q., Escher, G.C. and Urban, J.A.: The use of estrogens and androgens in advanced mammary cancer. J.Amer.med.Ass. 140:1193-1200, 1949

10. Nathanson, I.T.: Clinical investigative experience with steroid hormones in breast cancer. Cancer 5:754-762, 1952

11. Huggins, C. and Bergenstal, D.B.: Inhibition of human mammary and prostatic cancers by adrenalectomy. Cancer Res. 12:134-141, 1952

12. Segaloff, A., Carabasi, R., Horwitt, B.N., Schlosser, J.V. and Murison, P.J.: Hormonal therapy in cancer of the breast. VI. Effect of ACTH and cortisone on clinical course and hormonal excretion. Cancer 7:331-334, 1954

13. Koffman, S., Nagamani, D., Buenger, R.E., et al.: The use of prednisolone in the treatment of disseminated breast carcinoma. Cancer II, 226-232, 1958

14. Lemon, H.M.: Prednison therapy of advanced mammary cancer. Cancer 12:93-107, 1959

15. Council on Drugs. Androgen and estrogens in the treatment of disseminated mammary carcinoma. Retrospective study of nine-hundred-forty-four patients. Report to the Council. J.Amer.med.Ass. 172:1271, 1960

16. Cooperative Breast Cancer Group. Progress Report. Results of studies by the Cooperative Breast Cancer Group 1956-1960. Cancer Chemother.Rep. 11:109, 1961

17. Segaloff, A., Weeth, J.B., Rongone, E.L., Murison, P.J., Bowers, C.Y.: Hormonal therapy in cancer of the breast. XVI. The effect of 1-Testololactone on clinical course and hormonal excretion. Cancer 13:1017-1020, 1960

18. Gilse, H.A. van: Long-term treatment with corticosteroids of patients with metastatic breast cancer. Cancer Chemother.Rep. 16:293, 1962

19. Lacassagne, A.: Apparition de cancers de la mamelle chez la souris male, soumise à des injections de folliculine. C.R.Acad.Sci.(Paris) 195:630-632, 1932

20. Foulds, L.: Mammary tumours in hybrid mice: growth and progression of spontaneous tumours. Brit.J.Cancer 3:345-375, 1949

21. Mühlbock, O.: The hormonal genesis of mammary cancer. In: Advances in Cancer Research. J.P. Grenstein and A. Haddow, editors. New York, Academic Press, 4:371-391, 1956

22. Shay, H., Aegerter, E.A., Gruenstein, M. and Komarov, S.A.: Development of adenocarcinoma of the breast in the wistar rat following the gastric instillation of methylcholanthrene. J.Natl.Cancer Inst. 10:255-266, 1949

23. Huggins, C., Grand, L.C. and Brillantes, F.P.: Mammary cancer induced by a single feeding of polynuclear hydrocarbons, and its suppression. Nature 189:204, 1961

24. Soule, H.D., Vazquez, J., Long, A., Albert, S. and Brennan, M.: A human cell line from a pleural effusion derived from a breast carcinoma. J.Natl.Cancer Inst. 51:1409, 1973

25. Brooks, S.C., Locke, E.R. and Soule, H.: Estrogen receptor in a human receptor in a human cell line (MCF-7) from breast carcinoma. J.Biol.Chem. 248:6251, 1973

26. Pearson, O.H., West, C.D., Hollander, V.P. and Treves, N.: Evaluation of endocrine therapy for advanced breast cancer. J.Amer.med.Ass. 154:234, 1954

27. Nathanson, I.T.: Hormonal alteration of advanced cancer of the breast. Surg.Clin.N.Amer. 27:1144, 1947

28. Nathanson, I.T.: The effect of stilboestrol on advanced cancer of the breast. Cancer Res. 6:484, 1946

29. Glascock, R.F. and Hoekstra, W.G.: Selective accumulation of tritium-labelled hexoestrol by the reproductive organs of immature female goats and sheep. Biochem.J. 72:673-682, 1959

30. Jensen, E.V. and Jacobson, H.I.: Fate of steroid estrogens in target tissues. In: Biological activities of steroids in relation to cancer. G. Pincus and E.P. Vollmer, editors. New York, Academic Press, pp. 161-178, 1960

31. Jensen, E.V., De Sombre, E.R. and Jungblut, P.W.: Estrogen receptors in hormone-responsive tissues and tumors. In: Endogenous factors influenced host-tumor balance. R.W. Wissler, T.L. Dao and S. Wood, Jr., editors. Chicago, University of Chicago Press, pp. 18-30, 68, 1967

32. Huggins, C., Moon, R.C.: Extinction of experimental mammary cancer; β estradiol-17 and progesterone.
Proceedings of the National Academy of Sciences of the United States of America, 48:379, 1962

33. Stoll, B.A.: Progestin therapy of breast cancer: comparison of agents. Brit.med.J. 3:338-341, 1967

34. Babcock, J.C., Gutsell, E.S., Herr, M.E. et al.: 6 α-methyl-17 α-hydroxyprogesterone 17-acylates, a new class of potent progestins. J.Amer.Chem.Soc. 80:2904, 1958

35. Sala, G., Camerino, B. and Cavallero, C.: Progestational activity of 6 α-methyl-17 α-hydroxyprogesterone acetate. Acta Endocrinol. 29:508, 1958

36. Muggia, F.M., Cassileth, P.A., Ochoa Jr., M., Flatow, F.A., Gellhorn, A. and Hyman, G.A.: Treatment of breast cancer with medroxyprogesterone acetate. Ann.int.Med. 68:328-337, 1968

37. Pannuti, F., Martoni, A., Pollutri, E. et al.: Massive dose progestational therapy in oncology (medroxyprogesterone). Preliminary results. Pan.Minerva Med. 18:129, 1976

38. Pannuti, F., Martoni, A., Lenaz, G.R. et al.: A possible new approach to the treatment of metastatic breast cancer: massive doses of medroxyprogesterone acetate. Cancer Treat. Rep. 62:449, 1978

39. Mattsson, W.: High dose medroxyprogesterone-acetate treatment in advanced mammary carcinoma. Acta Radiol.Oncol. 17:387-400, 1978

40. Robustelli Della Cuna, G., Calciati, A., Strada, M.R.B., Bumma, C. and Campio, L.: High dose medroxyprogesterone acetate (MPA) treatment in metastatic carcinoma of the breast: a dose-response evaluation. Tumori 64:143-149, 1978

41. Ganzina, F.: High-dose medroxyprogesterone acetate (MPA) treatment in advanced breast cancer. A review. Tumori 65:563-585, 1979

42. Pagani, C.: 6-metil-6-deidro-17 α-idrossi progesterone acetato e dinamica uterina (Ricerche sperimentali). Ann.Ostet.Ginec. 4:387, 1961

43. Ansfield, F.J., Davis Jr., H.L., Ellerby, R.A., Ramirez, G.: A clinical trial of megestrol acetate in advanced breast cancer. Cancer 33:907-910, 1974

44. Ansfield, F.J., Davis Jr., H.L., Ramirez, G., Davis, T.E., Borden, E.C., Johnson, R.O., Bryan, G.T.: Further clinical studies with megestrol acetate in advanced breast cancer. Cancer 38:53-55, 1976

45. Morgan, L.R.: Tamoxifen versus megestrol acetate in breast cancer. Cancer Treat.Rep. 63 (7):1218. Abst.380, 1979

46. Blonk-v.d.Wijst, J., Alexieva-Figusch, J., Gilse, H.A. van: Megestrolacetate, a non toxic effective hormonal treatment of metastatic breast cancer. Abstr.42, p. S18: Medical Oncology; abstracts of the 3rd Annual Meeting of the Medical Oncology Society and of the Bi-Annual Meeting of the Immunology and Immunotherapy Group (C12G); Nice, December 4-6, 1977

47. Harper, M.J. and Walpole, A.L.: Contrasting Endocrine Activities of cis and trans Isomers in a series of Substituted Triphenylethylenes. Nature 212:87, 1966

48. Corner, G.W. and Allen, W.M.: Physiology of the corpus luteum. II. Production of a special uterine reaction (progestational proliferation) by extracts of the corpus luteum. Amer.J.Physiol. 88:326-339, 1929

49. Butenandt, A. and Westphal, U.: Uber die Darstellung des Corpus-luteum-Hormons aus Stigmasterin: die Konstitution des Corpus-luteum-Hormons. Ber.Deut.Chem.Gesellsch. 67:2085-2087, 1934

50. Allen, W.M., Butenandt, A., Corner, G.W. and Slotta, K.H.: Nomenclature of corpus luteum hormone. Science 82:153, 1935

51. Tausk, M.: Pharmacology of the endocrine system and related drugs: Progesterone, progestational drugs and antifertility agents. In: International encyclopedia of pharmacology and therapeutics. Executive editor: G. Peters, Lausanne. Section 48, Volume I, 1971

52. Balin, H. and Glasser, S. eds.: Reproductive Biology. Excerpta Med., Amsterdam, 1972

53. O'Malley, B.W. and Means, A.R.: The mode of action of the female sex steroids. In: Biochemistry Series One. Biochemistry of Hormones. H.V. Rickenberg, ed. Butterworths, London, Volume VIII, 1974

54. Martin, C.R.: Textbook of endocrine physiology. The Williams and Williams Company, Baltimore, 1976

55. Neumann, F.: The physiological action of progesterone and the pharmacological effects of progestogens - a short review. Postgraduate Medical Journal 54:11-24, 1978

56. Tausk, M.: Pharmacology of the endocrine system and related drugs: Progesterone, progestational drugs and antifertility agents. In: International encyclopedia of pharmacology and therapeutics. Executive editor: G. Peters, Lausanne. Section 48, Volume II, 1972

57. Briggs, M.H., Briggs, M.: Glucocorticoid properties of progestogens. Steroids 22:555-559, 1973

58. Gupta, C., Bullock, L.P., Bardin, C.W.: Further studies on the androgenic, anti-androgenic and synandrogenic actions of progestins. Endocrinology 120:736-744, 1978

59. Cooke, B.A. and Vallance, D.K.: Metabolism of 17 α-Acetoxy-6 α-methyl-pregna-4,6-diene-3,20-dione and related progesterone analogues by liver preparations in vitro. Biochem.J. 91:31, 1964

60. Cooper, J.M., Jones, H.E.H. and Kellie, A.E.: The metabolism of megestrol acetate (17 α-acetoxy-6-methylpregna-4,6-diene-3,20-dione) in the rabbit. Steroids 6:255, 1965

61. Cooper, J.M. and Kellie, A.E.: The metabolism of megestrol acetate (17 α-acetoxy-6-methylpregna-4,6-diene-3,20-dione) in women. Steroids 11:133-149, 1968

62. Adlercreutz, H. and Ervast, S.: Mass fragmentographic determination of megestrol acetate in plasma. Acta endocr.(Kbh.), Suppl.177, p.32, 1973

63. Adlercreutz, H., Nieminen, U. and Ervast, H.S.: A mass fragmentographic method for the determination of megestrol acetate in plasma and its application to studies on the plasma levels after administration of the progestin to patients with carcinoma corporis uteri. J.Ster.Biochem. 5:619-626, 1974

64. Adlercreutz, H., Martin, F., Wahlroos, O. and Soini, E.: Mass spectrometric and mass fragmentographic determination of natural and synthetic steroids in biological fluids. J.Ster.Biochem. 6:247-259, 1975

65. Adlercreutz, H., Martin, F., Järvenpää, P. and Fotsis, T.: Steroid absorption and enterohepatic recycling. Contraception, Vol.20 No.3, September 1979

66. Adlercreutz, H., Eriksen, P.B. and Christensen, M.S.: Plasma concentrations of megestrol acetate after single oral administration to healthy female subjects. Journal of Pharmaceutical & Biochemical Analysis, Vol.I, No.2, 1983

67. Martin, F., Järvenpää, P., Kosunen, K., Somers, C., Lindstrom, B., Adlercreutz, H.: Ring-A reduction of medroxyprogesterone acetate (17 α-acetoxy-6 α-methyl-4-pregnene-3,20-dione (MPA) in biological systems. J.Ster.Biochem. 12:491-497, 1980

68. McGuire, W.L.: Current status of estrogen receptors in human breast cancer. Cancer 36:638-644, 1975

69. Knight, W.A., Livingston, R.B., Gregory, E.J. and McGuire, W.L.: Estrogen Receptor as an Independent Prognostic Factor for Early Recurrence in Breast Cancer. Cancer Res. 37:4669-4671, 1977

70. Maynard, P.V., Blamey, R.W., Elston, C.W., Haybittle, J.L. and Griffiths, K.: Estrogen Receptor Assay in Primary Breast Cancer and Early Recurrence of the Disease. Cancer Res. 38:4292-4295, 1978

71. Bishop, H.M., Elston, C.W., Blamey, R.W., Haybittle, J.L., Nicholson, R.I., Griffiths, K.: Relationship of oestrogen-receptor status to survival in breast cancer. Lancet, Preliminary Communications, p.28, August 11, 1979

72. Martin, P.M., Rolland, P.H., Jacqmier, J., et al.: Estrogen receptor in human breast cancer. Correlation with anatomo-pathologic examination. Reviews on Endocrine Related Cancer, Supplement, April 1978

73. Griffiths, K., Maynard, P.V., Wilson, D.W. and Davies, P.: Endocrine aspects of primary breast cancer. Reviews on Endocrine Related Cancer, Supplement, April 1978

74. May Levin, F., Delarne, J.C., Bohnon, C. and Contesso, G.: Estrogen and progesterone cytosol receptors in human malignant and benign breast tumors. Reviews on Endocrine Related Cancer, Supplement, April 1978

75. Meyer, J.S., Rao, B.R., Stevens, S.C. and White, W.L.: Low incidence of estrogen receptor in breast carcinomas with rapid rates of cellular replication. Cancer 40:2290-2298, 1977

76. Kiang, D.T. and Kennedy, B.J.: Factors affecting estrogen receptors in breast cancer. Cancer 40:1571, 1977

77. McGuire, W.L.: Hormone Receptors: Their Role in Predicting Prognosis and Response to Endocrine Therapy. Seminars in Oncology, 5:428-433, 1978

78. Horwitz, K.B., Costlow, M.E. and McGuire, W.L.: MCF-7; a human breast cancer line with estrogen, androgen, progesterone, and glucocorticoid receptors. Steroids 26:785, 1975

79. McGuire, W.L. and Julian, J.A.: Comparison of macromolecular binding of estradiol in hormone-dependent and hormone-independent rat mammary carcinoma. Cancer Res. 31:1440-1445, 1971

80. Asselin, J., Labrie, F., Kelly, P.A., Philibert, D. and Raynand, J.P.: Specific progesterone receptors in dimethylbenz(a)anthracene (DMBA)-induced mammary tumors. Steroids 27:395-404, 1976

81. Horwitz, K.B. and McGuire, W.L.: Progesterone and progesterone receptors in experimental breast cancer. Cancer Res. 37:1733-1738, 1977

82. Goral, J.E. and Wittlif, J.L.: Comparison of glucocorticoid- binding proteins in normal and neoplastic mammary tissues of the rat. Biochemistry 14:2944-2951, 1975

83. Ip, M.M., Milholland, R.J., Kim, V. and Rosen, F.: Characterisation of androgen receptors in 7,12-dimethylbenz(a) anthracene-induced and transplantable rat mammary tumors. Cancer Res. 38:2879-2885, 1978

84. Allegra, J.C., Lippman, M.E., Thompson, E.B., Simond, R., Barlock, A., Green, L., Huff, K.K., Do, H.M.T. and Aitken, S.C.: Distribution, Frequency, and Quantitative Analysis of Estrogen, Progesterone, Androgen and Glucocorticoid Receptors in Human Breast Cancer. Cancer Res. 39:1447-1454, 1979

85. Saez, S., Martin, P.M. and Chouvet, C.D.: Estradiol and Progesterone Receptor Levels in Human Breast Adenocarcinoma in Relation to Plasma Estrogen and Progesterone Levels. Cancer Res. 38:3468-3473, 1978

86. Costlow, M.E., Buschow, R.A., Richert, N.J. and McGuire, W.L.: Prolactin and Estrogen Binding in Transplantable Hormone-dependent and Autonomous Rat Mammary Carcinoma. Cancer Res. 35:970-974, 1975

87. Turkington, R.W.: Prolactin Receptors in Mammary Carcinoma Cells. Cancer Res. 34:758-763, 1974

88. de Souza, I., Hobbs, J.R., Morgan, L. and Salih, H.: Localisation of prolactin in human breast tumours. J.Endocr., Vol.73, 1977

89. Holdaway, I.M. and Friesen, H.G.: Hormone binding by human mammary carcinoma. Cancer Res. 37:1946-1952, 1977

90. Morgan, L., Raggatt, P.R., de Souza, J., Salih, H. and Hobbs, J.R.: Prolactin receptors in human breast tumours. J.Endocr., Vol.73, 1977

91. Pearson, O.H., Manni, A., Chambers, M., Brodkey, J. and Marshall, J.S.: Role of Pituitary Hormones in the growth of Human Breast Cancer. Cancer Res. 38:4323-4326, 1978

92. Nowell, P.C.: The clonal evolution of tumor cell population. Science 194:23-28, 1976

93. Stoll, B.A.: Perspectives on Hormonal Therapy in Cancer. Clin.Oncol. 1:3-19, 1982

94. Adams, J.B. and Li, K.: Biosynthesis of 17β-oestradiol in human breast carcinoma tissue and a novel method for its characterization. Brit.J.Cancer 31:429, 1975

95. Couch, R.A.F., Skinner, S.J.M., Tobler, C.J.P. and Doouss, T.W.: The in vitro synthesis of 7-hydroxydehydroepiandrosterone by human mammary tissues. Steroids 26:1, 1975

96. Dao, T.L., Varela, R. and Morreal, C.: Metabolic transformation of steroids by human breast cancer. In: Estrogen Target Tissues and Neoplasia, p.163, T.L.Dao, Ed., University of Chicago Press, Chicago, 1972

97. Deshpande, N., Carson, P., Di Martino, L. and Tarquini, A.: Biogenesis of steroid hormones by human mammary gland in vivo and in vitro. Eur.J.Cancer 12:271, 1976

98. Hsueh, A.J.W., Peck, E.J. and Clark, J.H.: Progesterone antagonism of the estrogen receptor and estrogen-induced uterine growth. Nature 254:337, 1975

99. Di Carlo, F., Reboani, C., Conti, G., Portaleone, P., Viano, I. and Genazzani, E.: Changes in estrogen receptor levels induced by pharmacological agents. In: Pharmacological Modulation of Steroid Action, p.61, E. Genazzani, F. Di Carlo and W.I.P. Mainwaring, Eds., Raven Press, New York, 1980

100. Di Carlo, F., Conti, G. and Reboani, C.: Interference of gestagens and androgens with rat uterine oestrogen receptors. J.Endocr. 77:49-55, 1978

101. Bullock, L.P. and Bardin, C.W.: Androgenic, synandrogenic and antiandrogenic actions of progestins. Ann. NY Acad.Sci. 286:321, 1977

102. Bullock, L.P. and Bardin, C.W.: Regulation of androgen action in mouse kidney. In: Pharmacological Modulation of Steroid Action, p. 145, E. Genazzani, F. Di Carlo and W.I.P. Mainwaring, Eds., Raven Press, New York, 1980

103. Lippman, M.E., Bolan, G. and Huff, K.: The effect of glucocorticoids and progesterone on hormone-responsive human breast cancer in long-term tissue culture. Cancer Res. 36:4602, 1976

104. Ferenczy, A., Guralnick, M. and Gelfand, M.M.: Observed changes in endometrial carcinoma during hormone-induced regression. Reviews on Endocrine Related Cancer, 1:21-26, 1978

105. Nordqvist, S.R.B.: In Vitro Effects of Progestins on DNA Synthesis in Metastatic Endometrial Carcinoma. Gynecologic Oncology 2:415-428, 1974

106. Gerulath, A.H. and Borth, R.: Effect of progesterone and estradiol-17β on nucleic acid synthesis in vitro in carcinoma of the endometrium. Amer.J.Obstet.Gynec. 128:772, 1977

107. Sekiya, S., Takeda, B., Kikuchi, Y., Sakaguchi, S. and Takamizawa, H.: Morphologic and Enzyme-Cytochemical changes in Uterine Adenocarcinoma Cells of a Rat by Direct Application of Progesterone in Vitro. Gynecologic Oncology 5:5, 1977

108. Di Marco, A.: The Antitumor Activity of 6 α-Methyl-17 α-Acetoxy Progesterone (MPA) in Experimental Mammary Cancer. In: Role of Medroxyprogesterone in Endocrine-Related Tumors. S. Iacobelli and A. Di Marco, Eds. Raven Press, New York, 1980

109. David, A., Edwards, K., Fellowes, K.P. and Plummer, J.M.: Anti-ovulatory and other biological properties of megestrol acetate. 17 α-Acetoxy-6 Methyl Pregna 4:6-Diene-3:20-Dione (B.D.H.1298). J.Reprod.Fertil. 5:331-346, 1963

110. Albin, J., Vittek, J., et al.: On the mechanism of the anti-androgenic effect of MPA. Endocrinology 93:417-422, 1973

111. Anderson, D.C.: Sex-hormone-binding globulin. Clin.Endocr. 3:69, 1974

112. van Kammen, E., Thijssen, J.H.H., Rademaker, B., Schwarz, F.: The influence of hormonal contraceptives on sex hormone binding globulin (SHBG) capacity. Contraception 11:53, 1975

113. Lübbert, H., Pollow, K.: Correlation between the 17β-hydroxysteroid dehydrogenase activity and the estradiol and progesterone receptor concentrations of normal and neoplastic mammary tissue. J.Mol.Med. 3:175-183, 1978

114. Salander, H., Tisell, L.E.: Effects of megestrol on oestradiol induced growth of the prostatic lobes and the seminal vesicles in castrated rats. Acta endocr. 82:213-224, 1976

115. Osborne, C.K., Bolan, G., Monaco, M.E. and Lippman, M.E.: Hormone responsive human breast cancer in long term tissue culture: Effects of insulin. Proc.Natl.Acad.Sci. 73:4536, 1976

116. Monaco, M.E., Lippman, M.E., Knazek, R. and Kidwell, W.R.: Vasopressin stimulation of acetate incorporation into lipids in a dimethylbenz(a)-anthracene-induced rat mammary tumor cell line. Cancer Res. 38:4101, 1978

117. Osborne, C.K., Monaco, M.E., Lippman, M.E., and Kahn, C.R.: Correlation among insulin binding, degradation, and biological activity in human breast cancer cells in long-term tissue culture. Cancer Res. 38:94, 1978

118. Beck, P.: Progestin enhancement of the plasma insulin response to glucose in Rhesus monkeys. Diabetes 18:146-152, 1969

119. Costrini, N.V. and Kalkhoff, R.K.: Relative effects of pregnancy, estradiol and progesterone on plasma insulin and pancreatic islet insulin secretion. J.Clin.Invest. 50:992, 1971

120. Green, I.C., Howell, S.C., El Seifi, S., et al.: Binding of ^{3}H-progesterone by isolated rat islets of Langerhans. Diabetologia 15:349, 1978

121. Howell, S.L., Tyhurst, M. and Green, I.C.: Direct effects of progesterone on rat islets of Langerhans in vivo and in tissue culture. Diabetologia 13:579, 1977

122. Matute, M.L. and Kalkhoff, R.K.: Sex steroid influence on hepatic gluconeogenesis and glycogen formation. Endocrinology 92:762, 1973

123. Bhatia, S.K., Moore, D. and Kalkhoff, R.K.: Progesterone suppression of the plasma growth hormone response. J.Clin.Endocrinol.Metab. 35:364, 1972

124. Kalkhoff, R.K.: Metabolic effects of progesterone. Amer.J.Obstet.Gynec. 142:735, 1982

125. Sutter-Dub, M.Th., Dazey, B., et al.: Progesterone and insulin-resistance: studies of progesterone action on glucose transport, lipogenesis and lipolysis in isolated fat cells of the female rat. J.Endocr. 88:455, 1981

126. Rushakoff, R.J. and Kalkhoff, R.K.: Effect of pregnancy and sex steroid administration on skeletal muscle metabolism in the rat. Diabetes 30:545, 1981

127. Hervey, E. and Hervey, G.R.: The effects of progesterone on body weight and composition in the rat. J.Endocr. 37:361-381, 1967

128. Salans, L.B.: Influence of progestin and estrogen on fat cell size, number, glucose metabolism and insulin sensitivity. Proceedings of the 53rd Meeting, Endocrine Society, San Francisco, Calif., p. A-59, 1971

129. Kim, H.J. and Kalkhoff, R.K.: Sex steroid influence on triglyceride metabolism. J.Clin.Invest. 56:888, 1975

130. Dewar, A.D.: The nature of the weight gain induced by progesterone in mice. Quart.J.Exp.Physiol. 49:151-161, 1964

131. Song, C.S. and Kappas, A.: The influence of estrogens, progestins and pregnancy on the liver. Vitam.Horm. 26:147, 1968

132. Barbosa, J., Seal, U.S. and Doe, R.P.: Effects of steroids on Plasma Proteins - Progestational Agents. J.Clin.Endocrinol.Metab. 32:547, 1971

133. Landau, R.L. and Poulous, J.T.: The metabolic influence of progestins. Adv.Metab.Disord. 5:119, 1971

134. Landau, R.L., Bergenstal, D.M., Lugibihl, K. and Kascht, M.E.: The metabolic effects of progesterone in man. J.Clin.Endocrinol.Metab. 15:1194-1215, 1955

135. Corvol, P., Elkik, F., Feneant, M., Oblin, M.E., Michaud, A., Claire, M. and Menard, J.: Effect of Progesterone and Progestins on Water and Salt Metabolism. In: Progesterone and Progestins. C.W. Bardin, E. Milgröm, and P. Mauvais-Jarvis, Eds. Raven Press, New York, 1983

136. Oelkers, W., Schöneshöfer, M. and Blümel, A.: Effects of progesterone and four progestagens on sodium balane and the renin aldosterone system in man. J.Clin.Endocrinol.Metab. 39:882-890, 1974

137. Smithline, F., Sherman, L. and Kolodny, H.D.: Prolactin and Breast Carcinoma. The New Engl.J. of Med. 292:784-792, 1975

138. Welsch, C.W. and Gribler, C.: Prophylaxis of spontaneously devoping mammary carcinoma in C3H/HeJ female mice by suppression of prolactin. Cancer Res. 33:2939-2946, 1973

139. Meites, J.: Introduction: relation of prolactin to mammary tumorigenesis and growth in rat, Prolactin and Carcinogenesis: Fourth Tenovus Workshop. Edited by A.R. Boyns, K. Griffiths, Cardiff. Wales. Alpha Omega Alpha Publishing Company, pp.54-63, 1972

140. Sinha, D., Cooper, D., Dao, T.L.: The nature of estrogen and prolactin effect on mammary tumorigenesis. Cancer Res. 33:411-414, 1973

141. Wilson, R.G., Buchan, R., Roberts, M.M., Forrest, A.P.M., Boyns, A.R., Cole, E.N., Griffiths, K.: Plasma prolactin and breast cancer. Cancer 33:1325-1327, 1974

142. Sheth, N.A., Ranadive, K.J., Suraiya, J.N., Sheth, A.R.: Circulating levels of prolactin in human breast cancer. Br.J.Cancer 32:160-167, 1975

143. Boyns, A.R., Cole, e.N., Griffiths, K., Roberts, M.M., Buchan, R., Wilson, R.G., Forrest, A.P.M.: Plasma prolactin in breast cancer. Europ.J.Cancer 9:99-102, 1973

144. Kwa, H.G., De Jong-Bakker, M., Englesman, E., Cleton, F.J.: Plasma prolactin in human breast cancer. Lancet 1:433-435, 1974

145. Hill, P., Wynder, E.L., Kumar, H., Helman, P., Rona, G., Kuno, K.: Prolactin levels in populations at risk for breast cancer. Cancer Res. 36:4102-4106, 1976

146. Cole, E.N., Seelwood, R.A., England, P.G., Griffiths, K.: Serum prolactin concentrations in benign breast disease throughout the menstrual cycle. Europ.J.Cancer 13:597-603, 1977

147. Rolandi, E., Barreca, T., Masturzo, P., Polleri, A., Indiveri, F., Barabino, A.: Plasma prolactin in breast cancer. Lancet 2:845-846, 1974

148. Kwa, H.G., Cleton, F., Jong-Bakker, M., Bulbrook, R.D., Hayward, J.L., Wang, D.Y.: Plasma prolactin and its relationship to risk factors in human breast cancer. Int.J.Cancer 17:441-447, 1976

149. Kwa, H.G. and Wang, D.Y.: An abnormal luteal-phase evening peak of plasma prolactin in women with a family history of breast cancer. Int.J.Cancer 20:12, 1977

150. Bruning, P.F., Bonfrèr, H., de Jong-Bakker, M., Hart, A.A.M., Nooyen, W. and Kwa, H.G.: Prolactin and steroid hormones in premenopausal women at risk for breast cancer. 3rd EORTC Breast Cancer Working Conference, April 27-29, Amsterdam, 1983

151. McMahon, B., Cole, P. and Brown, J.: Etiology of human breast cancer. A review. J.Natl.Cancer Inst. 50:21, 1973

152. Henderson, B.E. and Pike, M.C.: Prolactin - An Important Hormone in Breast Neoplasia ? In: Hormone and Breast Cancer, Banbury Report No.8, pp. 115-127. M.C. Pike, P.K. Siiteri, C.W. Welsch, Eds. Cold Spring Harbor Laboratory, 1981

153. Posner, B.I., Kelly, P.A., Shiu, R.P.C., Friesen, H.G.: Studies of insulin, growth hormone and prolactin binding: tissue distribution, species variation and characterization. Endocrinology 96:521-531, 1974

154. Turkington, R.W.: Prolactin receptors in mammary carcinoma cells. Cancer Res. 34:758-763, 1974

155. Holdaway, I.M. and Friesen, H.G.: Hormone Binding by Human Mammary Carcinoma. Cancer Res. 37:1946-1952, 1977

156. Costlow, M.E., Buschow, R.A., McGuire, W.L.: Prolactin receptors in an estrogen receptor-deficient mammary carcinoma. Science 184:85-86, 1974

157. Walsh, R.J., Posner, B.I., Kopriwa, B.M., Brawer, J.R.: Prolactin binding sites in rat brain. Science 201, 1041-1043, 1978

158. Haug, E.: Progesterone Suppression of Estrogen-stimulated Prolactin Secretion and Estrogen Receptor Levels in Rat Pituitary Cells. Endocrinology 104:429, 1979

159. Danguy, A., Legros, N., Leclercq, G., Heuson, J.C.: Effects of medroxyprogesterone acetate on the development of dimethylbenzanthracene-induced mammary tumours: possible modes of action. In: F. Cavalli, W.L. Mcquire , F. Panutti, A. Pellegrini, G. Robustelli Della Cuna, Eds. Proceedings of the International Symposium on medroxyprogesterone acetate, Geneva, Switzerland, pp. 63-76, February 24-26, 1982

160. Lamberts, S.W.J., Janssens, E.N.W., Bons, E.G., Zuiderwijk, J.M., Uitterlinden, P., de Jong, F.H.: Effects of megestrol acetate on growth and secretion of a pituitary tumor. Eur.J.Cancer Clin.Oncol. 17:925-931, 1981

161. Rakoff, J.S. and Yen, S.S.S.: Progesterone induced acute release of prolactin in estrogen primed ovariectomized women. J.Clin.Endocrinol.Metab. 47:918-921, 1978

162. Beattie, C.W., Rodgers, C.H. and Soyka, L.F.: Influence of Ovariectomy and Ovarian Steroids on Hypothalamic Tyrosine Hydroxylase Activity in the Rat. Endocrinology 91:276-279, 1972

163. Beattie, C.W. and Soyka, L.F.: Influence of Progestational Steroids on Hypothalamic Tyrosine Hydroxylase in Vitro. Endocrinology 93:1453-1455, 1973

164. Soyka, L.F. and Long, R.J.: In vitro inhibition of drug metabolism by metabolites of progesterone. J.Pharmacol.Exp.Ther. 182:320, 1972

165. Parveen, L., Chowdhury, A.Q. and Chowdhury, Z.: Injectible contraception (medroxyprogesterone acetate) in rural Bangladesh. Lancet 11:946, 1977

166. Sala, G., Ianotta, F. and Facchinetti, A.: Endocrinological properties of medroxyprogesterone acetate. In: Proceedings of the International Symposium on Medroxyprogesterone Acetate. Geneva, Switzerland 1982. F. Cavalli, et al., Eds. Excerpta Medica, 1982

167. Welsch, C.W. and Nagasawa, H.: Prolactin and Murine Mammary Tumorigenesis: A Review. Cancer Res. 37:951-963, 1977

168. Nagasawa, H.: Prolactin and Human Breast Cancer: A Review. Europ.J.Cancer 15:267-279, 1979

169. Nagasawa, H. and Morii, S.: Prophylaxis of Spontaneous Mammary Tumorigenesis by Temporal Inhibition of Prolactin Secretion in Rats at Young Ages. Cancer Res. 41:1935-1937, 1981

170. Welsch, C.W., Goodrich-Smith, M., Brown, C.K. and Roth, L.: The prophylaxis of rat and mouse mammary gland tumorigenesis by suppression of prolactin secretion: a reappraisal. In: Breast Cancer Research and Treatment. Martinus Nijhoff Publishers, The Haque, 1:225-232, 1981

171. Welsch, C.W., Goodrich-Smith, M., Brown, C.K., Mackie, D. and Johnson, D.: 2-Bromo-d-Ergocryptine (CB-154) and Tamoxifen (ICI 46,474) Induced Suppression of the Genesis of Mammary Carcinomas in Female Rats Treated with 7,12-Dimethyl-benzanthracene (DMBA): A comparison. Oncology 39:88-92, 1982

172. L'Hermite-Balériaux, M., Vokaer, A., Loriaux, C., Noël, G. and L'Hermite, M.: Prolactin (Prl) and Prl-Receptors (Prl-R) in human breast disease. J.Steroid Biochem., Vol.19, Supplement 1983, p.139S

173. Dogliotti, L., Mussa, A. and Di Carlo, F.: Medroxyprogesterone Acetate High Dose and Bromocriptine: Results of a 4-year Study in Stage IV Breast Cancer. In: Role of Medroxyprogesterone in Endocrine-Related Tumors. Volume II. L. Campio, G. Robustelli Della Cuna and R.W. Taylor. Raven Press, New York, 1983

174. Ansfield, F.J.: Megace® and No Diabetes Mellitus. Ann.Int.Med. 86:365, 1977

175. Bottino, J.C. and Tashima, C.K.: Medroxyprogesterone Acetate and Diabetes Mellitus. Ann.Int.Med. 84:341-342, 1976

176. Gershberg, H., Zorrila, E., Hernandez, A., et al.: Effects of medroxyprogesterone acetate on serum insulin and growth hormone levels in diabetics and potential diabetics. Obstet.Gynecol. 33:383-389, 1968

177. Ganzina, F. and Robustelli Della Cuna, G.: Adverse events during high-dose medroxyprogesterone acetate therapy for endocrine tumours. Proceedings of the International Symposium on Medroxyprogesterone Acetate. Geneva 1982. F. Cavalli, et al.,Eds. Excerpta Medica, 1982

178. Lira, P., Rivera, L., Diaz, S. and Croxato, H.B.: Study of blood coagulation in women treated with megestrol acetate implants. Contraception Vol.12, No.6, 1975

179. Brema, F., Queirolo, M.A., Canobbio, L., Bruzzi, P., Puntoni, R., Campora, E. and Rosso, R.: Hematologic parameters during treatment with high-dose medroxyprogesterone acetate. Tumori 67:125-128, 1981

180. Stadel, B.V.: Oral contraceptives and cardiovascular disease. (First of Two Parts). The New England J. of Medicine 305:612-618, 1981. (Second of Two Parts) The New England J. of Medicine 305:672-677, 1981

181. Hayward, J.L., Carbone, P.P., Heuson, J.C., Kumaoka, S., Segaloff, A. and Rubens, R.D.: Assessment of Response to Therapy in Advanced Breast Cancer. Eur.J.Cancer 13:89-94, 1977

182. Goldenberg, I.S., and Haynes, M.A.: Hormonal therapy of metastatic female breast carcinoma. 1.9 x Fluoro-11-ketoprogesterone. Cancer 12:738-740

183. Escher, G.C. and Kaufman, R.J.: Advanced breast carcinoma - factors influencing survival. Acta UICC 19:1039-1043, 1963

184. Cooperative Breast Cancer Group. Testosterone propionate therapy in breast cancer. J.A.M.A. 188:1069-1072, 1964

185. Rozencweig, M. and Heuson, J.C.: Breast Cancer: Prognostic Factors and Clinical Evaluation. In: Cancer Therapy: Prognostic Factors and Criteria of Response. Staquet, M.J., Ed., Raven Press, New York, 1975

186. Cutler, S.J., Asire, A.J. and Taylor, S.G.: Classification of patients with disseminated cancer of the breast. Cancer 24:861-869, 1969

187. Fracchia, A.A., Farrow, J.H., De Palo, A.J., Conolly, D.P. and Huvos, A.G.: Castration for primary inoperable or recurrent breast carcinoma. Surg.Gynecol.Obstet. 128:1226-1234, 1969

188. Panutti, F., Camaggi, C.M., Strocchi, E., Giovanni, M., Canova, N., Tomasi, L. and Lippi, A.: High doses of medroxyprogesterone acetate (MAP) in advanced cancer treatment. Plasma levels and bioavailability after single and multiple dose administration. Preliminary results. Abstracts of II. International Symposium "Role of Medroxyprogesterone Acetate (MPA) in Endocrine-Related Tumors". Rome, 1981

189. Satyaswaroop, P.G. and Mortel, R.: Failure of Progestins to Induce Estradiol Dehydrogenase Activity in Endometrial Carcinoma, in Vitro. Cancer Res. 42:1322-1325, 1982

190. Martin, P.M.: Changing receptor status. In: Proceedings of the International Symposium on Medroxyprogesterone Acetate, Geneva, Switzerland 1982. Cavalli, F. et al., Eds., Excerpta Medica, 1982

CHAPTER II

Progestin Therapy in Advanced Breast Cancer:

Megestrol Acetate—An Evaluation of 160 Treated Cases

J. ALEXIEVA-FIGUSCH, MD, H. A. VAN GILSE, MD, W. C. J. HOP, PHD, C. H. PHOA, MD, J. BLONK-V.D. WIJST, MD, AND R. E. TREURNIET, MD

Progestin megestrol acetate treatment of 160 postmenopausal women with progressive metastatic breast cancer is evaluated. Objective remission was found in 48 patients (30%) and stabilization of disease occurred in 58 (36%) cases. In each of these response categories, the median time interval before subsequent progression took place was nine months. Patients with a postmenopausal age of more than five years showed a significantly higher remission rate than did younger patients. In addition, great differences in remission rates were apparent between groups of patients classified according to dominant sites and separate sites of lesions. The length of disease-free interval did not prove to be of great importance in predicting the remission. No great differences in remission rates were found between patients treated with megestrol acetate as the first hormonal treatment and those previously treated with hormones. Side effects were negligible; a moderate weight increase without demonstrated fluid retention was found during therapy in 10% of the cases. Toxicity was not encountered. Megesterol acetate therapy may be the first choice of treatment in late postmenopausal patients with soft tissue and lung metastases.

Cancer 46:2369–2372, 1980.

MEGESTROL ACETATE (17α-acetoxy-6-methyl-pregna-4,6-diene-3,20-dione) is a synthetic, highly potent progestin. Its metabolism in animals and man has been studied,[4–6] and it seemed to have mild glucocorticoid-like, antiandrogenic, and antiestrogenic properties. The mechanism of action is not clear.

Since the production of new synthetic progestins in recent years, a great variety of these agents have been used in clinical practice. Due to its relative lack of side effects, there is an increasing tendency to use progestin therapy as a first choice in the management of postmenopausal breast cancer.

Stoll[13] has found remissions in 2 of 11 patients with an oral dosage of 30 mg of megestrol acetate. Ansfield *et al.*[1] initially reported remissions in 7 of 30 patients (23.3%) with metastatic breast carcinoma, who were treated with daily oral doses of 160 mg of megestrol acetate. They suggested that megestrol acetate may be effective at any time in the sequence of therapy, including after chemotherapy.

In a later report,[2] Ansfield and co-workers verified the results of their previous study and often used megestrol acetate as the initial hormonal therapy in postmenopausal patients in sequential treatment of disseminated disease.

In our Institute, treatment with megestrol acetate in advanced mammary carcinoma was begun in 1969. The dosage was 60 mg, analogous to that used by Kuipers[10] in the treatment of endometrial carcinoma. Preliminary results have been reported.[3]

Intending to achieve a maximal clinical response, we gradually increased the dose over the years to the present 180 mg daily.

In this report, the statistically analyzed results of a retrospective evaluation of the treatment with megestrol acetate are presented. The emphasis is on identification of patients likely to respond, e.g., according to age, metastatic sites, optimal dose with minimal side effects, length of duration of the response, and ranking according to other treatment modalities.

Materials and Methods

Two hundred and twenty-seven postmenopausal women with progressive metastatic breast cancer

Partly presented at the 4th Congress of Medical Oncology Society, Nice, France, December 2–4, 1978.

From the Departments of Internal Medicine and Medical Statistics, Rotterdamsch Radio-Therapeutisch Instituut, Dr. Daniel den Hoed Kliniek, Rotterdam, The Netherlands.

Address for reprints: J. Alexieva-Figusch, MD, Rotterdamsch Radio-Therapeutisch Instituut, Groene Hilledijk 301, 3075 EA Rotterdam, The Netherlands.

The authors thank the members of the Breast Cancer Group for contributing their patients, and to Mr. L. Ries, Dr. A. C. Ford, Mr. P. van Assendelft, and Mrs. A. Sugiarsi for their assistance.

Accepted for publication December 31, 1979.

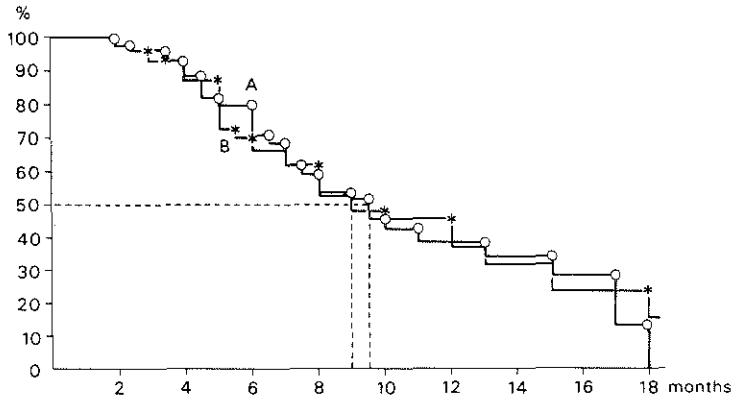


FIG. 1. Percentages of patients without progression according to treatment response. A = remission; B = stable disease.

received megestrol acetate therapy between 1969 and 1978. The effect of treatment could not be evaluated in 67 cases for a variety of reasons, including concurrent radiation therapy, surgical excisions, unmeasurable disease, or insufficient follow-up; 160 cases having measurable progressive disease at the start of the treatment, which means that new lesions had appeared or that the previously known lesions were increasing in size, were evaluable. These findings were objectively demonstrated by appropriate clinical and biochemical investigations (skeletal survey, bone and liver scans, color photographs with caliper measurement, hemoglobin, erythrocytes, leukocytes and platelet counts, sodium, potassium, calcium, phosphorus, BUN, creatinine, total protein, alkaline phosphatase with liver and bone fractions, SGOT, SGPT, LDH). These investigations were repeated after six weeks of treatment to assess response objectively. Criteria for assessment of response were those of Hayward *et al.*,[9] which have been adopted by the EORTC Cancer Group. The review and decision on the quality of response for each case were made by agreement between two independent reviewers.

TABLE 1. Response to Treatment According to Postmenopausal Age

	Postmenopausal age	
	<5 years	≥5 years
Response		
remission	5 (9%)	41 (40%)
stable	23	33
progression	25	28
Total number of patients*	53	102

Chi-square test: $P < 0.01$.

* The postmenopausal age was not clear in five patients because of prior gynecological operations.

Megestrol acetate (Niagestin® 15*) was given in daily oral doses varying from 60 to 180 mg in 4 equal fractions. The dose was gradually increased over the years to improve remission rates. There was no randomization in assigning the dosages. Follow-up investigations were carried out every six weeks or earlier if warranted. The drug was continued until the disease progressed.

Results

In 48 of 160 patients (30%), an objective remission was noted. Stabilization of disease occurred in 58 cases (36%). The median time-interval before progression of the disease was nine months in each category (Fig. 1).

Of the 54 patients with progressive disease at six weeks, two patients showed signs of activation of the process. Classification of patients according to postmenopausal age showed that those with a postmenopausal age of more than five years had achieved a significantly higher remission rate than did the younger group (Table 1).

Great differences in remission rates were observed among groups of patients classified according to the dominant site of the lesions. Table 2 shows that, for patients of postmenopausal age less than five years, the low remission rates according to dominant site did not greatly differ. For the elderly patients, significant differences were observed (Table 2). No relation could be established between remission rates and length of disease-free interval. For patients receiving megestrol acetate as the first hormonal therapy, a remission was noted in 14 of 59 cases (24%). When patients had experienced previous hormonal treatment, remission was found in 34 of 101 patients (34%).

* Niagestin® 15 (15 mg tablets of megestrol acetate) was supplied by the Novo Research Institute, Copenhagen, Denmark.

TABLE 2. Response to Treatment According to Postmenopausal Age and Dominant Site of Lesions

	Less than 5 years			More than 5 years		
	Viscera	Osseous	Soft tissue	Viscera	Osseous	Soft tissue
Response						
remission	2 (11%)	1 (5%)	2 (14%)	16 (67%)	5 (21%)	20 (37%)
stable	7	12	4	2	13	18
progression	9	8	8	6	6	16
Total number of patients	18	21	14	24	24	54

Differences in remission rates between dominant sites for patients with menopausal ages of more than 5 years: Chi-square test $P < 0.01$.

When adjusted for menopausal age and dominant site, this minor difference is far from statistical significance. No clear dose-effect relationship was apparent.

However, there was a suggestion of this in the group of patients with a postmenopausal age of less than five years. The few remissions in that group were achieved only with the higher doses of 160–180 mg daily. Because of small numbers and no randomization, definite conclusions are precluded at this time.

Table 3 gives treatment results according to pretreatment body weight. In the non-obese group, the percentage of patients with progressive disease in spite of treatment is about twice that of the obese group (adjusted for menopausal age and dominant site; $P = 0.05$; Mantel-Haenszel test).

Weight gain with megestrol acetate treatment has been reported.[1] In our series, a weight increase of more than 5% after six weeks of treatment was noted in 10% of the patients. This could be corrected by a dietary regimen of 1200 calories. Other side effects were negligible. No virilization was observed. Vaginal break-through and withdrawal bleeding occurred in 11 patients (seven during and four after megestrol acetate therapy), which could be easily managed. Toxicity was not encountered.

Discussion

The finding that late postmenopausal patients responded more favorably to megestrol acetate treatment agrees with general clinical experience with other hormonal treatments. In this study, evaluation of response was made according to the dominant site of the lesions,[11,12] classified as soft tissue, osseous, and visceral disease. For survival prognosis, patients with visceral lesions were considered to have the poorest, and those with soft tissue metastases the best prognosis. If more than one system was involved, the one with the poorest prognosis determined the classification. Surprisingly, the best results were in the gro of patients classified as visceral.

To investigate the reason for this unexpected finding, we studied the treatment responses of the separate anatomic sites (viscera, bones, and soft tissue). Treatment results for these sites in patients with a postmenopausal age of more than five years are given in Table 4. From this table, one can see that visceral and soft tissue lesions responded favorably to treatment, whereas only one remission was observed within the osseous group. Although 21% remissions were recorded for the group of patients classified "osseous" according to dominant site, all but one of these remissions were due to responses of coincident soft tissue metastases.

Further subdivision of patients with visceral disease showed that mainly lung metastases and involved mucous membranes responded favorably (the latter is very important for the subjective improvement of patients). Liver metastases, on the other hand, reacted poorly. Because of the poor reaction of bone and liver metastases in this series, it would be worthwhile to search for good treatment schemes that

TABLE 3. Pretreatment Body Weight and Response

	Remission	Stable	Progression	Total
Obesity ≥15% over kg/l(m)				
–	32 (28%)	38 (34%)	44 (38%)	114
+	14 (35%)	19 (45%)	8 (20%)	41
Total number of patients				155

Weight was not known in five patients (bedridden out-patients).

TABLE 4. Treatment Response of Separate Anatomical Sites: Postmenopausal Age of Patients more than 5 Years

	Remission	Stable	Progression
Visceral (24 patients)	10 (42%)	8	6
Bone (31 patients)	1 (3%)	24	6
Soft tissue (77 patients)	35 (45%)	22	20

Due to progression in another site, the response in one osseous and two soft tissue systems had not been documented.

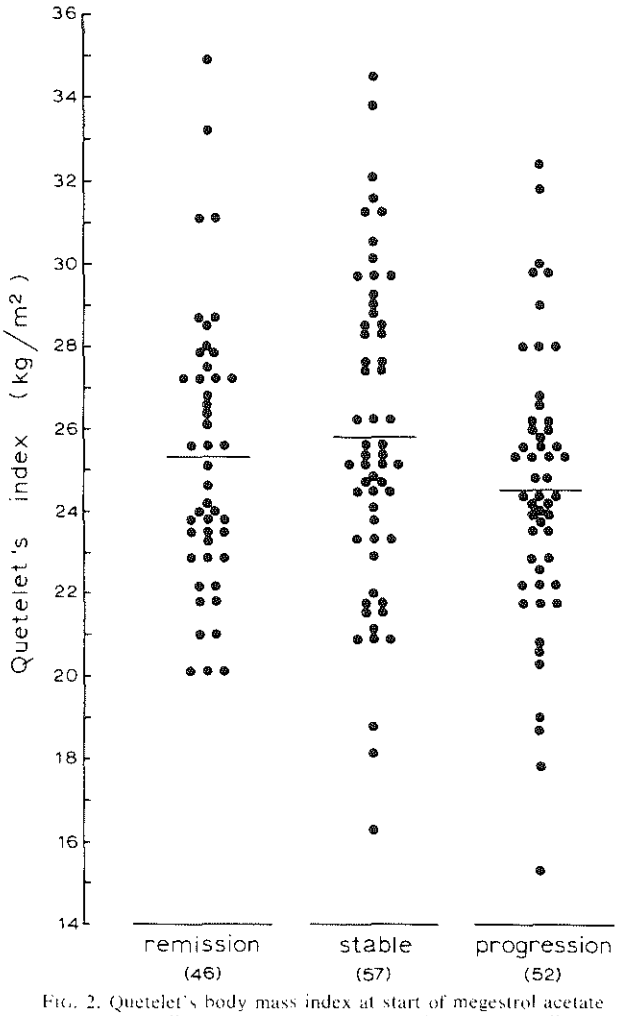


FIG. 2. Quetelet's body mass index at start of megestrol acetate treatment according to response category. (Means are indicated by bars.)

combine different therapeutic modalities, e.g., hormonal and chemotherapeutic agents, adjusted to the actual sites involved. It is hoped that more logical combination schemes can be developed in the future. Clearly, evaluation of treatment results classified according to "dominant site," originally designed for survival prognosis[1,2,7,8,11] and not for responses of the actual benefit to the patients, is not sufficient. An additional classification as to separate anatomical sites seems mandatory.

The observation that the obese group of patients had a higher response rate needs confirmation by other studies. If reproducible, the finding may lead to a better understanding of mechanisms of action of additional hormonal treatment. To suggest or to offer hypotheses seems premature at present. Our results confirm those of Ansfield[1]: Megestrol acetate may be used at any step of sequential treatment. As there are hardly any side effects, it may be the first choice of treatment in late postmenopausal patients with soft tissue and lung metastases. Studies to determine the optimal dose are now in progress.

Addendum

Since this paper was submitted, we reviewed case records to obtain precise heights and pretreatment body weights of patients because of the apparent relationship between obesity and response rate (Table 3). In plotting Quetelet's body mass index (weight/height^2), we found no great differences between the three response categories (Fig. 2). Although the findings listed in Table 3 could largely be reproduced when patients with an index greater than 27 were compared to those below that limit, a gradual increase of the percentage progressing with decreasing index was not apparent. On the basis of these results, it seems best to consider the suggested correlation between obesity and response as an incidental finding for now.

REFERENCES

1. Ansfield FJ, Davis HL Jr, Ellerby RA, Ramirez G. A clinical trial of megestrol acetate in advanced breast cancer. *Cancer* 1974; 33:907–910.

2. Ansfield FJ, Davis HL Jr, Ramirez G, *et al.* Further clinical studies with megestrol acetate in advanced breast cancer. *Cancer* 1976; 38:53–55.

3. Blonk-van der Wijst J, Alexieva-Figusch J, Gilse HA van. Megestrolacetate, a non toxic effective hormonal treatment of metastatic breast cancer. In: Medical Oncology: abstracts of the 3rd Annual Meeting of the Medical Oncology Society and of the Bi-Annual Meeting of the Immunology and Immunotherapy Group (C12G): Nice, December 4–6, 1977, Abstr. 42, p. S18.

4. Briggs MH, Briggs M. Glucocorticoid properties of progestogens. *Steroids* 1973; 22:555–559.

5. Cooper JM, Kellie AE. The metabolism of megestrol acetate (17α-acetoxy-6-methylpregna-4,6-diene-3,20-dione) in women. *Steroids* 1968; 11:133–148.

6. David A, Edwards K, Fellowes P, Plummer JM. Anti-ovulatory and other biological properties of megestrol acetate. 17α-acetoxy-6 methyl pregna 4:6 diene-3:20-dione (B.D.H. 1298). *J Reprod Fertil* 1963; 5:331–346.

7. Escher GC, Kaufman RJ. Advanced breast carcinoma-factors influencing survival. *Acta UICC* 1963; 19:1039–1043.

8. Goldenberg IS. Clinical trial Δ^1-testololactone (NSC 23759), medroxy progesterone acetate (NSC 26386) and oxylone acetate (NSC 47438) in advanced female mammary cancer. A Report of the Cooperative Breast Cancer Group. *Cancer* 1969; 23:109–112.

9. Hayward JL, Carbone PP, Heuson JC, Kumaoka S, Segaloff A, Rubens RD. Assessment of response to therapy in advanced breast cancer. *Eur J Cancer* 1977; 13:89–94.

10. Kuipers Tj. Megestrol acetate in the treatment of recurrent endometrial carcinoma. Proceedings of the 2nd International Symposium "New Developments in Gynaecological Endocrinology," R.C. Hospital, Sittard, The Netherlands. 1970:27–35.

11. Rozencweig M, Heuson JC. Breast cancer: prognostic factors and clinical evaluation. In: Staquet MJ, ed. Cancer Therapy: Prognostic Factors and Criteria of Response, New York: Raven Press, 1975:139–183.

12. Segaloff A, Weeth JB, Rongone EL, Murison PJ, Bowers CY. Hormonal therapy in cancer of the breast. XVI. The effect of Δ^1-Testololactone on clinical course and hormonal excretion. *Cancer* 1960; 13:1017–1020.

13. Stoll BA. Progestin therapy of breast cancer: comparison of agents. *Br Med J* 1967; 3:338–341.

[CANCER RESEARCH 40, 2557-2561, July 1980]
0008-5472/80/0040-0000$02.00

CHAPTER III

Estrogen, Androgen, Glucocorticoid, and Progesterone Receptors in Progestin-induced Regression of Human Breast Cancer[1]

F. A. G. Teulings,[2] H. A. van Gilse, M. S. Henkelman, H. Portengen, and J. Alexieva-Figusch

Departments of Biochemistry [F. T., M. S. H., H. P.] and Internal Medicine [H. van G., J. A-F.], Rotterdamsch Radio-Therapeutisch Instituut, Dr. Daniel den Hoed Kliniek, Groene Hilledijk 301, 3075 EA Rotterdam, The Netherlands

ABSTRACT

A study was made of basic mechanisms involved in regression of breast cancer exposed to high levels of synthetic progestins. The possibility that progestins act on breast cancer by way of the progesterone receptor mechanism and subsequent increase of estradiol 17β-dehydrogenase activity could not be confirmed in this investigation. It is demonstrated that the progestins megestrol acetate and medroxyprogesterone acetate are strong competitors for steroids which bind specifically to androgen, glucocorticoid, and progesterone receptors, indicating that the progestins are able to bind to these receptors with high affinity. In contrast, these progestins do not compete with estradiol for estrogen receptor binding. In 34 patients with progressive metastatic breast cancer, results of receptor studies have been correlated with clinical response during treatment with megestrol acetate. Statistically, regressions were significantly associated with tumors containing large amounts of androgen receptors. Clinical correlation with the quantities of glucocorticoid receptor was weak, while such correlations with estrogen and progesterone receptors were absent. However, we did demonstrate relationships between the quantities of the various receptors in breast cancer. Tumors containing a large amount of androgen receptors also generally contain estrogen receptors. It might be that a favorable response to progestins is confined to the group of patients with hormone-responsive breast cancers, as such characterized by the presence of estrogen receptors, and that within this group the actual androgen receptor levels determine response.

INTRODUCTION

Results of clinical trials have demonstrated that synthetic progestins such as MA[3] are useful in the management of metastatic breast cancer (3, 4, 15). At our Institute, 160 patients with metastatic breast cancer have been treated with MA. Objective remission was found in 48 patients (30%), with median time until progression of 9.5 months (1).

It has been suggested that steroid hormone receptors are involved in additive steroid therapy in breast cancer. In patients treated with estrogens, androgens, or glucocorticoids, objective tumor regressions were obtained in 60% of the patients with positive ER values, whereas only 8% of the patients with negative ER values responded favorably (12). There is some clinical evidence, however, that breast cancer patients who respond to progestin therapy do not belong to exactly the same group as do those who respond to the conventional androgenic or estrogenic hormones (15). Furthermore, patients whose tumors were unresponsive to prior therapy with estrogens alone subsequently responded to a combination of estrogen and progesterone (5, 14).

In addition to ER, many breast cancer specimens contain AR, GR, and PR (7, 18, 19). The role of the various receptor sites in progestin therapy has not been established yet, and the mechanisms of action by which additive endocrine therapies cause breast cancer regression are still not understood. In endometrial cancer, however, the observation that the activity of E_2DH increased during progestin therapy provided a basis for understanding the action of progestins (6, 13). E_2DH activation results in a lower intracellular estradiol level and, consequently, reduction of estrogenic activity. Pollow *et al.* (13) suggested that the PR mechanism is involved in E_2DH activation by progestins. Furthermore, a decline of cytosolic ER levels was found during progestin therapy of endometrial cancer (6, 11). Lübbert and Pollow (9) have recently demonstrated the presence of E_2DH in human breast cancers.

In this investigation, the E_2DH activity in breast tumors was studied, and the effect of MA administration on E_2DH activity was examined in 6 patients. To gain some insight into the interactions of progestins with the various receptors of breast cancer, the relative affinity of some progestins for these receptors was determined. In a clinical study, tumor responses during MA therapy were correlated with the amounts of each of the various receptors in the tumors of a series of breast cancer patients.

MATERIALS AND METHODS

Breast Cancer Tissues. Breast cancer specimens were placed on ice immediately after surgery and transported to the pathology department where nontumorous tissue was removed and representative samples were taken for histological examination. Within 30 min after surgery, samples were frozen and stored until analysis in a −70° freezer or under liquid nitrogen.

Chemicals. 17β-[2,4,6,7-^{3}H]Estradiol (90 to 115 Ci/mmol), 17β-[4-^{14}C]estradiol (50 mCi/mmol), [1,2,4,5,6,7-^{3}H]DHT (110 to 150 Ci/mmol), and [6,7-^{3}H]dexamethasone (35 to 50 Ci/mmol) were obtained from New England Nuclear Chemicals, Dreieich, W. Germany. [17α-*methyl*-^{3}H]R5020 (promegestone; 70 to 87 Ci/mmol) and unlabeled R5020 were kindly donated by Dr. J. P. Raynaud, Roussel-Uclaf, Romainville, France. Unlabeled steroids and other chemicals of analytical grade were obtained from Merck AG, Darmstadt, W. Germany, Serva Feinbiochemica, Heidelberg, W. Germany, and Sigma Chemi-

[1] This work has been financially supported by the Dutch National Cancer League, the "Koningin Wilhelmina Fonds."

[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: MA, megestrol acetate; ER, estrogen receptor; AR, androgen receptor; GR, glucocorticoid receptor; PR, progesterone receptor; E_2DH, 17β-estradiol dehydrogenase; DHT, 5α-dihydrotestosterone; PB, phosphate buffer (0.15 M Na_2HPO_4:KH_2PO_4, pH 7.4); MPA, medroxyprogesterone acetate.

Received December 26, 1979; accepted April 8, 1980.

cal Co., St. Louis, Mo. Dextran T70 was obtained from Pharmacia, Uppsala, Sweden, and Noble's agar was from Difco Laboratories, Inc., Detroit, Mich.

Receptor Assays. Low-temperature agar gel electrophoresis according to the method of Wagner (19) was used, enabling simultaneous assessment of the various receptors. For analysis, frozen tissue was pulverized under liquid nitrogen (Microdismembrator; Braun, Melsungen, W. Germany) and, after thawing, homogenized in 10 mM Tris-HCl:1.5 mM EDTA:0.5 mM dithioerythritol buffer, pH 7.4. Cytosol (supernatant) was obtained by ultracentrifugation (100,000 × *g*, 60 min, 2°). Samples were prepared by incubation of cytosol with ^{3}H-steroids for 2 hr at 4°. Incubation concentrations of ^{3}H-ligands were: 17β-estradiol, 10 nM; DHT, 30 nM; dexamethasone, 10 nM; and R5020, 10 nM. Samples for correction of nonspecific binding were prepared by adding a 100-fold excess of unlabeled 17β-estradiol, DHT, dexamethasone, or R5020, respectively, to duplicate samples. To reduce albumin binding, 17β-estradiol and DHT samples were treated after incubation with charcoal:dextran [0.5% (w/v) Norit A, 0.05% Dextran T70, and 0.1% gelatin in 10 mM Tris-HCl:1.5 mM EDTA:0.5 mM dithioerythritol buffer, pH 7.4] for 90 min, while R5020 samples were treated for 15 min (17). Agar gel electrophoresis was performed on 0.05-ml samples for 90 min (21 V/cm, 130 ma, 4°). After electrophoresis, agar slabs were cut into 9 fractions at the cathodic side and 7 fractions at the anodic side of the origin. ^{3}H content of the fractions was determined by liquid scintillation counting. Results of receptor assays are expressed as fmol/mg protein.

Competition Experiments. In a typical experiment, an excess of tritiated ligand was added to a cytosol:buffer mixture together with varying amounts of unlabeled competitor in a range between 1 and 1000 times molar excess over the radioactively labeled ligand. To enable correction for nonspecific binding, parallel incubations were made containing a 100-fold molar excess of unlabeled ligand. Binding data, corrected for nonspecific binding, were expressed as percentage of the amount specifically bound in the absence of competitors. The results were plotted on a logit scale against the log competitor excess, and linear regressions were fitted to the data. The molar excess of competitor which causes 50% displacement of radioactive ligand was estimated as a measure of affinity of the competitor for the receptor, expressed as relative affinity.

Assay of E_2DH Activity. E_2DH activity was estimated in 800 × *g* supernatants according to the method of Lübbert and Pollow (9) with some modifications. Frozen tissue was pulverized and extracted at 4° for 10 min with 4 parts PB (w/v). An 800 × *g* supernatant was prepared by centrifugation (10 min). The standard reaction mixture contained 2 nmol [^{14}C]estradiol (dissolved in 0.025 ml methanol), 26 nmol unlabeled estradiol (dissolved in 0.01 ml methanol), 0.5 to 2.0 ml 800 × *g* supernatant, and PB to make a total volume of 5.0 ml. This mixture was preincubated at 37° for 10 min. The reaction was started by the addition of NAD^+ (0.025 ml of an 80 mM solution in PB) and continued for 30 min at 37°. [^{14}C]Estradiol conversion was stopped by the addition of 0.05 ml of a mixture of 0.02 M estradiol and 0.02 M estrone in methanol. The reaction mixture was then extracted 3 times with 5 ml chloroform:ether (1:3, v/v). Extracts were pooled and evaporated to dryness under nitrogen. The residue was redissolved in 0.2 ml chloroform and transferred quantitatively to a thin-layer plate (precoated Silica Gel 60 F254, 0.25 mm; Merck AG, Darmstadt, W. Germany). Estrone and estradiol were separated with a solvent system of chloroform:ethyl acetate (4:1, v/v). Radioactivity of separated steroids was quantitated on a radiochromatogram scanner (LB 2721; Berthold, Wildbad, W. Germany). E_2DH activity is expressed as nmol estrone formed per 30 min per mg 800 × *g* supernatant protein.

RESULTS

Competition Studies. Eight mammary cancers with known receptor concentrations provided cytosols for studying the affinity of MA for the various receptors. For comparative purposes, the competitive effects of MPA and progesterone for AR and GR were also estimated. DHT, dexamethasone, and promegestone (R5020) bind with high affinity to AR, GR, and PR, respectively, although cross-affinity may exist to some degree. To minimize interference of PR binding in experiments with ER, AR, and GR, cytosols were selected containing small amounts of PR (Table 1).

With ER, no significant displacement of radiolabeled estradiol by MA or MPA could be detected. MA competed well for PR, AR, and GR (Chart 1). The relative affinities of MA for PR, AR, and GR were 3, 4, and 8, respectively. MPA was found to compete slightly more strongly for AR and GR (relative affini-

Table 1

Concentrations of various receptors in breast cancer cytosols used in the competition studies

ER, AR, GR, and PR were estimated by low-temperature agar gel electrophoresis.

Tumor	Used in experiments for	fmol/ml cytosol[a]			
		ER	AR	GR	PR
198	ER	6,200	675	700	ND[b]
217	PR	1,470	810	200	2,620
253[c]	PR	630	380	470	21,800
368	AR	2,180	780	770	120
424	GR	1,080	75	525	285
475	GR	290	ND	810	ND
503	AR, GR	635	370	400	920
777	ER	1,150	180	300	760

[a] Protein concentrations of the cytosols ranged from 8.5 to 19.5 mg/ml cytosol.
[b] ND, not detectable.
[c] Tumor 253 was obtained from a patient receiving low-dose estrogen therapy.

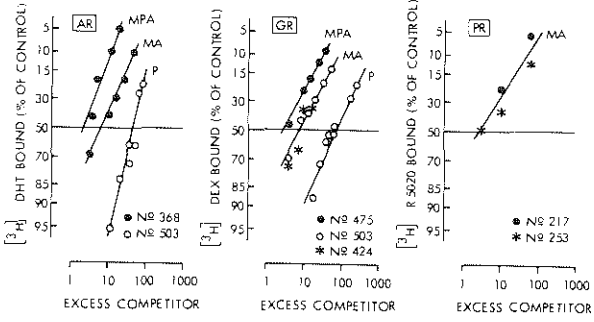


Chart 1. Competition of MA, MPA, and progesterone (*P*) for AR, GR, and PR of human breast cancers. Numbers refer to tumor numbers. For each type of receptor, the binding data, expressed as percentage of specifically bound tritiated ligand, are plotted on a logit scale as function of the log molar excess of competitor. These progestins did not compete significantly for the ER. *DEX*, dexamethasone.

ties, 2 and 3, respectively). The capacity of progesterone to compete for AR and GR (relative affinities, 45 and 71, respectively) was considerably lower.

E_2DH Activities in Breast Cancer Specimens. To demonstrate the presence of E_2DH in breast cancer, the capacity of 800 × *g* supernatants to oxidize estradiol in the presence of NAD^+ was determined in some arbitrarily chosen specimens. E_2DH activity was observed in 10 of 10 primary tumors and in 2 of 4 metastatic deposits.

We were in the position to make a pilot study of the E_2DH activity in metastatic tissues of 6 advanced breast cancer patients before as well as during MA therapy. The E_2DH determinations in the consecutive samples, which were kept at −70° until analysis, were performed simultaneously. The results are shown in Table 2. Prior to therapy, E_2DH activity was present in 4 tumors and absent in the tumors of 2 patients. No significant increase in E_2DH activity during therapy was observed in any of the patients, although tumor regression was reported in 4 of them.

Correlations between Receptors and Clinical Response. The response to MA administration (daily dose, 160 to 180 mg p.o.) was assessed in 34 postmenopausal breast cancer patients with progressive disease in whom one or more of the receptors had been determined. Therapies lasted for at least 6 weeks, and objective criteria for the assessment of response could be used inasmuch as these patients had other lesions to document the response. Clinicians who evaluated response were unaware of the receptor contents of the tumors. Objective remissions, defined according to the criteria of the EORTC Breast Cancer Cooperative Group, were obtained in 17 patients. The duration of remission varied from 2 months to more than 23 months.

In Chart 2, the distributions of the respective receptors are compared in patients in whom remission occurred (responders) and in those with stable or progressive disease (nonresponders).

Statistical tests revealed a significant difference between these groups in the distributions of the amounts of AR (p = 0.01, Wilcoxon test). A weak correlation was also found with GR (p = 0.07), but there were no statistically significant relationships between clinical responses and quantities of ER and PR (p = 0.34 and 0.24, respectively). While 7 of 17 tumors of responding patients did not contain a measurable amount of PR, this was the case in only 1 of 12 tumors of the nonresponders. This difference is of borderline statistical significance (p = 0.1).

As reported earlier (16), we found relationships between the amounts of ER, AR, and GR in breast cancer. In this paper, these associations, including those with PR, are demonstrated with results of all receptor analyses in breast cancer as performed at present in this laboratory. Associations between the quantities of the several receptors were evaluated using the rank correlation coefficient of Kendall (Table 3). All relationships, except GR-PR, were found to correlate in a statistically significant manner, although the associations are not strong.

Similar associations occur in the receptor values of the present study. All tumors without PR contained no ER or only small amounts of ER (<60 fmol/mg protein). It is of interest to note that all patients with AR levels above a cutoff level of 30 fmol/mg protein were responders. Their tumors also contained ER (range, 12 to 750 fmol/mg protein) and GR (range, 15 to 30 fmol/mg protein), but PR was present in only 5 of 7 cases (range, 0 to 250 fmol/mg protein). In our larger tumor material, 44 of 111 tumors obtained from postmenopausal women contained over 30 fmol AR per mg protein. Of these, 40 specimens also contained ER in varying amounts.

Two remissions were associated with malignant tumors devoid of AR. The best explanation for an unexpected finding of a clinical remission associated with a tumor lacking receptors might be that the specimen was obtained from a patient having receptor-containing as well as receptor-lacking metastases.

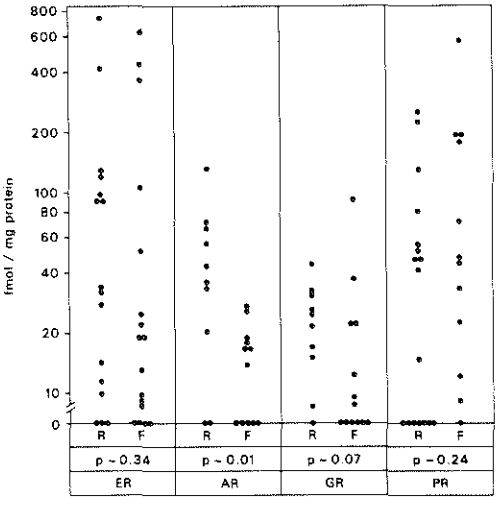


Chart 2. For each type of steroid hormone receptor, the quantities, expressed as fmol/mg protein, are compared between responders (*R*) and nonresponders (*F*) to MA administration. The *p* values given (2-sided) are from the Wilcoxon test, corrected for ties.

Table 2

E_2DH activity in breast cancer specimens taken subsequently before and during MA administration

Patient	Clinical response	No. of days[a]	E_2DH (nmol estrone/30 min/mg protein) Before	During
540	Regression	7	0.17	0.19
569	Regression	5	0.13	0.15
591	Regression	7	0.13	0.14
564	Regression	6	ND[b]	ND
581	Progression	7	0.24	0.24
532	Stable	84	ND	ND

[a] Number of days between start of therapy and taking of second biopsy.
[b] ND, not detectable.

Table 3

Relationships between the quantities of the various receptors in breast cancer

ER, AR, GR, and PR were estimated by low-temperature agar gel electrophoresis. Total number of patients, 237; mean age, 59 years. Premenopausal, 19%; postmenopausal, 64%; castrated, 16%. Primary tumors, 32%; metastatic tumors, 68%.

Set	No. of patients	Kendall rank correlation coefficient	*p*
ER-PR	155	0.23	<0.0001
ER-AR	174	0.29	<0.0001
ER-GR	180	0.26	<0.0001
AR-PR	113	0.22	0.0003
AR-GR	164	0.31	<0.0001
GR-PR	118	0.03	0.33

DISCUSSION

The possibility that tumor regression in breast cancer during MA administration proceeds by mechanisms as postulated for endometrial cancer is contradicted by some observations in the present study. As demonstrated, many but not all breast tumors possess *in vitro* capacity to oxidize estradiol. However, no significant increase in E_2DH activity was observed during MA therapy. Furthermore, in the clinical correlation study, 7 of 17 responders lacked PR in their tumors, which contradicts the suggestion that the presence of PR is required for MA action on breast cancer.

From the competition studies, it may be concluded that MA competes strongly with DHT, R5020, and dexamethasone for AR, PR, and GR, respectively. The high-affinity character of binding was also demonstrated recently by MacLaughlin and Richardson (10), who, using tritiated MPA, found dissociation constants of 6×10^{-10} and 5×10^{-10} M, respectively, for the binding to PR and AR. It appears that MA and MPA have a broad receptor specificity, while other steroids interact mainly with only one of the receptors. Therefore, part of the specificity of binding of steroids is determined by their configuration and not solely by the configuration of the receptor binding sites.

In the present study, an estimate of the receptor quantities was made using a single concentration method. With such a method, underestimation of the available number of receptor sites will occur due to equilibrium kinetics. However, no attempt was made to correct for underestimation. In the present study, the receptor amounts are handled by rank-order statistics.

As demonstrated, the quantities of the various receptors found in breast cancers are positively associated with each other, although the relationships are rather weak. With increasing amounts of ER, there is a corresponding increase in the quantities of the other receptors. Recently, Allegra *et al.* (2) reported similar observations with their population of breast cancer patients. In contrast to their study, we found no correlation between the quantities of GR and PR.

There may be a link in the regulation of synthesis of the various steroid hormone receptors, but secondary regulation mechanisms also appear to be present. This is demonstrated by Patient 253 (Table 1), who received low-dose estrogen therapy preceding receptor determinations. The tumor contained a very high level of PR's, but low or moderate levels of the other receptors. It was hypothesized by Horwitz *et al.* (8) that PR synthesis in breast cancer is estrogen dependent.

It may be doubted that ER is actively involved in the mechanism of action of progestins, since the competition studies ruled out the importance of ER binding. However, due to the above-mentioned relationships in the quantities of the various receptors, tumors with high AR levels may largely coincide with ER-containing tumors. Indeed, most (40 of 44) of these tumors contained varying amounts of ER.

In view of these data, it would appear that ER may be a marker for hormone responsiveness of a tumor, irrespective of its role in the regression mechanism. Responsiveness to a particular type of endocrine therapy, however, may actually be determined by one (or more) of the various receptors.

Data from the present study suggest that AR is directly involved in MA-induced regression and that AR acts as a receptor for MA. Furthermore, a high concentration of AR, and not merely its presence, appears to reflect the sensitivity of mammary cancer to MA. A similar observation was made for endometrial cancer, inasmuch as remissions during progestin treatment were reported to be associated with high PR contents of the tumors (20).

This suggests that suppression of tumor growth occurs in cases where a sufficiently high number of receptor:progestin complexes are translocated to the tumor cell nuclei. One may hypothesize that a high nuclear concentration of these complexes is necessary to compete adequately with nuclear receptors for acceptor sites. As a result, some transcription processes operative in hormone responsive tumor cells may be inhibited.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to J. Blonk-van der Wijst who participated in this study, and to W. C. J. Hop, statistician of the Rotterdamsch Radio-Therapeutisch Instituut, for his advice and valuable discussions. The contributions of P. H. Baggerman-Lems, J. van der Meij, H. A. Peters, and J. Wike to this study and to the preparation of the manuscript are highly appreciated.

REFERENCES

1. Alexieva-Figusch, J., van Gilse, H. A., Hop, W. C. J., Phoa, C. H., Blonk-van der Wijst, J., and Treurniet, R. E. Progestin therapy in advanced breast cancer: megestrol acetate, an evaluation of 160 treated cases. Cancer (Phila.), in press, 1980.
2. Allegra, J. C., Lippman, M. E., Thompson, E. B., Simon, R., Barlock, A., Green, L., Huff, K. K., Do, H. M. T., and Aitken, S. C. Distribution, frequency, and quantitative analysis of estrogen, progesterone, androgen, and glucocorticoid receptors in human breast cancer. Cancer Res., *39*: 1447–1454, 1979.
3. Ansfield, F. J., Davis, H. L., Ellerby, A., and Ramirez, G. A clinical trial of megestrol acetate in advanced breast cancer. Cancer (Phila.), *33*: 907–910, 1974.
4. Ansfield, F. J., Davis, H. L., Jr., Ramirez, G. Davis, T. E., Borden, E. C., Johnson, R. O., and Bryan, G. T. Further clinical studies with megestrol acetate in advanced breast cancer. Cancer (Phila.), *38*: 53–55, 1976.
5. Crowley, L. G., and MacDonald, I. Delalutin and estrogens for the treatment of advanced mammary carcinoma in the post-menopausal women. Cancer (Phila.), *18*: 436–446, 1965.
6. Gurpide, E., Tseng, L., and Gusberg, S. B. Estrogen metabolism in normal and neoplastic endometrium. Am. J. Obstet. Gynecol., *129*: 809–816, 1977.
7. Horwitz, K. B., and McGuire, W. L. Specific progesterone receptors in human breast cancer. Steroids, *25*: 497–505, 1975.
8. Horwitz, K. B., McGuire, W. L., Pearson, O. H., and Segaloff, A. Predicting response to endocrine therapy in human breast cancer: a hypothesis. Science (Wash. D. C.), *189*: 726–727, 1975.
9. Lübbert, H., and Pollow, K. Correlation between the 17β-hydroxysteroid dehydrogenase activity and the estradiol and progesterone receptor concentrations of normal and neoplastic mammary tissue. J. Mol. Med., *3*: 175–183, 1978.
10. MacLaughlin, D. T., and Richardson, G. S. Specificity of medroxyprogesterone acetate binding in human endometrium: interaction with testosterone and progesterone binding sites. J. Steroid Biochem., *10*: 371–377, 1979.
11. Martin, P. M., Rolland, P. H., Gammerre, M., Serment, H., and Taga, M. Estradiol and progesterone receptors in normal and neoplastic endometrium: correlations between receptors, histopathological examinations and clinical responses under progestin therapy. Int. J. Cancer, *23*: 321–329, 1979.
12. McGuire, W. L., Carbone, P. P., Sears, M. E., and Escher, G. C. Estrogen receptors in human breast cancer: an overview. *In*: W. L. McGuire, P. P. Carbone and E. P. Vollmer (eds.), Estrogen Receptors in Human Breast Cancer, pp. 1–7. New York: Raven Press, 1975.
13. Pollow, K., Boquoi, E., Lübbert, H., and Pollow, B. Effect of gestagen therapy upon 17β-hydroxysteroid dehydrogenase in human endometrial adenocarcinoma. J. Endocrinol., *67*: 131–132, 1975.
14. Stoll, B. A. Effect of Lyndiol, an oral conceptive, on breast cancer. Br. Med. J., *1*: 150–153, 1967.
15. Stoll, B. A. Progestin therapy of breast cancer—comparison of agents. Br. Med. J., *3*: 338–341, 1967.
16. Teulings, F. A. G. Estrogen, androgen, corticoid and progesterone receptors in human mammary cancers. *In*: J. M. Castellanos Llorens (ed.), Advances in Steroid Analysis, Vol. 3, pp. 47–63. Barcelona, Spain: Graficas PER, 1977.
17. Teulings, F. A. G., Blonk-van der Wijst, J., Portengen, H., Henkelman, M. S., Treurniet, R. E., and van Gilse, H. A. Quantitation of estrogen receptors

in human breast cancer by agar gel electrophoresis. Clin. Chim. Acta, *64:* 27–38, 1975.
18. Teulings, F. A. G., and van Gilse, H. A. Demonstration of glucocorticoid receptors in human mammary carcinomas. Hormone Res., *8:* 107–116, 1977.
19. Wagner, R. K. Characterization and assay of steroid hormone receptors and steroid-binding proteins by agar-gel electrophoresis at low temperature. Hoppe-Seyler's Z. Physiol. Chem., *353:* 1235–1245, 1972.
20. Young, P. C. M., Ehrlich, C. E., and Cleary, R. E. Progesterone binding in human endometrial carcinomas. Am. J. Obstet. Gynecol., *125:* 353–360, 1976.

Recent Results in Cancer Research, Vol. 91

CHAPTER IV

Steroid Receptors in Megestrol Acetate Therapy

J. Alexieva-Figusch, F. A. G. Teulings, W. C. J. Hop,
J. Blonk-van der Wijst, and H. A. van Gilse*

Department of Internal Medicine, Rotterdamsch Radio-Therapeutisch Instituut,
Dr. Daniel den Hoed Kliniek, Groene Hilledijk 301, 3075 EA Rotterdam, The Netherlands

Introduction

The role of megestrol acetate (17α-acetoxy-6-methyl-pregna-4,6-diene-3,20-dione), a synthetic progestin derived from hydroxyprogesterone, in the treatment of advanced breast cancer has long been established: generally, remission can be achieved in ca. 30% of postmenopausal patients [1–3, 12, 13, 16, 18, 19]. It is difficult to define the physiological and pharmacological effects of progesterone and synthetic progestins, since virtually none of them are due exclusively to these hormones. Nevertheless, while estrogens and androgens have mainly growth-stimulating effects, the action of progestins is directed more towards modification and differentiation [14]. Megestrol acetate has been studied in both man and animals and seems to have antiestrogenic, antiandrogenic, and glucocorticoid-like properties [4–8, 10, 15] (Fig. 1). There is little information correlating the effect of megestrol acetate treatment and receptor content in human tumor tissue. Morgan reported response to megestrol acetate in seven of 16 patients with ER+ and in two of five patients with ER− [13]. In our previous study on steroid receptors in megestrol acetate-induced regression of human breast cancer [17], it was demonstrated that megestrol and medroxyprogesterone acetates are strong competitors for steroids which bind specifically to androgen, glucocorticoid, and progesterone receptors, indicating that these progestins

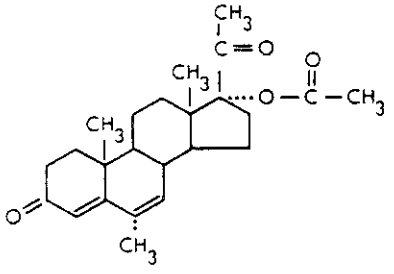


Fig. 1. Megestrol acetate 17 α - acetoxy - 6 - methyl - pregna - 4,6 - diene - 3,20 - dione

* The authors wish to thank Mr. M. S. Henkelman and Mr. H. Portengen for performing the receptor analysis, and Mrs. A. Sugiarsi, Mr. P. van Assendelft, and Mrs. J. L. Wike-Hooley for their assistance

are able to bind to these receptors with high affinity. In contrast, they do not compete with estradiol for estrogen receptor binding. Regressions were significantly associated with tumors containing large amounts of androgen receptors, tumors which also generally contain estrogen receptors.

The possibility that megestrol acetate acts on breast cancer via the progesterone receptor mechanism with subsequent increase in estradiol 17β-dehydrogenase could not be confirmed, although the presence of estradiol 17β-dehydrogenase in human breast cancer has been demonstrated [11].

The present study correlates steroid receptors to response in primary and metastatic breast tumor tissues of 60 postmenopausal patients treated with megestrol acetate.

Materials and Methods

One or more steroid receptors were assessed in 22 primary and 38 metastatic breast cancer specimens. Estrogen receptor was determined in 59 patients, androgen in 32, glucocorticoid in 34, and progesterone in 45. Tumors were classified as receptor negative if they contained less than 10 fmol/mg protein. The receptor assay methods used have been reported previously [17].

Some characteristics of the patients in this study are shown in Table 1. At the start of treatment all patients received megestrol acetate for progressive disease as objectively demonstrated by appropriate clinical and biochemical investigation. After 6 weeks of

Table 1. Characteristics of the 60 patients whose tumors were assayed for steroid hormone receptors

Mean age in years (range)	60 (32–86)
Menopause age	
1–5 years	25
> 5 years	35
Disease-free interval	
0 months	10
1–12 months	14
13–24 months	19
25–36 months	7
> 36 months	10
Previous hormonal therapy	21
No previous hormonal therapy	39
Dominant sites	
Soft tissue	28
Bones	21
Viscera	11
Response to megestrol acetate therapy	
Remission	20 (33%)
Stable disease	24 (40%)
Progression	16 (27%)

treatment, response was assessed according to EORTC criteria [9]. The clinicians who evaluated response were unaware of the receptor contents of the tumors.
Megestrol acetate (Niagestin 15) was given in daily oral doses varying from 120 to 180 mg in four equal fractions. The drug was continued until the disease progressed.

Results

In our series of 60 patients treated with megestrol acetate, we found the following: estrogen receptor positivity in 64%, progesterone receptor positivity in 56%, glucocorticoid and androgen receptor positivity in 53% and 56% respectively. With increasing amounts of estrogen receptor there was a corresponding increase in the quantities of the other receptors.
Figure 2 gives estrogen receptor data according to response to treatment. Neither the presence of the receptor nor its amount was demonstrably related to the outcome of treatment. However, the duration of remission in patients with estrogen receptor-positive tumors was significantly longer (log-rank test: $P < 0.01$) than in those who lacked estrogen receptor (Fig. 3).
Figure 4 gives data on androgen, glucocorticoid, and progesterone receptors in relation to treatment outcome. A statistically significant higher amount of androgen receptor was noted in the group with remission as compared with the group with stable or progressive disease (Wilcoxon test: $P < 0.05$). No correlation between response to treatment and the amount of glucocorticoid or progesterone receptor was demonstrated. Remission in the group of patients with androgen receptor exceeding 30 fmol/mg protein was 60% (six of 10), while in the group with androgen receptor below that limit the figure was 23% (five of

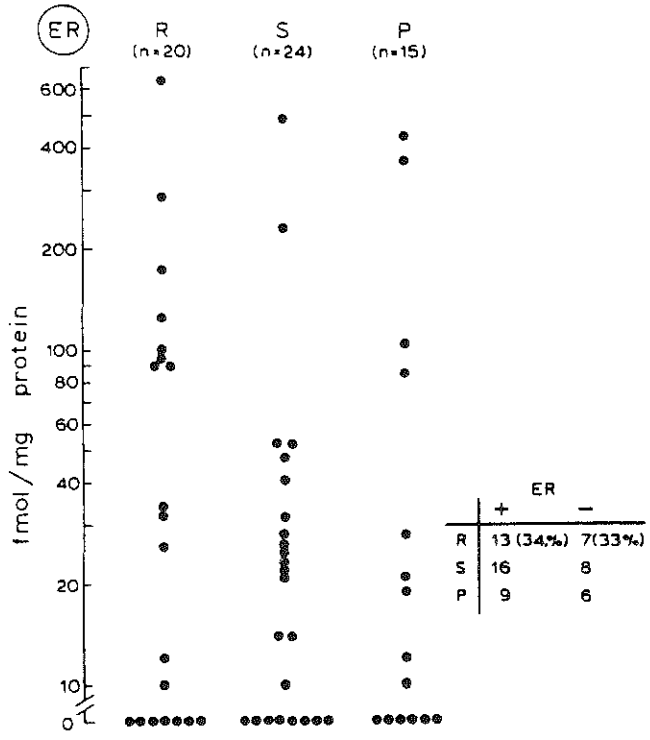


Fig. 2. Response to megestrol acetate treatment according to estrogen receptor values. *R*, remission; *S*, stable; *P*, progression; *ER*, estrogen receptor

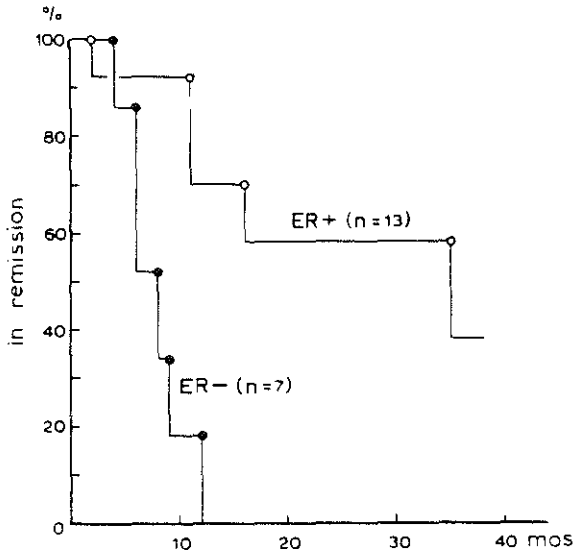


Fig. 3. Acturial percentages of patients in remission according to presence of estrogen receptor *(ER)*

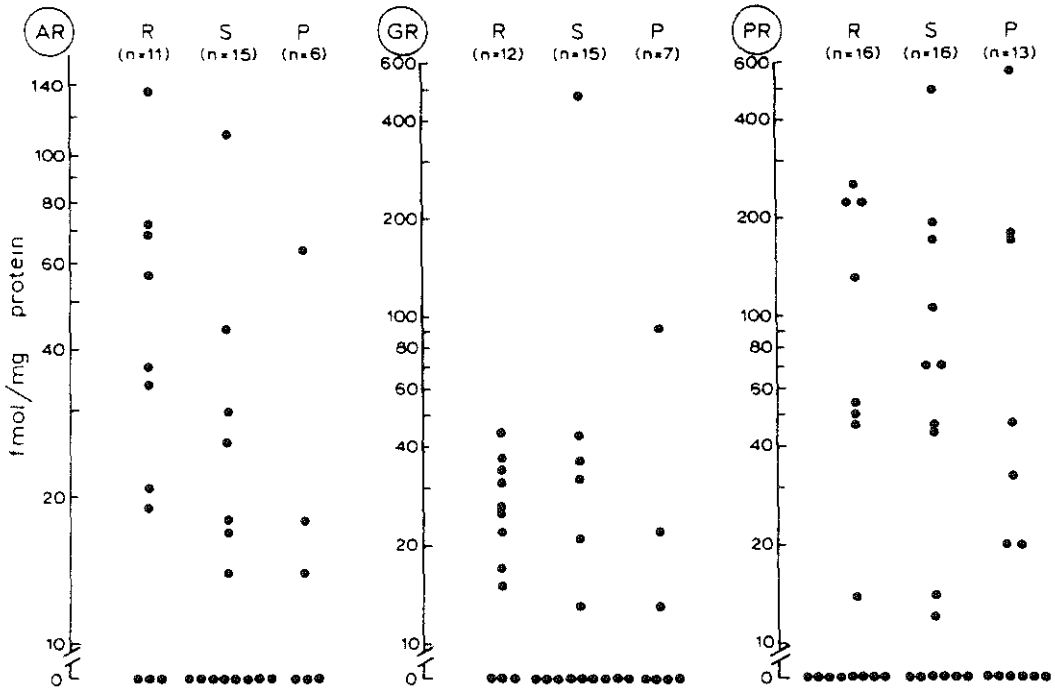


Fig. 4. Response to megestrol acetate therapy according to androgen *(AR)*, glucocorticoid *(GR)*, and progesterone *(PR)* receptor values. *R*, remission; *S*, stable; *P*, progression

22). Moreover, the duration of remission in the group with a high androgen receptor level (> 30 fmol/mg protein) was significantly (log-rank test: $P = 0.05$) longer than that in the group with a low value (< 30 fmol/mg protein) (Fig. 5). The results given in this section did not appreciably differ between patients according to whether receptors were evaluated in primary or in metastatic tumor.

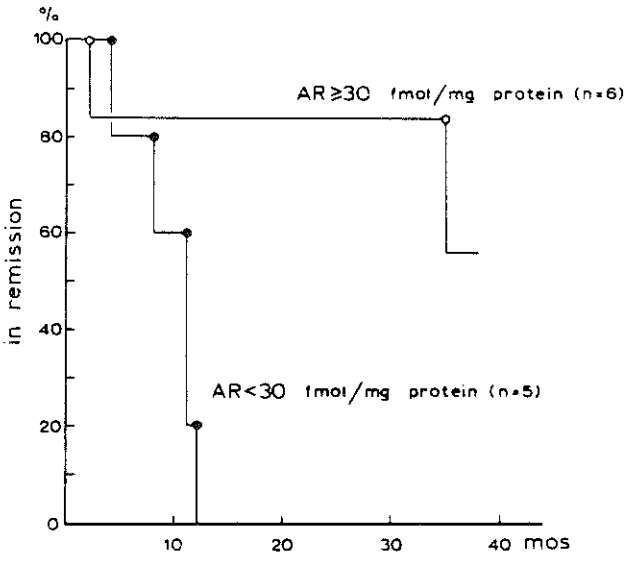


Fig. 5. Actuarial percentages of patients in remission according to amount of androgen receptor *(AR)*

Discussion

Steroid hormone receptors are found in a variety of cancers, including human breast cancer. For megestrol acetate therapy it seems that only the androgen receptor may be useful as an indicator of response. Moreover, this study strongly suggests that a satisfactory remission (i.e., of long duration) may be expected in cases with a large amount of androgen receptor. None of the other receptors appear to predict the probability of remission to megestrol acetate.

The fact that long remissions are associated with estrogen receptor-positive tumors might be explained by the correlation between amounts of androgen and estrogen in breast tumors. All tumors high in androgen receptor were also estrogen receptor positive. However, the number of patients studied is too small to assess fully the additional value of estrogen receptor once the androgen receptor level is known.

References

1. Alexieva-Figusch J, Van Gilse HA, Hop WCJ, Phoa CH, Blonk-v d Wijst J, Treurniet RE (1980) Progestin therapy in advanced breast cancer: Megestrol acetate – An evaluation of 160 treated cases. Cancer 46: 2369–2372
2. Ansfield FJ, Davis HL Jr, Ellerby RA, Ramirez G (1974) A clinical trial of megestrol acetate in advanced breast cancer. Cancer 33: 907–910
3. Ansfield FJ, Davis HL Jr, Ramirez G, Davis TE, Borden EC, Johnson RO, Bryan GT (1976) Further clinical studies with megestrol acetate in advanced breast cancer. Cancer 38: 53–55
4. Briggs MH, Briggs M (1973) Glucocorticoid properties of progestogens. Steroids 22: 555–559
5. Cooper JM, Jones HEH, Kellie AE (1973) The metabolism of megestrol acetate (17α-acetoxy-6-methylpregna-4,6-diene-3,20-dione) in the rabbit. Steroids 22: 255–275
6. Coooper JM, Kellie AE (1968) The metabolism of megestrol acetate (17α-acetoxy-6-methylpregna-4,6-diene-3,20-dione) in women. Steroids 11: 133–148

7. David A, Edwards K, Fellowes KP, Plummer JM (1963) Anti-ovulatory and other biological properties of megestrol acetate. 17α-acetoxy-6 methyl pregna 4:6-diene-3:20-dione (B.D.H. 1298). J Reprod Fertil 5: 331–346
8. Gupta C, Bullock LP, Bardin CW (1978) Further studies on the androgenic, anti-androgenic, and synandrogenic actions of progestins. Endocrinology 120: 736–744
9. Hayward JL, Carbone PP, Heuson JC, Kumaoka S, Segaloff A, Rubens RD (1977) Assessment of response to therapy in advanced breast cancer. Eur J Cancer 13: 89–94
10. Lee AE, Williams PC (1964) Oestrogen antagonists: assay by inhibition of vaginal cornification. J Endocrinol 28: 199–203
11. Lübbert H, Pollow K (1978) Correlation between the 17β-hydroxysteroid dehydrogenase activity and the estradiol and progesterone receptor concentrations of normal and neoplastic mammary tissue. J Mol Med 3: 175–183
12. Morgan LR (1979) Tamoxifen versus megesterol acetate in breast cancer. Cancer Treat Rep 63: 1218 (Abstr 380)
13. Morgan LR, Donley PJ (1981) Tamoxifen versus megestrol acetate in breast cancer. In: Anti-hormones and breast cancer. Reviews on Endocrine-Related Cancer [Suppl] 9: 301–310
14. Neumann F (1978) The physiological action of progesterone and the pharmacological effects of progestogens – a short review. Postgrad Med J 54: 11–24
15. Salander H, Tisell LE (1976) Effects of megestrol on oestradiol induced growth of the prostatic lobes and the seminal vesicles in castrated rats. Acta Endocrinol (Kbh) 82: 213–224
16. Stoll BA (1967) Progestin therapy of breast cancer: Comparison of agents. Br Med J 3: 338–341
17. Teulings FAG, Van Gilse HA, Henkelman MS, Portengen H, Alexieva-Figusch J (1980) Estrogen, androgen, glucocorticoid, and progesterone receptors in progestin-induced regression of human breast cancer. Cancer Res 40: 2557–2561
18. Weeth JB (1974) Large dose progestin palliation. Valuable in solid tumor patients. Proc Am Assoc Cancer Res 15: 726 (Abstr)
19. Weeth JB (1975) Megestrol palliation of breast cancer. Proc Am Assoc Cancer Res 16: 38 (Abstr)

Eur J Cancer Clin Oncol, Vol. 20, No. 1, pp. 33–40, 1984.
Printed in Great Britain.

CHAPTER V

Treatment of Metastatic Breast Cancer Patients with Different Dosages of Megestrol Acetate; Dose Relations, Metabolic and Endocrine Effects*

J. ALEXIEVA-FIGUSCH,† M. A. BLANKENSTEIN,† W. C. J. HOP,† J. G. M. KLIJN,†
S. W. J. LAMBERTS,‡ F. H. DE JONG,‡ R. DOCTER,‡ H. ADLERCREUTZ§ and H. A. VAN GILSE†

†*Department of Internal Medicine and Clinical Endocrinology, Biochemistry and Biostatistics, Rotterdamsch Radio-Therapeutisch Instituut, Groene Hilledijk* 301, 3075 *EA Rotterdam, The Netherlands,* ‡*Department of Internal Medicine III and Clinical Endocrinology, Erasmus University, Rotterdam, The Netherlands and* §*Department of Clinical Chemistry, University of Helsinki, Finland*

Abstract—*Megestrol acetate (MA) is of therapeutic value in breast cancer patients. This study was designed to evaluate the effects of different dosages of MA on endocrine events potentially influenced by the drug in relation to plasma level of MA and clinical effects in patients with advanced breast cancer. Eighteen postmenopausal patients were randomly distributed over six groups to receive daily* 90, 180 *or* 270 mg *of MA (Niagestin®) orally in a cross-over study consisting of* 3 *periods of* 6 weeks. *Complete remission was observed in* 1 *patient, partial remission in* 9, *no change in* 4 *and failure in* 4 *patients. During the* 18 weeks *of treatment plasma levels of MA gradually increased, irrespective of the dose administered. Significant rises of the basal and TRH-stimulated plasma PRL and basal insulin levels were observed, whereas LH and FSH, estradiol, SHBG and the pituitary–adrenal axis were suppressed. None of these metabolic effects showed a correlation with the clinical response. We concluded that treatment of metastatic breast cancer with* 180 mg *MA/*day *is effective and causes minimal adverse effects.*

INTRODUCTION

THE SYNTHETIC progestin megestrol acetate (MA) (17α-acetoxy-6α-methyl-pregna-4,6-diene-3,20-dione) is effective in the treatment of patients with advanced breast cancer [1, 2]. The mechanism through which MA exerts its effects on mammary tumors is unclear. The drug has been reported to display progestin-, glucocorticoid-like and androgenic properties [3, 4]. An attempt to explain the action of MA through a progesterone-receptor-mediated increase in estradiol dehydrogenase activity in the tumor specimens of postmenopausal breast cancer patients was unsuccessful. Only a very low affinity of MA to estrogen receptors was observed, but the drug bound relatively well to progesterone, androgen and glucocorticoid receptors [5]. This study was designed to evaluate the effect of oral administration of 3 different dosages of MA on the plasma concentrations of gonadotropins, prolactin (PRL), growth hormone, estradiol, sex hormone binding globulin (SHBG), insulin and glucose, on adrenocortical function and to investigate relationships between plasma levels of MA and clinical responses during the treatment of metastatic breast cancer.

Accepted 11 June 1983.
*Partly presented at VIII[th] Dutch-British Endocrine Meeting 25th–27th August 1982, Noordwijkerhout, The Netherlands.

MATERIALS AND METHODS

Patients

Eighteen patients with evaluable and measurable advanced breast cancer, who had not yet otherwise been treated, were selected for this study. Informed consent to participate in this study was obtained from all patients. All were more than 2 yr after their natural menopause and between 54 and 75 yr of age. All patients except one (ascites with gastrointestinal discomfort) were in good general condition, without gastrointestinal-, hepatic- or renal disease, diabetes mellitus or malabsorption. The patients received three 6-week treatments with daily doses of 90, 180 and 270 mg of megestrol acetate (Niagestin®,

supplied by NOVO, Denmark) in a cross-over regimen. Medication was taken orally with 8-hr intervals (07.00–15.00–23.00). When definite progression of metastatic cancer occurred, the drug was withdrawn. At the start and end of each treatment period, routine clinical and biochemical investigations were performed. In addition, blood was collected for the estimation of the plasma concentration of MA (at 14.30), basal gonadotropins, growth hormone, estradiol, SHBG, prolactin (PRL) and TSH before and after administration of 200 μg of TRH intravenously, 11-deoxycortisol before and after 6 × 750 mg of metyrapone and insulin and glucose during a glucose tolerance test (100 g of glucose orally). Fifteen fully evaluable patients completed the study, which allowed statistical analysis with respect to the endocrine parameters. Clinical evaluation was done after each period of 6 weeks based on UICC criteria of response with regard to visceral, osseous and soft tissue metastases [6].

Laboratory methods

For the radioimmunological assay of MA in plasma, an anti-MPA-3-(*o*-carboxymethyl)oxime-bovine serum albumin serum, which cross-reacts extensively with MA, was used [7, 8]. Blood samples were taken before and at the end of each treatment period just before the administration of the drug. LH and FSH were measured by specific radioimmunoassay, using materials from KABI (Stockholm, Sweden). Results were expressed as U/l; normal values for postmenopausal women were for LH more than 21.0 and FSH more than 5.0 U/l respectively. Plasma PRL levels were determined by a double-antibody radioimmunoassay method using the kit from IRE (Antwerp, Belgium). One nanogram of the standard employed is equivalent to 1 ng of the standard VLS/1 of the NIH. The upper limit of normal in women is 15 ng/ml. Blood samples were taken at 0, 20, 30, 60 and 120 min after 200 μg of TRH given i.v. before and at the end of each treatment period (at 09.00–11.00 a.m.). In addition, TSH estimation was performed. TSH was determined by radioimmunoassay, using the kit from DPC (U.S.A.). Normal values are lower than 10 μU/ml. Plasma levels of growth hormone were determined by a double-antibody radioimmunoassay using the CEA kit (Paris, France). Normal basal fasting levels are 1–5 ng/ml. Estradiol was measured by radioimmunoassay [9]. 11-Deoxycortisol (compound S) was estimated in plasma by a competitive protein binding assay following a simple solvent extraction before and 24 hr after 6 × 750 mg metyrapone given orally. The normal value after metyrapone exceeds 15 μg/100 ml [10]. The capacity of the serum to bind [^{3}H]-dihydrotestosterone was used as a measure for the concentration of SHBG and was determined by agar gel electrophoresis [11]. Total immunoreactive insulin was determined using ethanol extraction, whereby antibody-bound immunoreactive insulin is dissociated and separated together with the free insulin from the serum proteins and antibodies [12]. Glucose was estimated enzymatically.

Statistics

Dose-effects for the various parameters were in the first instance investigated by multivariate methods appropriate for change-over designs [13]. These methods, in addition to dose-effects, allow for individual differences, differences between the 3 treatment periods and residual effects from the treatment with a particular dose in the previous period for periods 2 and 3. These analyses were supplemented by standard non-parametric tests (the Friedman test, the signed rank test and the rank sum test). *P* values given in the text and figures arose from these non-parametric tests.

RESULTS

Clinical response

Complete remission after 18 weeks was achieved in 1/18, partial remission in 9/18 and stable disease in 4/18 patients while progression occurred in 4/18 patients. Favourable effects of MA have been found in all types of metastases, e.g. in soft tissue, bone and viscera. No patient had liver metastases. During the study period no

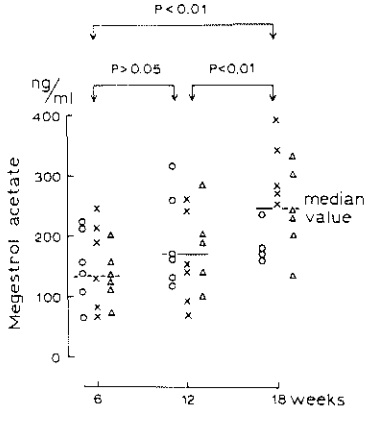


Fig. 1. Concentration of megestrol acetate (MA) in plasma of breast cancer patients treated with different daily doses of MA for 3 periods of 6 weeks. Individual values are given as a function of the time after the start of the experiment, and with respect to the dosage of MA received during the 6 weeks immediately preceding the blood sampling. ○ : 90 mg/day; × : 180 mg/day; △ 270 mg/day.

Table 1. Plasma concentrations of megestrol acetate and endocrine parameters in patients with metastatic breast cancer responsive (CR+PR) or refractory (NCH+F) to treatment with megestrol acetate

Mean value of plasma concentration at 6 weeks	Responders ($n=10$)	Non-responders ($n=8$)	P
Megestrol acetate (ng/ml)	125	174	0.1
Basal prolactin (ng/ml)	10.7	18.6	0.1
Basal TSH (μU/ml)	7.1	5.1	0.2
Basal growth hormone (μg/l)	4.4	9.3	0.4
LH (U/l)	20.8	15.0	0.8
FSH (U/l)	15.9	10.0	0.9
Estradiol (pmol/l)	57.8	67.0	0.06
11-Deoxycortisol (μg/100 ml) after metyrapone	1.9	2.8	0.9
SHBG (nmol/l)	57.8	52	0.5
Basal insulin (μU/ml)	23.1	20	0.4
Basal glucose (mmol/l)	5.1	4.8	0.4

adverse effects of the drug have been observed; MA was tolerated excellently at all 3 dosages.

Megestrol acetate concentration in plasma

The concentrations of MA in plasma increased with time during the treatment (Fig. 1, $P<0.01$). No relation could be demonstrated between dose and plasma level, even if corrected for body surface. Therefore the results for the different dosages were combined to give median values of 134, 170 and 243 ng/ml after 6, 12 and 18 weeks respectively. No relation to the clinical response has been found; plasma levels of failures did not differ from those in responders (Table 1).

In 5 other patients who gradually discontinued long-term treatment with 180 mg MA (within 18 days), plasma levels of MA 3 days after complete cessation appeared decreased from 243 ± 25 (S.E.M.) during the steady state to 17 ± 7 ng/ml.

Endocrine effects

Prolactin. Before the start of the treatment basal plasma PRL levels were normal in all patients, while there was a normal reaction of PRL to TRH (Fig. 2). During treatment with MA the mean basal PRL levels increased significantly from 7.1 ± 0.8 to 13.9 ± 2.2 ng/ml (Table 2, $P<0.001$). There was also a hyper-response of PRL to TRH during treatment with MA. The range of the response of plasma PRL to TRH varied widely (Fig. 2).

TSH and growth hormone. Plasma levels of TSH were measured before and after TRH stimulation. There was no change in the basal and stimulated TSH levels during MA treatment. Basal mean TSH level was 6.6 ± 1.8 μU/ml before treatment and 6.2 ± 0.6 μU/ml after 6 weeks of treatment. In addition, TSH response to TRH was not altered during MA treatment. Basal

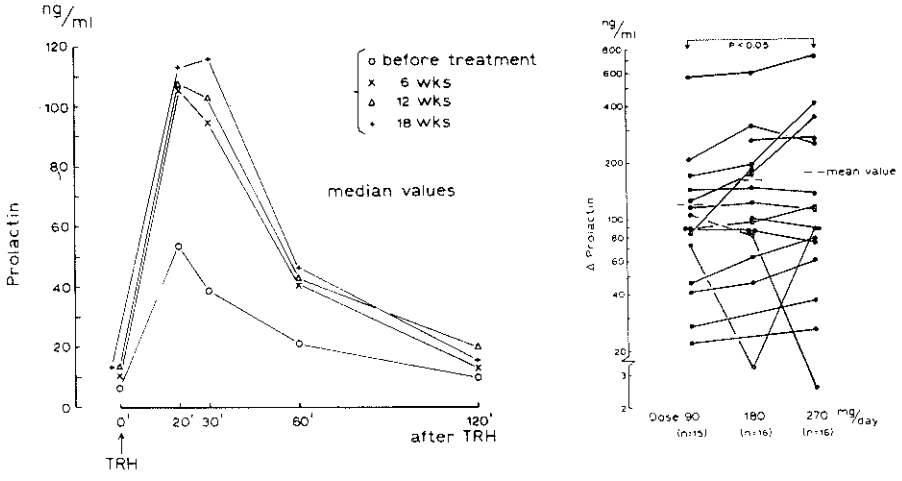


Fig. 2. Concentration of prolactin (PRL) in plasma of breast cancer patients treated with different dosages of megestrol acetate (MA) before and after injection of 200 μg TRH as a function of time after administration of TRH (left) and dosage of MA received during 6 weeks prior to the test (right). Δ prolactin: increase in PRL 20 min after injection of TRH.

Table 2. Response of plasma prolactin to TRH administration (200 μg i.v.) in patients with metastatic breast cancer before and after a 6-week treatment with megestrol acetate

Time after TRH (min)	Prolactin (ng/ml) (mean ± S.E.M. $n = 18$) Before treatment	After treatment	P
0	7.1 ± 0.8	13.9 ± 2.2	0.001
20	78.9 ± 15.2	157.2 ± 41.1	NS
30	60.5 ± 11.0	128.4 ± 29.4	NS
60	28.4 ± 4.2	51.5 ± 9.3	NS
120	12.1 ± 2.4	17.0 ± 2.3	0.005

plasma growth hormone was not influenced by the use of MA. The mean value before the treatment was 5.8 ± 1.2 μg/l; after a 6-week period the plasma concentration of growth hormone was 6.4 ± 1.8 μg/l.

Gonadotropins. All doses of MA used caused a significant decrease ($P < 0.001$) of the basal plasma concentrations of LH and FSH after 6 weeks (Fig. 3). Before treatment the mean concentrations of LH and FSH were 46.6 ± 4.5 and 28.0 + 3.0 U/l respectively. After 6 weeks of treatment these mean values were 18.4 ± 2.9 and 13.4 ± 2.6 U/l respectively. The inhibition of gonadotropin levels was identical with all three doses of MA without exception during the whole period.

Estradiol and SHBG. All doses of MA caused a significant decrease ($P < 0.001$) of plasma estradiol concentrations after 6 weeks of treatment (Fig. 3), which persisted throughout the whole treatment period. The mean concentration was 79 ± 6.3 pmol/l before and 62 ± 2.5 pmol/l after the 6-week period. This inhibition did not differ in the three dose-groups.

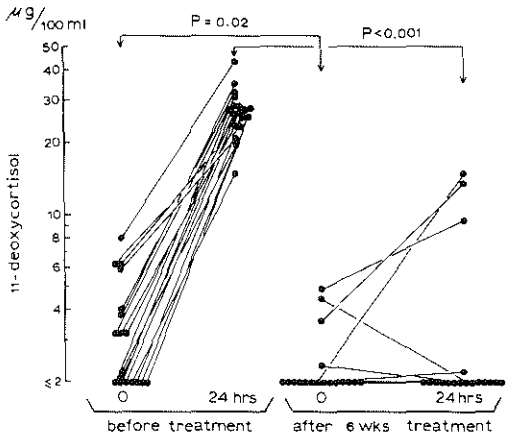


Fig. 4. Concentration of 11-deoxycortisol (compound S) before and 24 hr after the start of oral administration of 6 × 750 mg of metyrapone in patients with advanced breast cancer. The metyrapone test was performed before and after a 6-week treatment course with megestrol acetate.

A significant decrease ($P < 0.001$) in the concentration of SHBG was found after 6 weeks (Fig. 3). During the study periods a weak correlation ($P < 0.02$) between SHBG concentration and the dose administered appeared to be present.

11-Deoxycortisol (compound S). Plasma levels of 11-deoxycortisol before as well as after 6 × 750 mg metyrapone orally showed a significant decrease during MA therapy (Fig. 4). During treatment with 90 mg daily 3 out of 6 patients still showed a detectable increase of plasma compound S (to 9.0, 14.9 and 13.6 μg/100 ml), while these levels remained completely suppressed after metyrapone administration during treatment with 180 and 270 mg MA/day.

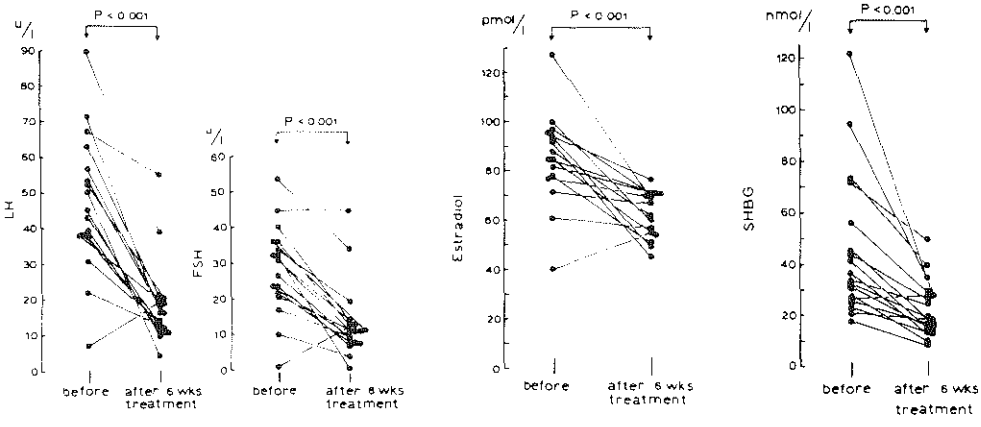


Fig. 3. Concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E_2) and sex hormone binding globulin (SHBG) in plasma of patients with advanced breast cancer before and after a 6-week treatment course with megestrol acetate.

Table 3. Results of a prolonged glucose tolerance test in patients with metastatic breast cancer before and after a 6-week treatment course with megestrol acetate (MA)

	Glucose (mmol/l)				Insulin (μU/ml)			
	0 hr	1 hr	2 hr	5 hr	0 hr	1 hr	2 hr	5 hr
Before treatment	5.5 (0.2)	9.7 (0.6)	8.4 (0.7)	4.3 (0.2)	17 (1.6)	122 (21.4)	147 (23.7)	20 (2.7)
After 6 weeks of MA	4.9 (0.1)	9.7 (0.8)	8.8 (0.5)	4.4 (0.2)	22 (1.7)	162 (30.3)	203 (27.2)	32 (6.4)
P	0.002				0.01			0.04
After 6 weeks of:								
90 mg of MA	5.0 (0.1)	9.7 (2.1)	8.6 (0.9)	4.8 (0.3)	18 (2.8)	127 (45.7)	155 (36.6)	19 (4.2)
180 mg of MA	4.7 (0.2)	8.8 (0.7)	8.7 (0.7)	4.4 (0.6)	22 (3.4)	96 (11.7)	151 (18.1)	54 (24.3)
270 mg of MA	5.2 (0.2)	10.5 (1.1)	9.1 (0.9)	4.2 (0.3)	26 (3.3)	256 (72.8)	305 (58.8)	34 (7.4)

Results are expressed as means ($n = 18$).
Standard errors of the mean (S.E.M.) are given in parentheses.

Insulin and glucose

Insulin and glucose in plasma were estimated before and at 1, 2 and 5 hr after oral administration of 100 g of glucose. Mean basal plasma glucose and insulin levels before the treatment were 5.5 mmol/l and 17 μU/ml respectively (Table 3). After 6 weeks of MA treatment the mean basal glucose was significantly decreased ($P < 0.002$), whereas the concentration of insulin before ($P < 0.01$) and 5 hr after ($P < 0.04$) glucose administration was significantly increased. The increase of basal plasma insulin appeared dose dependent ($r_s = 0.71$, $P = 0.002$).

DISCUSSION

The results of this study demonstrate that treatment of metastatic breast cancer with megestrol acetate (MA) induces multiple changes in the hormonal environment of the tumor. The drug caused significant increases in basal plasma prolactin and insulin and decreases in basal plasma LH, FSH, estradiol and SHBG, and a suppression of the pituitary–adrenal axis. Some of these parameters changed slightly in a dose-dependent way. There was no relation between these changes and the clinical response.

The study was designed as a cross-over regimen in order to obtain extensive information from a limited number of patients. Treatment periods of six weeks were chosen since this period is the minimum time span required for the evaluation of the clinical reponse. Based on data from the available literature obtained after administration of a single dose of 60 mg of MA [14–16], it was anticipated that steady-state plasma levels of MA would be achieved very soon after initiation of treatment or cross-over to a different dose. The excretion of a single dose of MA has been reported to be essentially complete within 4–7 days [17]. Our observation that plasma MA 3 days after complete withdrawal was decreased by 93 ± 2% of the steady-state level further justified the choice of a 6-week cross-over interval. In contrast to our expectation, however, we found an accumulation of MA in the plasma of the patients, irrespective of the order in which the different dosages were administered (Fig. 1). Moreover, for the doses used, no relation was found between the plasma concentration of MA and the clinical response (Table 1). Based on these observations, an exact therapeutic level for MA cannot be derived from plasma levels of MA alone. The lowest plasma level of MA associated with an objective response was 65 ng/ml. When the observed metabolic and endocrine effects are taken into account an optimal dose with minimal adverse effects can be defined. Treatment with a daily dose of 270 mg of MA caused an undesirably high basal concentration of insulin, whereas treatment with 90 mg daily was not sufficient to completely suppress the pituitary–adrenal axis. Such a suppression seems to be desirable, because a glucocorticoid effect may be important for the mechanism of action. Therefore, we advocate the use of 180 mg of MA/day. This dose is in agreement with the doses reported by other investigators [2, 18, 19]. Our results are the first to provide a rationale for this dose. When compared to the structurally related progestin medroxyprogesterone acetate (MPA), which is also frequently used, MA has several

advantages. Firstly, much lower oral doses of MA than of MPA are required [20, 21] to reach therapeutic levels of the drugs (roughly, above 100 ng/ml). This is probably due to the presence of a C-6,7 double bond in the MA molecule, which prevents it from breakdown by the intestinal bacterial enzymes [22, 23]. A second advantage of MA is the oral way of application in contrast to MPA, which is routinely administered intramuscularly and thus may cause local irritation. Thirdly, the intramuscular administration of MPA leads to the formation of a depot which may interfere with the next kind of treatment at the time of progression [24–26].

The metabolic effects of MA observed in this study are similar to those reported for high-dose progesterone and MPA [27–34]. Our results are in agreement with a hypothesis in which the primary effect of MA would be an effect on hypothalamic–pituitary function resulting in decreased secretion of ACTH, LH, FSH and estradiol and increased secretion of prolactin. Animal experiments, however, were not unequivocal since administration of MPA or MA to rats bearing DMBA-induced mammary tumors [35] or estrogen-induced prolactinomas [36] caused a decrease in the circulating prolactin. Atrophy of pituitary adrenocorticotrophs after administration of progestins [35] is probably the explanation for the suppression of the pituitary–adrenal axis, as found during long-term treatment with pharmacological doses of corticosteroids. The observed decrease in the plasma concentration of SHBG may be attributed to the (anti-) androgenic properties of MA [37, 38].

The observed increase in basal plasma insulin levels was not unexpected since progestins have been reported to induce hyperinsulinaemia [39–42]. This is possibly a direct action on pancreatic islets [43], whereas an additional glucocorticoid-like effect on gluconeogenesis cannot be excluded. Progestins promote glycogen storage in the liver and stimulate deposition of body fat. Paradoxically, they antagonize the effects of insulin on glucose metabolism in adipose tissue and skeletal muscle [44–46]. The most relevant expression of these actions appears to reside in the physiology of normal pregnancy. Based on our observations, it is difficult to identify the increase of insulin as a primary or secondary effect. In conclusion, megestrol acetate induced multiple endocrine and metabolic changes, which could be attributed to the progestational, (anti-)androgenic and glucocorticoid properties of the drug. None of these changes, nor the plasma concentration of MA, were related to the clinical response. This may be explained by the occurrence of hormone-resistant tumor. Decrement of plasma estradiol may be important for hormone-dependent tumors, but we found no clear relationship between response of the tumors and the presence of the estrogen receptors [5]. Also, estrogen-receptor-negative tumors can respond to MA. Besides an indirect effect, MA may have a direct cytotoxic effect because of glucocorticosteroid properties of the drug. On the other hand, high doses of progestins cause at least twice as many regressions as comparable doses of glucocorticoids (roughly, 35 vs 15%). So a glucocorticoid-like anti-tumor effect cannot be the sole mechanism of action. On the basis of a relation with the androgen receptor [5, 38], an extra anti-tumor effect may be attributed to the (anti-)androgenic effect of MA.

Acknowledgements—The authors thank Mrs. M. S. Christensen, NOVO, Copenhagen, Denmark for support, Mr. P. van Assendelft and Mrs. A. Sugiarsi for their assistance, and the members of the Breast Cancer Group for contributing their patients.

REFERENCES

1. ALEXIEVA-FIGUSCH J, VAN GILSE HA, HOP WCJ, PHOA CH, BLONK-VAN DER WIJST J, TREURNIET RE. Progestin therapy in advanced breast cancer: megestrol acetate—an evaluation of 160 treated cases. *Cancer* 1980, **46**, 2369–2372.
2. ROSS MB, BUZDAR AU, BLUMENSCHEIN GR. Treatment of advanced breast cancer with megestrol acetate after therapy with tamoxifen. *Cancer* 1982, **49**, 413–417.
3. BRIGGS MH, BRIGGS M. Glucocorticoid properties of progestogens. *Steroids* 1973, **22**, 555–559.
4. DAVID A, EDWARDS K, FELLOWES P, PLUMMER JM. Antiovulatory and other biological properties of megestrol acetate 17 α-acetoxy-6 methyl pregna 4:6 diene-3:20-dione (B.D.H. 1298). *J Reprod Fertil* 1963, **5**, 331–346.
5. TEULINGS FAG, VAN GILSE HA, HENKELMAN MS, PORTENGEN H, ALEXIEVA-FIGUSCH J. Estrogen, androgen, glucocorticoid, and progesterone receptors in progestin-induced regression of human breast cancer. *Cancer Res* 1980, **40**, 2557–2561.
6. HAYWARD JL, CARBONE PP, HEUSON JC, KUMAOKA S, SEGALOFF A, RUBENS RD. Assessment of response to therapy in advanced breast cancer. *Eur J Cancer* 1977, **13**, 89–94.
7. MARTIN F, ADLERCREUTZ H. Aspects of megestrol acetate and medroxyprogesterone acetate metabolism. In: GARATTINI S, BERENDES HW, eds. *Pharmacology of Steroid Contraceptive Drugs*. New York, Raven Press, 1977, 99–115.
8. LAATIKAINEN T, NIEMINEN U, ADLERCREUTZ H. Plasma medroxyprogesterone acetate levels following intramuscular or oral administration in patients with endometrial adenocarcinoma. *Acta Obstet Gynecol Scand* 1979, **58**, 95–99.
9. DE JONG FH, HEY AH, VAN DER MOLEN HJ. Effect of gonadotropins on the secretion of oestradiol-17β and testosterone by the rat testis. *J Endocrinol* 1973, **57**, 277–284.
10. MEIKLE AW, JUBIZ W, HUTCHINGS MP, WEST CD, TYLER FH. A simplified metyrapone test with determination of plasma 11-deoxycortisol (metyrapone test with plasma S). *J Clin Endocrinol Metab* 1969, **29**, 985–987.
11. KRIEG M, ARNING C. Quantitative determination of the binding capacity of the sex hormone-binding globulin, using agar gel electrophoresis. *J Clin Chem Clin Biochem* 1978, **16**, 429–434.
12. HEDING LG. Determination of total serum insulin (IRI) in insulin-treated diabetic patients. In: *Diabetologia*. Berlin, Springer, 1972, Vol. 8, 260–266.
13. JONS PWM. Change-over designs balanced for residual effects. In: *Statistical Design and Analysis of Experiments*. New York, Macmillan, 1971, 117–120.
14. ADLERCREUTZ H, ERVAST HS. Mass fragmentographic determination of megestrol acetate in plasma. *Acta Endocrinol* 1973, Suppl. 177, 32.
15. ADLERCREUTZ H, NIEMINEN U, ERVAST HS. A mass fragmentographic method for the determination of megestrol acetate in plasma and its application to studies on the plasma levels after administration of the progestin to patients with carcinoma corporis uteri. *J Steroid Biochem* 1974, **5**, 619–626.
16. ADLERCREUTZ H, MARTIN F, WAHLROOS O, SOINI E. Mass spectrometric and mass fragmentographic determination of natural and synthetic steroids in biological fluids. *J Steroid Biochem* 1975, **6**, 247–259.
17. COOPER JM, KELLIE AR. The metabolism of megestrol acetate (17α-acetoxy-6-methylpregna-4,6-diene-3,20-dione) in women. *Steroids* 1968, **11**, 133–149.
18. ANSFIELD FJ, DAVIS HL JR, RAMIREZ G *et al.* Further clinical studies with megestrol acetate in advanced breast cancer. *Cancer* 1976, **38**, 53–55.
19. MORGAN LR. Tamoxifen versus megestrol acetate in breast cancer. *Cancer Treat Rep* 1979, **63**, Abstr. 380, 1218.
20. MASKENS AP, HAP B, KOZYREFF VN, CALLEWAERT W, LION G, VAN DEN ABBEELE KG. Serum levels of medroxyprogesterone acetate under various treatment schedules. *Proc Am Assoc Cancer Res* 1980, **21**, 165.

21. HESSELIUS I, JOHANSSON EDB. Medroxyprogesterone acetate (MPA) plasma levels after oral and intramuscular administration in a long-term study. *Acta Obstet Gynecol Scand Suppl* 1981, **101**, 65–70.
22. ADLERCREUTZ H, MARTIN F, JÄRVENPÄÄ P, FOTSIS T. Steroid absorption and enterohepatic recycling. *Contraception* 1979, **20**, 201–223.
23. MARTIN F, JÄRVENPÄÄ P, KOSUNEN K, SOMERS C, LINDSTROM B, ADLERCREUTZ H. Ring-A reduction of medroxyprogesterone acetate (17α-acetoxy-6α-methyl-4-pregnene-3,20-dione (MPA) in biological systems. *J Steroid Biochem* 1980, **12**, 491–497.
24. MATTSSON W, VON EYBEN F, HALLSTEN L, TENNVALL L. A trial of tamoxifen versus high-dose medroxyprogesterone acetate in advanced postmenopausal breast cancer. A final report. In: CAVALLI F, MCGUIRE WL, PANNUTI F, PELLEGRINI A, ROBUSTELLI DELLA CUNA G, eds. *Proceedings of the International Symposium on Medroxyprogesterone Acetate. Geneva (February* 24–26). Amsterdam, Excerpta Medica, 1982, 276–284.
25. BERETTA G, TABIADON D, LUPORINI G. Clinical experience with medroxyprogesterone acetate in advanced breast cancer. In: CAVALLI F, MCGUIRE WL, PANNUTI F, PELLEGRINI A, ROBUSTELLI DELLA CUNA G, eds. *Proceedings of the International Symposium on Medroxyprogesterone Acetate. Geneva (February* 24–26). Amsterdam, Excerpta Medica, 1982, 285–289.
26. ROBUSTELLI DELLA CUNA G, BERNARDO-STRADA MR, GANZINA F. High-dose medroxyprogesterone acetate in metastatic breast cancer. A critical review. In: CAVALLI F, MCGUIRE WL, PANNUTI F, PELLEGRINI A, ROBUSTELLI DELLA CUNA G, eds. *Proceedings of the International Symposium on Medroxyprogesterone Acetate. Geneva (February* 24–26). Amsterdam, Excerpta Medica, 1982, 290–305.
27. CAMANNI F, MASSARA F, MOLINATTI GM. The cortisone-like effect of 6α-methyl-17α-acetoxyprogesterone in the adrenalectomized man. *Acta Endocrinol* 1963, **43**, 477–483.
28. CAVALLI F, GOLDHIRSCH A, KAPLAN E, ALBERTO P. High (H) versus low (L) dose medroxyprogesterone acetate (MPA) in advanced breast cancer. II International Symposium "Role of Medroxyprogesterone Acetate (MPA) in Endocrine-related Tumors", Rome, Italy, 30 September–1 October 1981, Abstract.
29. SADOFF L, LUSK W. The effect of large doses of medroxyprogesterone acetate (MPA) on urinary estrogen levels and serum levels of cortisol, T^4, LH and testosterone in patients with advanced cancer. *Obstet Gynecol* 1974, **43**, 262–267.
30. KALKHOFF RK. Metabolic effects of progestins. In: JAMES VHT, ed. *Endocrinology*. Amsterdam, Exerpta Medica, 1977, Vol. 1, 360–365.
31. HELLMAN L, YOSHIDA K, ZUMOFF B, LEVIN J, KREAM J, FUKUSHIMA K. The effect of medroxyprogesterone acetate on the pituitary–adrenal axis. *J Clin Endocrinol Metab* 1976, **42**, 912–917.
32. RAKOFF JS, YEN SSC. Progesterone induced acute release of prolactin in estrogen primed ovariectomized women. *J Clin Endocrinol Metab* 1978, **47**, 918–921.
33. WORTSMAN J, SINGH KB, MURPHY J. Evidence for the hypothalamic origin of the polycystic ovary syndrome. *Obstet Gynecol* 1981, **58**, 137–141.
34. VESTERINEN E, BACKAS NE, PESONEN K, STENMAN UH, LAATIKAINEN T. Effect of medroxyprogesterone acetate on serum levels of LH, FSH, cortisol and estrone in patients with endometrial carcinoma. *Arch Gynecol* 1981, **230**, 205–211.
35. DANGUY A, LEGROS N, LECLERCQ G, HEUSON JC. Effects of medroxyprogesterone acetate on the development of dimethylbenzanthracene-induced mammary tumours: possible modes of action. In: CAVALLI F, MCGUIRE WL, PANUTTI F, PELLEGRINI A, ROBUSTELLI DELLA CUNA G, eds. *Proceedings of the International Symposium on Medroxyprogesterone Acetate, Geneva (February* 24–26). Amsterdam, Excerpta Medica, 1982, 63–76.
36. LAMBERTS SWJ, JANSSENS ENW, BONS EG, ZUIDERWIJK JM, UITTERLINDEN P, DE JONG FH. Effects of megestrol acetate on growth and secretion of a pituitary tumor. *Eur J Cancer Clin Oncol* 1981, **17**, 925–931.

37. ANDERSON DC. Sex-hormone-binding globulin. *Clin Endocrinol* 1974, **3**, 69–96.
38. ALEXIEVA-FIGUSCH J, TEULINGS FAG, HOP WCJ, BLONK-VAN DER WIJST J, VAN GILSE HA. Steroid receptors in megestrol acetate therapy. *Recent Results Cancer Res* In press.
39. KALKHOFF RK. Effects of oral contraceptive agents on carbohydrate metabolism. *J Steroid Biochem* 1975, **6**, 949–956.
40. ADAMS PW, WYNN V. The effects of a progestogen, megestrol acetate, on carbohydrate and lipid metabolism. *J Obstet Gynaecol Cwlth* 1972, **79**, 744–752.
41. KALKHOFF RK, JACOBSON M, LEMPER D. Progesterone, pregnancy and the augmented plasma insulin response. *J Clin Endocrinol Metab* 1970, **31**, 24.
42. MATUTE ML, KALKHOFF RK. Sex steroid influence on hepatic gluconeogenesis and glycogen formation. *Endocrinology* 1973, **92**, 762–768.
43. AERTS L, VAN ASSCHE FA, FAURE A *et al.* Effects of treatment with progesterone and oestradiol-17β on the endocrine pancreas, in ovariectomized rats: ultrastructural variations in the B cells. *J Endocrinol* 1980, **84**, 317.
44. DAHM CH JR, MINAGAWA J, JELLINEK M. Effects of progesterone on some enzymes of fat and carbohydrate metabolism in rat liver. *Am J Obstet Gynecol* 1977, **129**, 130–132.
45. SUTTER-DUB MTH, DAZEY B *et al.* Progesterone and insulin-resistance, studies of progesterone action on glucose transport, lipogenesis and lipolysis in isolated fat cells of the female rat. *J Endocrinol* 1981, **88**, 455.
46. RUSHAKOFF RI, KALKHOFF RK. Effects of pregnancy and sex steroid administration on skeletal muscle metabolism in the rat. *Diabetes* 1981, **30**, 545.

CHAPTER VI

SEQUENTIAL TREATMENT OF METASTATIC BREAST CANCER WITH TAMOXIFEN AFTER MEGESTROL ACETATE THERAPY VICE VERSA (A RETROSPECTIVE STUDY).

J. Alexieva-Figusch[*], W.L.J. van Putten[**], H.A. van Gilse[*], J. Blonk-v.d.Wijst[*], J.G.M. Klijn[*]

[*] Department of Internal Medicine and Endocrinology, Rotterdamsch Radio-Therapeutisch Instituut, Rotterdam, The Netherlands.

[**] Department of Biostatistics, Rotterdamsch Radio-Therapeutisch Instituut Rotterdam, The Netherlands.

Running title

Sequence of megestrol acetate and tamoxifen in metastatic breast cancer.

Partly presented at Second International Congress on Hormones and Cancer, Monte Carlo, September 18-23, 1983.

Submitted for publication

SUMMARY

The progestin megestrol acetate and the anti-estrogen tamoxifen are used as effective and minimally harmful drugs in the treatment of metastatic breast cancer. The mechanism of action of either are not clearly understood, they may act directly on tumor cells, their receptors, or indirectly by changing the hormonal environment. The sequence and indications for use in practice still need to be defined. Of 219 postmenopausal patients with metastatic breast cancer and measurable lesions, 136 were treated with megestrol acetate (MA) per os (180 mg daily) and followed by tamoxifen (TAM) (40 mg daily) in cases with progression, and 83 patients were treated with the inverse drug regimen. The interval between the treatment with these two drugs was less than 8 weeks, median 3 weeks. In the first line treatment they showed similar effects: MA caused remission in 31/136 patients (23%) and TAM in 17/80 patients (22%) (mean duration 12 and 13 months respectively), while as a second treatment line MA caused remission in 14/83 patients (17%) and TAM in 12/132 patients (9%), which was not significant ($p=0.10$). Also with respect to survival there was no significant difference between the two treatment modalities. When the groups were divided according to menopause (< 5 ; ≥ 5 years) and age (< 60 ; ≥ 60 years), significantly better results were obtained using the sequence MA/TAM in the less than 5 years postmenopause group, while in the 5 years or more postmenopause and younger than 60 years group the sequence TAM/MA was better.

INTRODUCTION

Anti-estrogens and synthetic progestins, widely used in the treatment of advanced breast cancer, both give a similar response rate of approximately 30% in groups of patients, unselected as to biological and postmenopausal

age and receptor status, although the two drugs differ in chemical structure and in mechanism of action (Mouridsen and Palshof, 1978; Alexieva-Figusch, 1980, 1984).

Since the early seventies, both kinds of drugs are used to replace the first line additive therapy with estrogens for late- and androgens for early-postmenopausal patients, which have several undesirable effects. A choice between anti-estrogens and progestins as first or second line treatment with regard to postmenopausal age and localisation of metastases has as yet not been established.

Recently two randomized prospective studies were reported (Mattsson et al., 1982; Beretta et al., 1982) on the use of the progestin medroxyprogesterone acetate (MPA) versus tamoxifen (TAM) in the treatment of metastatic breast cancer, with the possibility of cross-over in case of tumor progression. In both studies MPA and TAM appeared to be equally effective as the first line treatment. Mattsson et al. (1982) reported a MPA induced response in 10/17 patients with tumors resistant to TAM, while only 2/13 patients with tumors resistant to MPA (intramuscularly) responsed to TAM. Beretta et al. (1982) reported equal response rates in second line with the sequence oral MPA/TAM (19%) and TAM/MPA (17%) in a not reported number of patients. On the other hand, not any patient resistant to intramuscular MPA responded to subsequent TAM.

It should be mentioned that MPA administered intramuscularly leads to the formation of a depot (Camaggi, 1982) which may interfere with subsequent therapy if tumor progression occurs. For, when treating sequentially with these kinds of drugs, the antitumor effect of the second treatment may be influenced by changes in hormonal environment and receptor status caused by the first treatment and thus the effect of one drug could be potentiated or negated by the other.

We have reviewed our own data on 219 postmenopausal patients with metastatic breast cancer, treated sequentially with TAM after MA vice versa, with the purpose of establishing which was the better sequence.

MATERIALS AND METHODS

From the postmenopausal patients with metastatic breast cancer treated in our clinic between 1975 and 1981 we evaluated all patients (219) with measurable progressive disease who had been sequentially treated with MA (180 mg daily orally) and then TAM (40 mg daily orally) or vice versa, with an interval between these two treatment regimens varying from 2 to 8 weeks. The treatment had been changed if the first therapy had appeared to be ineffective or if progression had occurred after a temporary response. The characteristics of the patients are shown in Table 1.

Table 1

Patient characteristics

	Megestrol acetate / Tamoxifen	Tamoxifen / Megestrol acetate	
Number of patients	136	83	
Median age (at diagnosis)	62	49	
Menopause < 5 yrs	12	50	
≥ 5 yrs , age < 60 yrs	38	11	
age ≥ 60 yrs	86	22	
Median disease-free interval in months	12	19	p=0.04
Localisation of metastases (dominant site)			
- Soft tissue	50 (37%)	27 (32%)	
- Bone	45 (33%)	32 (39%)	
- Viscera	41 (30%)	24 (29%)	
Previous hormonal therapy			
- Ovariectomy	3	37	
- Estrogen	9	12	
- Other	1	2	
Previous chemotherapy			
- CMF	4	3	
- Other (single drug)	3	2	

One-hundred-thirty-six patients had been treated with MA followed by TAM (group A) and 83 patients with the inverse drug regimen (group B). Twenty patients in group A and 56 in group B had previously been treated with other ablative/additive hormonal- or chemotherapy. After cessation of previous estrogen therapy patients were not treated for a period of at least 8 weeks because of the possibility of a withdrawal regression.

Patients with initial rapid progression during MA or TAM therapy as first line treatment, were treated with chemotherapy, and excluded from the study.

According to the policy at that time, patients less than one year postmenopausal were treated by ovariectomy, and later at the time of progression with the anti-estrogen TAM.

Thus, the 2 groups differ not only in age, but may be in hormonal sensitivity. This difference is also reflected by the fact that in the group of patients treated with MA/TAM 10% (13/136) of the patients had been previously treated by hormonal therapy, whereas in the group TAM/MA 61% (51/83), with disease-free interval 12 and 19 months respectively.

The distribution of metastases was similar in both groups. The receptor status was known in only 22 patients and therefore not further considered.

Before the start of treatment, a routine assessment of disease had been performed (physical examination, laboratory blood investigation, X-skeletal survey, bone- and liver scan, and fine needle biopsy of lymph-skin-liver metastases if necessary for diagnosis of metastatic disease. The results were assessed using the UICC criteria for response (Hayward et al., 1977).

The response was not evaluable in 3 patients treated with TAM initially and in 4 patients treated with MA subsequently.

Statistics:

The overall survival and remission data were analysed statistically using life tables and the logrank test (Peto et al., 1977). Wilcoxon's two-sample test was used to compare the effectiveness of the agents.

RESULTS

The overall results are shown in Table 2.

Table 2 Response to megestrol acetate and/or tamoxifen given as the 1st and/or the 2nd line treatment in metastatic breast cancer

Response	1st Therapy Megestrol acetate		Median time to progression in months	2nd Therapy Megestrol acetate		Median time to progression in months
C.R.	8/136			6/83		
		(23%)	12		(17%)	9
P.R.	23/136			8/83		
N.CH.	73/136	(54%)	7	38/83	(46%)	7
F.	32/136	(23%)	-	31/83	(37%)	-
Overall response (CR,PR, N.CH)	104/136		8	52/83		8
	1st Therapy Tamoxifen			2nd Therapy Tamoxifen		
C.R.	6/80			3/132		
		(22%)	13		(9%)	33
P.R.	11/80			9/132		
N.CH.	41/80	(51%)	9	61/132	(46%)	6
F.	22/80	(28%)	-	59/132	(45%)	-
Overall response (CR,PR, N.CH)	58/80		9	73/132		7

CR = complete remission
PR = partial "
N.CH = no change
F = failure

Results of the "first line" of this treatment regimen:

1) Megestrol acetate

In the group of 136 patients treated with megestrol acetate before tamoxifen 31 patients (23%) showed an objective remission (CR+PR) with a median duration of 12 months, 73 patients (54%) showed no change (N.CH) with a median duration of 7 months, while 32 patients (23%) did not respond.

2) Tamoxifen

In the group of 80 patients treated with tamoxifen before megestrol acetate 17 patients (22%) showed an objective remission with a median duration of 13 months, 41 patients (51%) showed no change with a median duration of 9 months, while progression occurred in 22 patients (28%).

Results of the "second line" treatment of this treatment regimen:

1) Megestrol acetate

In the group of 83 patients treated with megestrol acetate after tamoxifen 14 patients (17%) showed an objective remission with a median duration of 9 months, 38 patients (46%) showed no change with a median time to progression of 7 months, while 31 patients (37%) did not respond.

2) Tamoxifen

In the group of 132 patients treated with tamoxifen after megestrol acetate 12 patients (9%) showed an objective remission with a median duration of 33 months, 61 patients (46%) showed no change with a median time to progression of 6 months, while progression occurred in 59 patients (45%).

Comparison with regard to response rate, duration of response and survival

Megestrol acetate had a higher response rate as a first line agent than as a second line one (Wilcoxon's two sample test: $p = 0.05$). For tamoxifen ($p < 0.01$) this was equally true (Table 2).

There was no significant difference between the percentage of patients in remission for "first line" MA or TAM (Fig. 1A) and "second line" MA or TAM (Fig. 1B). Also there was no significant difference for overall remission (Fig. 1C) and survival (Fig. 1D) for both treatment modalities.

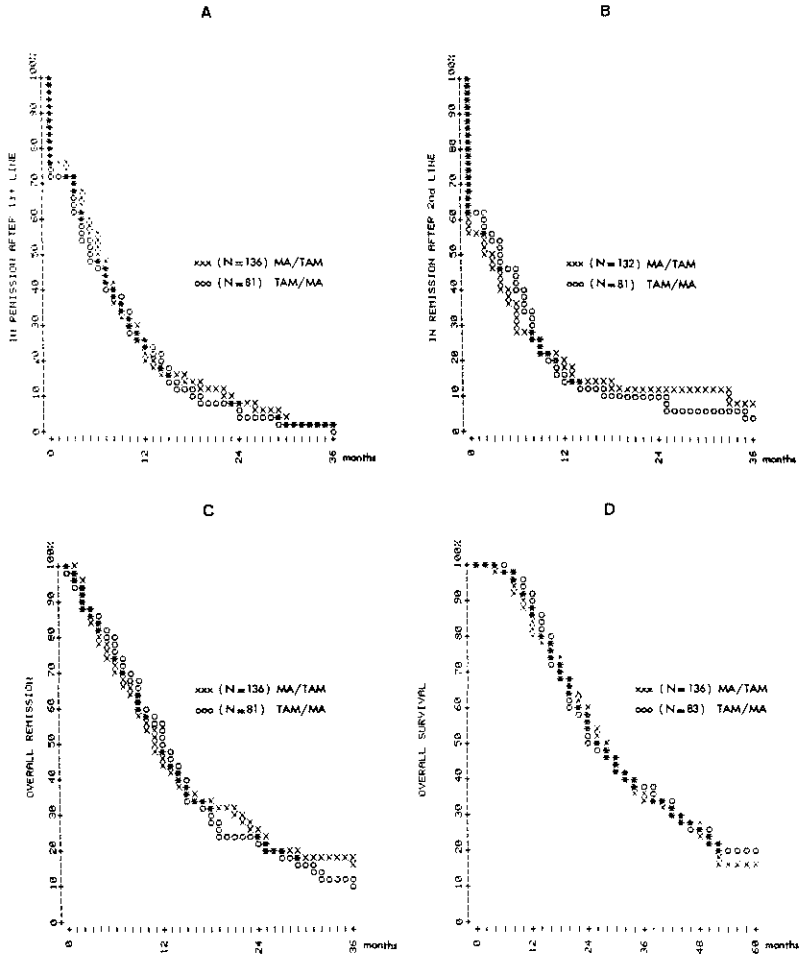


Fig.1 The treatment modalities MA/TAM and TAM/MA in relation to :

A) Remission rate of 1st line MA or TAM

B) Remission rate of 2nd line MA or TAM

C) Overall remission of MA/TAM and TAM/MA

D) Overall survival

There was no significant difference.

However, in the group of patients treated with MA after TAM the response to the second line agent was better than the response to the first line agent in 15 out of 80 patients (19%) (Fig.2a), whereas in the group of patients treated with TAM after MA the response to the second line agent was better than the response to the first line treatment in only 11 out of 132 (8%) (Fig.2b). In this sense MA as second line agent was significantly better ($p = 0.03$) than TAM.

Further, when either therapy sequence was considered in relation to the duration of the first and second response, the first response in the group MA/TAM (Fig.3a) was significantly longer than the second one ($p = 0.02$), while in the group TAM/MA there was the same tendency, but non-significant (Fig.3b).

a

Response to 2nd line MA

Response to 1st line TAM	CR	PR	NCH	F	
CR	3	1	2		6
PR	1	4	3	3	11
NCH	1	3	21	16	41
F	1	.	9	12	22
	6	8	35	31	80

b

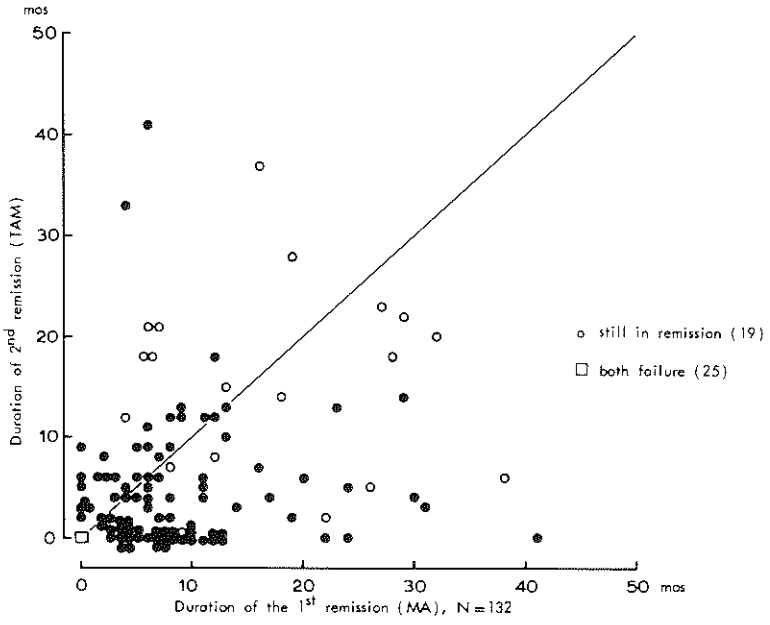
Response to 2nd line TAM

Response to 1st line MA	CR	PR	NCH	F	
CR	2	1	3	2	8
PR	.	5	9	7	21
NCH	1	3	42	25	71
F	.	.	7	25	32
	3	9	61	59	132

Fig.2 Overall results (CR = complete remission; PR = partial remission; N.CH = no change; F = failure) of :

a) 83 patients treated with the 1st line tamoxifen (TAM) and their response to the 2nd line treatment with megestrol acetate (MA). Fifteen out of 80 patients (19%) responded better to the 2nd line (circled area).

b) 136 patients treated with the 1st line MA and their response to the 2nd line TAM. Better response to the 2nd line occurred in 11 out of 132 patients (8%).



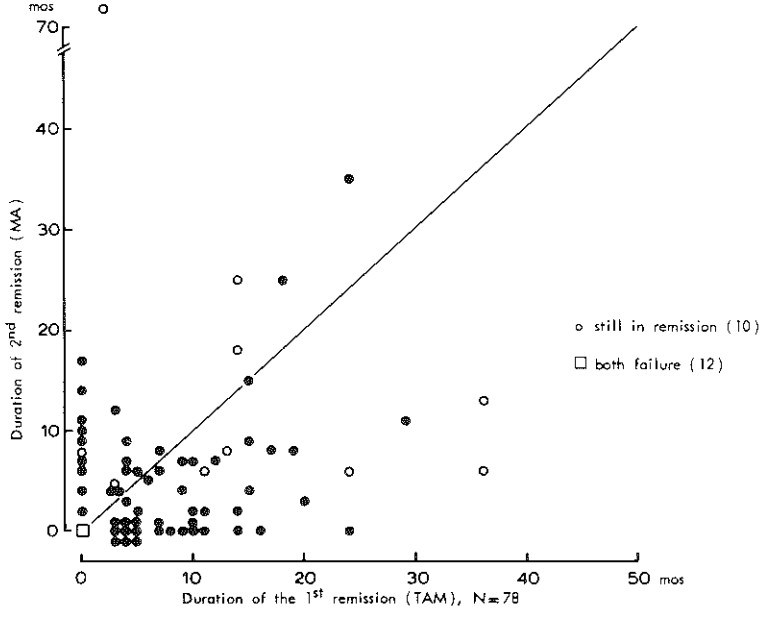


Fig.3 The two treatment modalities in relation to duration of the remission:

a) The first remission in the group MA/TAM was significantly longer than the second one (p=0.02).

b) In the group TAM/MA there was the same tendency, but this was not significant.

Evaluation with regard to menopausal status

Since the treatment groups differed with regard to age and years after menopause, a detailed analysis was done after subdivision into three groups of patients:

i) under 5 years postmenopause

ii) over 5 years postmenopause aged under 60 years

iii) over 5 years postmenopause aged 60 years or more

(Table 1)

In these subgroups disease-free interval and previous therapy did not appear to influence the response to TAM/MA or to MA/TAM treatment and equally not the time to progression on 2nd line treatment, measured from start of 1st line, and were therefore not further considered.

The following conclusions could be drawn from an extensive analysis (Figs.4A,B):

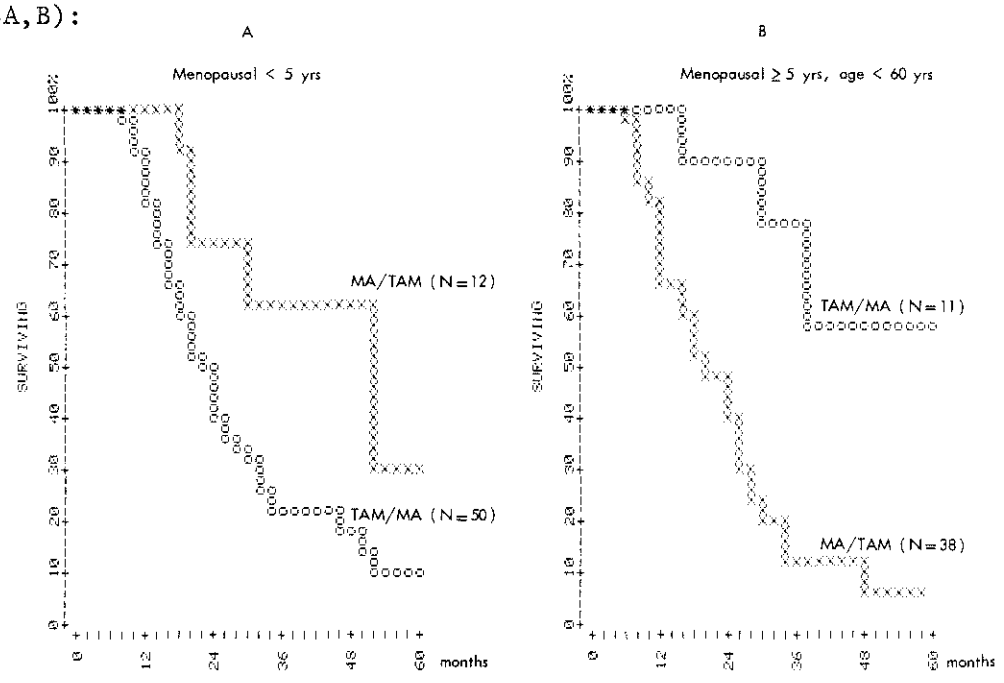


Fig.4 Survival in relation to menopausal status and age :

A) Group of less than 5-year postmenopausal patients; sequence MA/TAM compared with sequence TAM/MA (p=0.02).

B) Group of more than 5-year postmenopausal patients aged under 60 years; sequence MA/TAM compared with sequence TAM/MA (p=0.001).

For the less than 5-year postmenopausal group of patients the best results were obtained with the sequence MA/TAM compared with the sequence TAM/MA: the percentage of failures in the first line was smaller with MA/TAM.
The duration of response for the non-failures tended to be longer in the first line (n.s.) and the second line ($p = 0.05$) with MA/TAM in comparison with TAM/MA.
Also the overall survival was longer ($p = 0.02$) in the MA/TAM group (Fig.4A). Only the percentage of failures in the second line tended to be larger for MA/TAM than for TAM/MA.

In the group of patients over 5 years after menopause, but younger than 60 years, the sequence TAM/MA showed the best results: smaller percentage of failures in first and second lines, longer duration of response for the non-failures in the first line ($p = 0.02$) and a tendency for this in the second line (n.s.); further a longer survival ($p = 0.001$) (Fig.4B).

For the group of patients aged 60 years or more no differences were found in response rate, duration of response or survival.

Side effects

Serious side effects in either group were not numerous (Table 3). The most dangerous one, hypercalcemia, did not occur during the first line treatment, but did so in the second line treatment with TAM in 6 patients. Weight gain more than 2 kg was significantly higher during MA-therapy.

Table 3 Side effects of megestrol acetate and tamoxifen

	1st line		2nd line	
	MA	TAM	TAM	MA
Skin rash or itch	-	1	-	-
Thrombophlebitis	-	-	3	-
Lung embolism	1	-	1	-
Hypertension	2	-	-	-
Intolerance	1	4	3	-
Hypercalcemia	-	-	6	-
Weight gain > 2 kg	53	21	6	30
Vaginal bleeding	1	-	-	-

DISCUSSION

From our results we may conclude that the overall results of the sequential treatments MA/TAM and TAM/MA are comparable with findings of Beretta et al. (1982), using oral MPA instead of MA.
In general, a bias in sequential studies may be the fact that some patients already need second line therapy before the first drug and metabolites are eliminated. For oral drugs such as we have used, the elimination time is shorter than for intramuscularly administered depot-ones. Plasma levels of MA, TAM, MPA given orally have fallen substantially 3 weeks after discontinuation of the treatment (which disappearance is dose dependent) whereas plasma levels after discontinuation of MPA i.m. remain unaltered for at least 6 weeks (Alexieva-Figusch et al., 1984; Fabian and Sternson, 1982; Camaggi, 1982).

Since the postmenopausal status and biologic age could influence responses to the treatment of metastatic breast cancer, the patient-material was further statistically analysed in these directions in order to find indications for the choice between sequences MA/TAM or TAM/MA. Our data showed that the sequence MA/TAM gave significantly better results in the group of patients under 5 years postmenopause (Fig.4A). This may suggest that, since tumor growth is generally more aggressive in early - as opposed to late - postmenopausal patients a hormonal agent with a broad spectrum of activity such as MA might be more effective. For MA has not only anti-estrogenic, but also androgenic and glucocorticoid properties (Alexieva-Figusch, 1984), and is effective in ER-negative patients (Teulings et al. 1980).

We would like to emphasize again that the results of MA and TAM as first line treatment (response rates of 23 and 22%) may be lower as reported before (Alexieva-Figusch, 1980; Mouridsen, 1978), because of the selection of less favourable patients who needed second line treatment because of tumor progression, in contrast to those patients who are still in long-term remission on "first line" treatment and therefore not considered in this study.

CONCLUSION

In conclusion:

We are aware of the limitations of a retrospective study, which holds equally for prospective studies with small numbers of patients (Mattsson et al., 1982; Beretta et al., 1982). However, our analysed results lead us to propose the following policy for choice between MA and TAM as a first line drug: MA in patients less than 5 years postmenopause and TAM in patients over 5 years postmenopause and younger than 60 years.
Randomized studies have to confirm the validity of these proposals.

ACKNOWLEDGEMENTS

The authors thank Mr. P. van Assendelft and Mrs. A. Sugiarsi for their assistance.

REFERENCES

1. Alexieva-Figusch, J., Gilse, H.A. van, Hop, W.C.J, Phoa, C.H., Blonk-v.d.Wijst, J. and Treurniet, R.E., Progestin therapy in advanced breast cancer: Megestrol Acetate - an evaluation of 160 treated cases. Cancer, 46, 2369 (1980).

2. Alexieva-Figusch, J., Blankenstein, M.A., Hop, W.C.J., Klijn, J.G.M., Lamberts, S.W.J., de Jong, F.H., Docter, R., Adlercreutz, H. and van Gilse, H.A., Treatment of metastatic breast cancer patients with different dosages of megestrol acetate; dose relations, metabolic and endocrine effects. Europ.J.Cancer (1984) Vol. 20, No 1, pp 33-40.

3. Beretta, G., Tabiadon, D. and Luporini, G., Clinical experience with medroxyprogesterone acetate in advanced breast cancer. Intern. Symposium on MPA. Geneva, February 24-25, 1982. Excerpta Medica, 285 (1982).

4. Camaggi, C.M., In: Round Table. MPA pharmacokinetics Proceedings of the International Symposium on Medroxyprogesterone Acetate. Geneva, Switzerland, February 24-26, 1982. F. Cavalli et al., eds. Excerpta Medica, pp.185-189 (1982).

5. Fabian, C., and Sternson L.A., Tamoxifen pharmacokinetics in patients with breast cancer: comparison of traditional and loading dose schedules. Reviews on Endocrine-Related Cancer Supplement 9, October 1981, pp. 155-168.

6. Hayward, J.L., Carbone, P.P., Heuson, J.C., Kumaoka, S., Segaloff, A., Rubens, R.D., Assessment of response to therapy in advanced b r e a s t cancer. Europ.J.Cancer, 13, 89-94 (1977).

7. Mattsson, W., Von Heyben, F., Hallsten, L., and Tennvall, L., A phase III study of tamoxifen versus high dose medroxyprogesterone a c e t a t e (HD-MPA) in postmenopausal advanced breast cancer. Intern. Symposium on MPA. Geneva, February 24-25 (1982).

8. Mouridsen, H. and Palshof, T. Tamoxifen in advanced breast cancer. Cancer Treatment Reviews, 5, 131 (1978).

9. Peto, R., Pike, M.C., Armitage, P., Breslow, N.E., Cox, D.R., Howard, S.V., Mantel, N., McPherson, K., Peto, J. and Smith, P.G. Design and analysis of randomised clinical trials requiring prolonged observation of each patient. Brit.J.Urol., 45, 586 (1977).

10. Teulings, F.A.G., van Gilse, H.A., Henkelman, M.S., Portengen, H., Alexieva-Figusch, J. Estrogen, androgen, glucocorticoid, and progesterone receptors in progestin-induced regression of human breast cancer. Cancer Res. 1980, 40, 2557.

CHAPTER VII

ENDOCRINE EFFECTS OF THE COMBINATION OF MEGESTROL ACETATE AND TAMOXIFEN IN THE TREATMENT OF METASTATIC BREAST CANCER

J. Alexieva-Figusch*, M.A. Blankenstein*, F.H. de Jong+, S.W.J. Lamberts+

* Departments of Internal Medicine and Biochemistry, Rotterdam Radiotherapy Institute / Dr. Daniel den Hoed Cancer Center, Rotterdam, The Netherlands

\+ Department of Internal Medicine III and Clinical Endocrinology, Erasmus University, Rotterdam, The Netherlands

Running head: Endocrine effects of megestrol acetate + tamoxifen

Submitted for publication.

ABSTRACT

Six postmenopausal patients with metastatic breast cancer, who responded to megestrol acetate after 6 weeks of treatment, were treated with the combination of megestrol acetate and tamoxifen during the next 6 weeks. The study was oriented towards the endocrine effects of this combination, since it was known from our previous studies that megestrol acetate induces suppression of plasma gonadotropins and of the pituitary-adrenal axis, a decrease of peripheral concentration of SHBG and of estradiol, and an increase of basal and TRH-stimulated plasma prolactin concentration. Tamoxifen, on the other hand, produces a decrease of prolactin and gonadotropins, whilst estradiol remains unaffected.

Although the role of prolactin in the growth of human breast cancer has not been elucidated yet and there is no unequivocal evidence that a decrease in plasma prolactin could be of benefit for treatment of metastatic breast cancer, we tested if addition of tamoxifen to the treatment regimen eliminated megestrol acetate-induced hyperprolactinaemia.

The results show that addition of tamoxifen to megestrol acetate treatment annihilated the hyperresponse of prolactin to TRH stimulation, while basal prolactin levels remained unaffected. The negative effect on plasma gonadotropin concentration appeared to be amplified, while estradiol and cortisol were not affected, and SHBG increased. The results of these endocrine investigations merit a further study, directed to antitumor effects of this combination modality.

INTRODUCTION

Approximately 30% of patients with metastatic breast cancer will respond to a single agent endocrine therapy. The mechanism through which tumor regression is achieved has not been completely resolved for any modality of

endocrine treatment. Anti-estrogens are thought to act mainly by interference with the action of estrogens on estrogen-target cells [1,2]. Progestins on the other hand may act through their progestagenic- but also through glucocorticoid- and/or (anti-)androgenic properties [3]. Based on these differences in the presumed mechanism of action of anti-estrogens and progestins, an additional benefit might be expected from a combination of these two modalities.

Iacobelli et al. [4] studied the interaction of the progestin medroxyprogesterone acetate (MPA) and the anti-estrogen tamoxifen (TAM) on the growth of the human breast cancer cell line CG-5 (an estrogen-supersensitive variant of the MCF-7 cell line). They observed that addition of MPA and TAM together caused a stronger inhibition of cell growth than either agent alone. This potentiating effect might be achieved through steroid receptors. The biosynthesis of the progestin receptor is known to be under estrogenic control. Antiestrogens deplete cytoplasmic estrogen receptor sites but also stimulate the synthesis of cytoplasmic progesterone receptors [5,6]. In addition, for the action of synthetic progestins with androgenic and glucocorticoid properties the presence of other than estrogen and progestin receptors could be important [7,8]. Alternatively, the potentiating effect of progestin and anti-estrogen treatment might be attributed to differences in their influences on the endocrine system. Both agents induce a decrease in the circulating concentrations of gonadotropins. Megestrol acetate (MA) induces a suppression of the pituitary-adrenal axis, a decrease in the circulating levels of sex-hormone-binding-globulin (SHBG) and estradiol and an increase in the plasma concentration of prolactin (PRL), both under basal conditions and after TRH stimulation [3]. Tamoxifen (TAM) has been reported to cause an increase of plasma SHBG and a decrease of PRL, while circulating estradiol levels remain unaffected in postmenopausal women [9,10,11].

Therefore, it may be expected that a combination of progestin and anti-estrogenic drugs further suppresses the level of circulating gonadotropins, whereas the effects of progestins on SHBG and PRL are partly eliminated by antiestrogens. The present study was undertaken to evaluate this possibility.

MATERIALS AND METHODS

Seven patients with evaluable and measurable metastatic breast cancer, who were not yet treated otherwise for their metastatic disease, were selected for this study. All patients gave informed consent before participating in the study. They all were more than 4 years postmenopausal (two of them after adjuvant ovariectomy) and between 43 and 76 years of age. All patients were in good general condition, without gastrointestinal- hepatic- or renal disease, diabetes mellitus or malabsorption. Five patients had bone metastases and lung metastases were observed in 2 patients. None of the patients had soft tissue metastases only. The patients received a 6-week treatment with megestrol acetate (MA) 180 mg daily orally and after the clinical assessment of the response [12] only the 6 responders continued the treatment with MA in combination with tamoxifen (TAM) 40 mg daily orally during the next period of 6 weeks.

Routine clinical and biochemical investigations were performed at the start of the study, after 6 weeks of treatment with MA and after 6 weeks of treatment with the combination of MA + TAM. In addition, blood was collected for the estimation of estradiol, SHBG, cortisol, LH and FSH. PRL and TSH were estimated before and after administration of 200 µg TRH intravenously. PRL, TSH and estradiol were measured with commercial radioimmunoassay kits and SHBG by agar gel electrophoresis as described before [3]. Normal values: PRL: < 15 ng/ml TSH: <5 µU/ml. Cortisol was measured by RIA, using the gammacoat kit from Clinical Assays (Cambridge, MA, USA); normal values at 9.00 a.m. were > 220 nmol/l. LH and FSH-levels were estimated using the Coat RIA solid phase radioimmunoassays from Biomerieux (Marcy l'Etoile, France). Results of these assays are expressed as ng/ml; 1 ng LH corresponds to 5 mIU (MRC 68/40) and 1 ng FSH corresponds to 2.7 mIU (MRC 78/579). The plasma level of tamoxifen was measured by thin-layer densitometry before and 6 weeks after the addition of TAM to MA [13].

During the whole period of 12 weeks there was no change in other medications in order to avoid any influence on the endocrine parameters.

RESULTS

The results obtained are summarized in Table 1 and indicate that the addition of tamoxifen to megestrol acetate annihilated the effects of MA on TRH-induced prolactin release and on SHBG. The effects of MA on basal PRL and cortisol levels appeared to be unaffected by addition of TAM, whereas the suppressive effect of MA on gonadotropins appeared to be amplified.

Table 1 Effect of administration of megestrol acetate (MA) alone and in combination with tamoxifen (MA+TAM) on endocrine parameters in postmenopausal patients with metastatic breast cancer.
(Results are given as means ± S.D., N=6)

Parameter	Before Treatment		After 6 weeks MA		After 6 weeks MA + 6 weeks MA + TAM	
	Absolute	% of pre-treatment value	Absolute	% of pre-treatment value	Absolute	% of pre-treatment value
Basal PRL (ng/ml)	6,6 ± 3,5	100	10,0 ± 4,3	165 ± 79*	10,0 ± 7,3	153 ± 74
PRL (ng/ml) (20' after TRH)	44,9 ± 16,7	100	66,9 ± 29,1	147 ± 21*	40,3 ± 15,2**	96 ± 36**
LH (ng/ml)	6,9 ± 2,4	100	3,3 ± 1,6*§	49 ± 26*§	1,5 ± 0,7*	22 ± 11*
FSH (ng/ml)	18,9 ± 6,2	100	7,8 ± 4,7*	41 ± 20*	3,4 ± 1,8*	19 ± 8**
Estradiol (pmol/l)	53 ± 21	100	41 ± 10	90 ± 43	40 ± 24	75 ± 22
SHBG (nmol/l)	19 ± 14	100	14 ± 16	66 ± 15*	23 ± 15	141 ± 99**
Cortisol (nmol/l)	431 ± 159	100	97 ± 141*	19 ± 26*	125 ± 138*	35 ± 45
Tamoxifen (nmol/l)	N.D.		0	N.D.	610 ± 125**	N.D.

* p < 0,05 vs pretreatment value (Wilcoxon's test)

** p < 0,05 vs value obtained after MA alone (Wilcoxon's test)

N.D. not determined

§ n = 4

Estradiol values were not affected by addition of TAM to MA. Because of a large heterogeneity observed in the magnitude of the parameters studied, the results were expressed not only in absolute values, but also as a percentage of the pretreatment values (Table 1). In addition, results of individual patients are shown in Figures 1 and 2. Sera, obtained after 6 weeks on MA treatment served as blanks for estimation of TAM serum levels, since it was not known whether MA or its metabolites are capable of interfering with the TAM assay. These blanks were essentially zero and thus the possibility of interference was ruled out. The combination was excellently tolerated without any signs of side-effects e.g. glucose tolerance deterioration, fluid retention or increase in blood pressure.

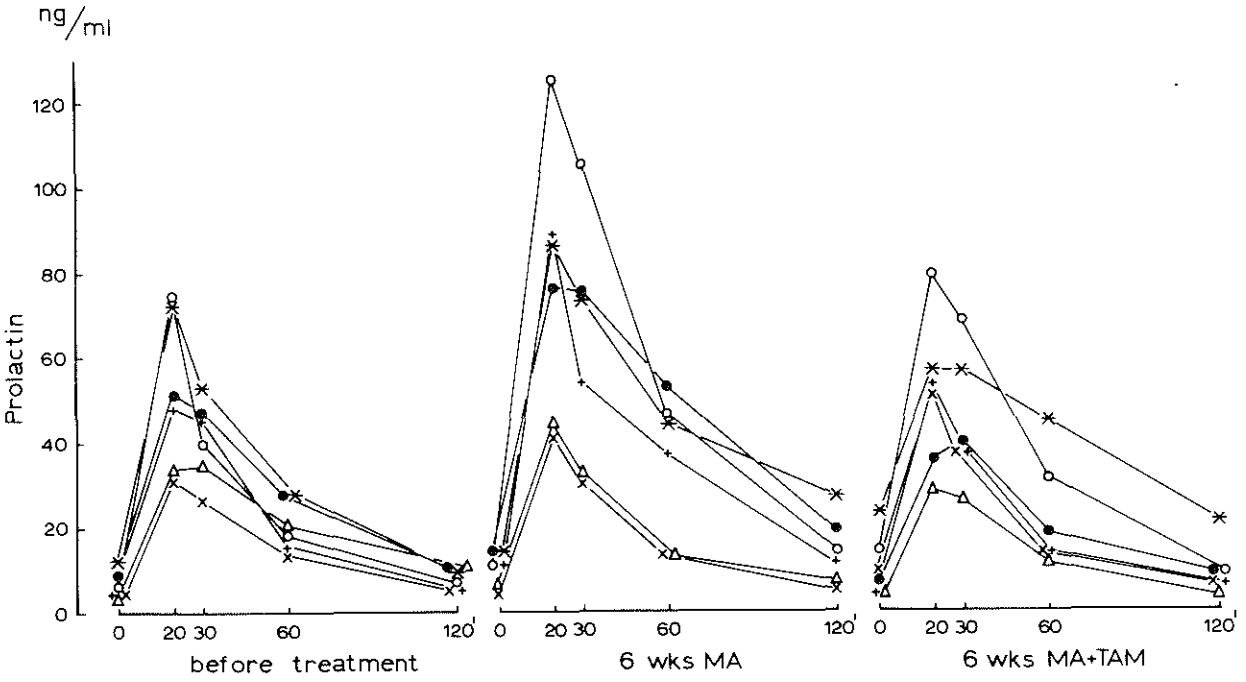


Figure 1 The concentration of prolactin in plasma before and after administration of 200 µg TRH in 6 breast cancer patients before, after 6 weeks on megestrol acetate therapy (MA) and after 6 weeks on the combination of megestrol acetate and tamoxifen (MA + TAM).
Different symbols have consequently been used for the same patient in Figures 1 and 2.

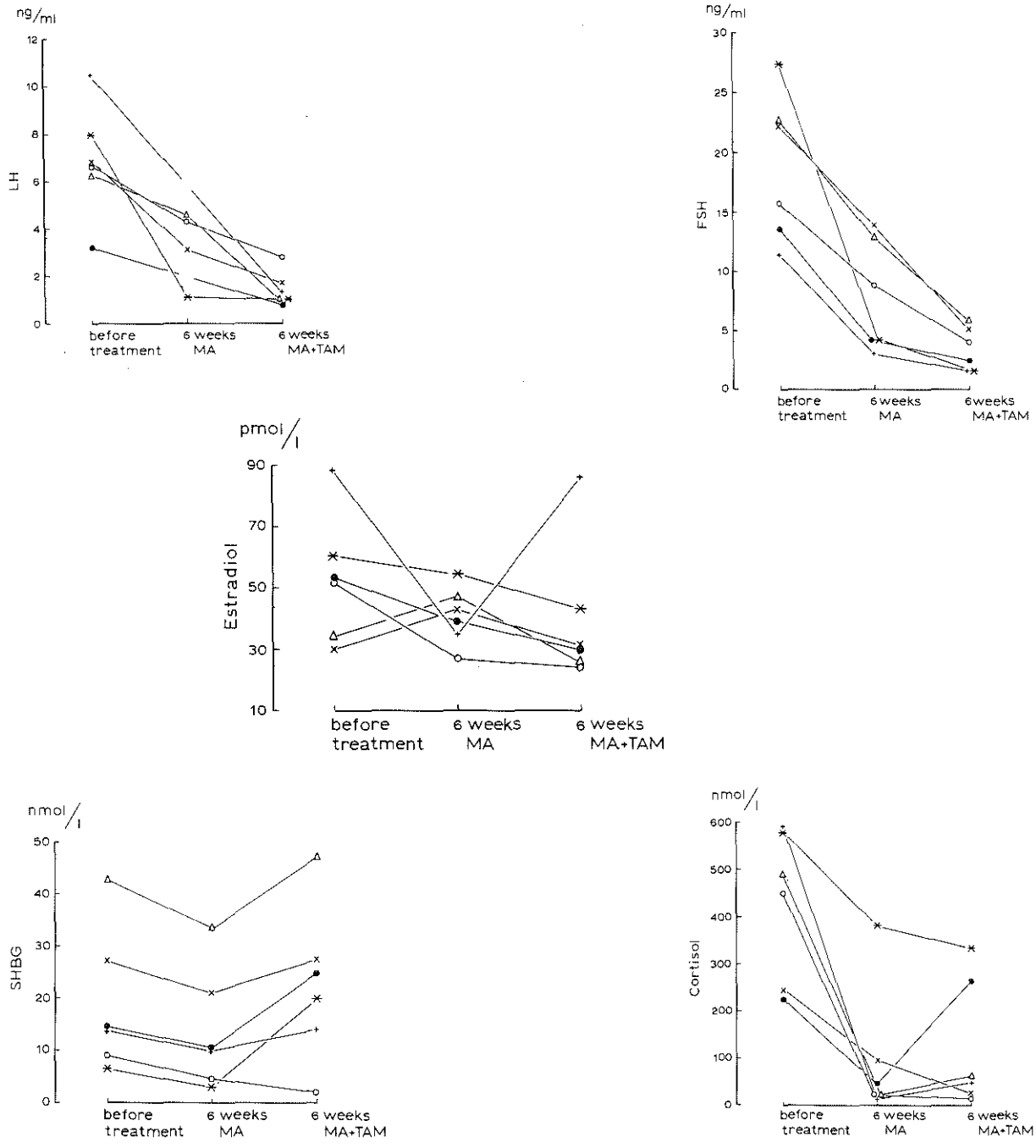


Figure 2 The concentrations in the plasma of:
a) LH, b) FSH, c) estradiol, d) SHBG and e) cortisol before, after 6 weeks on megestrol acetate therapy (MA) and after 6 weeks on combination of megestrol acetate and tamoxifen (MA + TAM) in 6 patients with metastatic breast cancer. Different symbols have consequently been used for the same patient in Figures 1 and 2.

DISCUSSION

The present study confirms the results obtained in a previous series of breast cancer patients in which the endocrine effects of MA treatment were studied [3]. The hyperresponse of prolactin to TRH stimulation on MA treatment was eliminated by addition of TAM to MA, while basal PRL level tended to be lower in patients receiving the combination of both drugs (Table 1, Fig.1). There is no equivocal evidence, however, that lowering of plasma PRL concentrations in itself is of benefit in the treatment of metastatic breast cancer. PRL could be involved, at least in a supportive role, in the development and growth of breast cancer, not only in animal models but also in humans [14,15]. Even though conflicting data exist about the involvement of prolactin in malignant breast tumor growth in humans, the presence of prolactin receptors in human breast tumors and the relation between prolactin and steroid hormone receptors indirectly supports an importance of this hormone as a growth factor in malignant neoplasia [16-20]. The inhibition of PRL secretion with the PRL-release inhibitor bromocriptine has recently been reported as a prophylaxis for spontaneous mammary tumorigenesis in rats [21] and of DMBA-induced mammary tumors in rats and mice [22]. TAM produced the same inhibition of tumor growth, while a further inhibition occurred when both TAM and bromocriptine were combined [23]. In human breast cancer bromocriptine as a single drug was ineffective [24] while after the addition of bromocriptine to medroxyprogesterone acetate a better response was suggested [25]. The influence of combination treatment of a steroid hormone and a prolactin inhibitor demands further investigation.

A further lowering of gonadotropins elicited by TAM could have an additional inhibitory effect to that of MA. All 6 patients showed a decrease during MA therapy in plasma SHBG concentrations, while in only 1 of them no increase was observed after addition of TAM to the regimen. To assure that this could not be due to protocol violation or poor absorption, the serum levels of TAM were estimated. The patient whose SHBG did not increase after addition of TAM, had a plasma tamoxifen level comparable to that found in

the other patients. The TAM levels observed (Table 1) agree with those reported [13].

The glucocorticoid effect of MA seemed to be unaffected by addition of TAM. Cortisol levels remained very low in 4 patients; in 1 patient the suppression was very slow, and in 1 patient plasma cortisol recovered (Fig.2e). However, no data on MA levels were collected in this particular study and therefore a possible inadequately low level of MA, allowing an escape of suppression of the adrenal axis cannot be ruled out.

Tumor response of this combination of drugs could not be considered in this study which was directed to the endocrine effects since only the responders to MA were treated with combination of both drugs. It can be mentioned, however, that all 6 patients are still in remission at the present moment (with a follow-up of 6-12 months). The fact that 6 out of 7 patients (an extremely high "percentage" of $\pm$ 85%), responded beneficially to MA simply demonstrates again that trials in small numbers of patients may be very useful for endocrine studies to indicate outlines for further research, but cannot be used for demonstration of the effectiveness of drugs.

This combination treatment could become a new therapeutic approach and therefore a large study directed to its tumor effects has to be done. Also the possibility of dose reduction of both drugs, when given simultaneously, should be investigated.

ACKNOWLEDGEMENTS

The authors thank Mrs. A. Sugiarsi and Mr. P. van Assendelft for their assistance.

REFERENCES

1. Clark JH, Anderson JN and Peck EJ Jr. Estrogen receptor- anti-estrogen complex: a typical binding by uterine nuclei and effects on uterine growth. Steroids 1973, 22:707.

2. Lippman M, Bolan G and Huff K. Interactions of antiestrogen with human breast cancer in long-term tissue culture. Cancer Treatment Reports 1976, 60:1421.

3. Alexieva-Figusch J, Blankenstein MA, Hop WCJ, Klijn JGM, Lamberts SWJ, de Jong FH, Docter R, Adlercreutz H, van Gilse HA. Treatment of metastatic breast cancer patients with different dosages of megestrol acetate; dose relations, metabolic and endocrine effects. Eur.J.Cancer Clin.Oncol. 1984, 20:33-40.

4. Iacobelli S, Natoli C, Sica G and Marchetti P. Common and distinctive features in the growth-inhibitory activity of medroxyprogesterone acetate and tamoxifen on oestrogeen-sensitive human breast cancer cells. In: Proceedings of the International Symposium on Medroxyprogesterone Acetate. Geneva 1982. Eds., F. Cavalli et al. Excerpta Medica 1982, pp.80-87.

5. Horwitz KB and McGuire WL. Estrogen control of progesterone receptor in human breast cancer. J.Biol.Chem. 1978, 253:2223.

6. Horwitz KB and McGuire WL. Nuclear mechanisms of estrogen action. Effects of estradiol and anti-estrogens on estrogen receptors and nuclear receptor processing. J.Biol.Chem. 1978, 253:8185.

7. Teulings FAG, van Gilse HA, Henkelman MS, Portengen H, Alexieva-Figusch J. Estrogen, androgen, glucocorticoid, and progesterone receptors in progestin-induced regression of human breast cancer. Cancer Res. 1980, 40:2557.

8. Alexieva-Figusch J, Teulings FAG, Hop WCJ, Blonk-van der Wijst J, van Gilse HA. Steroid receptors in megestrol acetate therapy. ("Clinical interest of steroid hormonereceptors in breast cancer; European experience"). In: Recent Results in Cancer Research. Eds.: Leclereq G., Toma S., Paridaens R, Heuson J.C., Springer-Verlag Berlin, Heidelberg, 1984, 91:253-258.

9. Willis KJ, London DR, Ward HWC, Butt WR, Lynch SS, Rudd BT. Recurrent breast cancer treated with the antioestrogen tamoxifen: correlation between hormonal changes and clinical course. Brit.med.J. 1977, 1:425.

10. Groom GV and Griffiths K. Effect of the anti-oestrogen tamoxifen on plasma levels of luteinizing hormone, follicle-stimulating hormone, prolactin, oestradiol and progesterone in normal pre-menopausal women. J.Endocr. 1976, 70:421.

11. Sakai F, Cheix F, Clavel M, Colon J, Mayer M, Pommatau E and Saez S. Increases in steroid binding globulins induced by tamoxifen in patients with carcinoma of the breast. J.Endocr. 1978, 76:219.

12. Hayward JL, Carbone PP, Heuson JC, Kumaoka S, Segaloff A, Rubens RD. Assessment of response to therapy in advanced breast cancer. Eur.J.Cancer 1977, 13:89-94.

13. Adam HK, Gay MA and Moore RH. Measurement of tamoxifen in serum by thin-layer densitometry. J.Endocr. 1980, 84:35-42.

14. Welsch CW and Nagasawa H. Prolactin and Murine Mammary Tumorigenesis. A Review. Cancer Res. 1977, 37:951-963.

15. Nagasawa H. Prolactin and Human Breast Cancer: a Review. Eur.J.Cancer 1979, 15:267-279.

16. Pearson OH, Manni A, Chambers M, Brodkey J and Marshall JS. Role of Pituitary Hormones in the growth of Human Breast Cancer. Cancer Res. 1978, 38:4323-4326.

17. de Souza I, Hobbs JR, Morgan L and Salih H. Localization of prolactin in human breast tumours. J.Endocr. 1977, 73:17P.

18. Morgan L, Raggatt PR, de Souza I, Salih H and Hobbs JR. Prolactin receptors in human breast tumours. J.Endocr. 1977, 73:17P.

19. Bonneterre J, Peyrat JPh, Vandewalle B, Beuscart R, Vie MC and Cappelaere P. Prolactin Receptors in Human Breast Cancer. Eur.J.Cancer Clin.Oncol. 1982, 18:1157-1162.

20. L'Hermite-Balériaux M, Vokaer A, Loriaux C, Noël G and L'Hermite M. Prolactin (PRL) and PRL-Receptors (PRL-R) in Human Breast Disease. J.Steroid Biochem., Supplement 1983, volume 19, 139 S.

21. Nagasawa H and Morii S. Prophylaxis of Spontaneous Mammary Tumorigenesis by Temporal Inhibition of Prolactin Secretion in Rats at Young Ages. Cancer Res. 1981, 41:1935-1937.

22. Welsch CW, Goodrich-Smith M, Brown CK and Roth L. The prophylaxis of rat and mouse mammary gland tumorigenesis by suppression of prolactin secretion: a reappraisal. In: Breast Cancer Research and Treatment 1981, 1:225-232.

23. Welsch CW, Goodrich-Smit M, Brown CK, Mackie D, Johnson D. 2-Bromo-α-Ergocryptine (CB-154) and Tamoxifen (ICI 46,474) Induced Suppression of the genesis of Mammary Carcinoma in Female Rats Treated with 7,12-Dimethylbenzanthracene (DMBA): A comparison. Oncology 1982, 39:88-92.

24. European Breast Cancer Group: Clinical Trial of α Bromoergocryptine in Advanced Breast Cancer. Eur.J.Cancer 1972, 8:155-156.

25. Dogliotti L, Mussa A and DiCarlo F. Medroxyprogesterone Acetate High Dose and Bromocriptine. Results of a 4-year Study in Stage IV Breast Cancer. In: Role of Medroxyprogesterone in Endocrine Related Tumors. Volume II. Eds.: L. Campio, Robustelli Della Cuna, G. and R.W. Taylor. Raven Press, New York, 1983, pp.115-129.

GENERAL DISCUSSION, SUMMARY AND CONCLUSIONS

In the course of time between the first publication on the subject, our experience, knowledge and sight has evolved. They have been incorporated in this discussion that is centered on the items of the introduction:

1. How effective is megestrol acetate in the treatment of metastatic breast cancer? (Chapter II)

Similar to what can be expected from any hormonal treatment, objective remissions (CR+PR) were found in 30% of the patients with a median duration of 9 months.

Some comments are useful, since the results of our study show the importance of defining relevant parameters for the evaluation of clinical trials to serve the final goal for doing them: to enable a choice for the best treatment modalities in individual patients.

Our best results in the "dominant visceral" category which supposedly should be worst underlines Cutlers'[186] opinion that the category "visceral" is too broad and should be assessed by the involvement of separate anatomical sites, e.g. lung and liver. It was also found that metastases in the lungs and pleura have a definite better prognosis during megestrol acetate administration than those in the liver.

In addition to the data mentioned in Chapter II we tabelized the evaluation of these data in a comparative way (Table 1):

Pleural and peritoneal metastases responded excellent to megestrol acetate administration, which could especially of benefit for those patients needing frequent aspirations of the effusions. This finding is in agreement with Panutti et al.'s (1981)[188] observations that medroxyprogesterone acetate concentrates nearly as highly in pleural and

Table 1 MEGESTROL ACETATE THERAPY

Dominant site compared to Anatomical site of metastases

	remission	stable	progr.
Viscera	11/25 44%	7/25	7/25
Bone	10/57 18%	29/57	18/57
Soft tissue	26/77 34%	25/77	26/77

		remission	stable	progr.
Viscera	lung	5/18 29%	8/18	5/18
	liver	0/ 7 0%	5/ 7	2/ 7
	muccous membr.	8/14 57%	1/14	5/14
	other	5/20 25%	10/20	5/20
Bone		1/57 2%	38/57	18/57
Soft tissue		40/111 36%	40/111	31/111

peritoneal effusions as in plasma. The very low response found in bone metastases (2%), could be explained by the mentioned heterogeneity of dominant site classification (Chapter I,8) and also by multiple bone involvement at the start of the therapy in many patients, in whom complete or partial remission could hardly be expected. On the other hand, a subjective improvement, such as diminishing of pain, improving of validity, sense of being well, occurred frequently, but they are not expressed in figures that concern objective evaluation, how important they may be for the patients.

2. **What is the role of steroid receptors in the mechanism of action of megestrol acetate in tumor regression ? (Chapter III and IV)**

By virtue of the capability of steroid receptors to bind steroids and thereby facilitate the transport of the steroid-receptor complex to the intracellular site of action, steroid receptors have been implemented in the mechanism of action of additive steroid therapy in breast cancer (Chapter I,3). The way in which the antitumor effect is reached, is not exactly understood yet.

When patients were selected, with regard to ER status, for the treatment with estrogens, androgens or glucocorticoids, objective responses were found in $\pm$ 60% of patients with ER+ tumors while an additional improve-

ment was found if also PgR was present.[68,77] On the other hand, some patients with ER+ tumors did not respond.

Also, other mechanisms of action could be involved such as a direct cytotoxic effect of pharmacological dosages of steroid hormones and activation or inhibition of enzymes.

Tumor response during MA treatment showed no clear correlation with the ER content. However, the duration of remission of patients with ER+ tumors was significantly longer ($p < 0.01$) than in those whose tumors lacked the estrogen receptor. Also the suggestion that progestins would act on the breast cancer cell by means of the progesterone/estrogen receptor mechanism and subsequent increase of 17β dehydrogenase activity (E_2DH)[113] could not be confirmed in our study. However, Satyaswaroop et al.[189] warned that the ineffectiveness of progestins to induce E_2DH in endometrial cancer specimens in vitro is related to instability of PgR under culture conditions. Also, it is possible that for the appearance of specific PgR estrogen-priming is necessary.

Megestrol acetate and medroxyprogesterone acetate are strong competitors for steroids which bind specially to androgen, glucocorticoid and progesterone receptors, indicating that the progestins are able to bind to these receptors with high affinity. As mentioned before (Chapter I,2), the chemical structure of individual progestins provides androgenic or glucocorticoid activity and the binding to other receptors such as androgen and glucocorticoid might be expected.

The observed tumor regressions were significantly associated indeed, with the presence of large amounts of androgen receptors in the tumors, while correlations with the glucocorticoid receptor, ($p=0.07$), with the estrogen ($p=0.34$) and the progestin receptors ($p=0.24$) were not significant. On the other hand, with increasing amount of ER there was also an increasing amount of the other receptors.

It is not possible nor feasible to estimate ER, PgR, AR and GR in tumor specimens of all patients. However, the presence of high contents of ER predicts the simultaneous presence of other receptors, increasing chance to a beneficial response to any hormonal treatment.

3. Has the effect of megestrol acetate on some endocrine parameters any relation to tumor regression? What is the optimal dose? (Chapter V)

The effect of MA on some endocrine parameters is most intervential. MA may centrally inhibit hypothalamic-pituitary functions resulting in a decreased secretion of LH, FSH, ACTH and an increased secretion of prolactin. This finally results in a decrease of plasma estradiol and cortisol concentrations.

An increase of the basal plasma insulin level could be attributed to the action of progesterone, in the case of progestins, however, also to their additional glucocorticoid-like effects (Chapter I,5).

In our study these endocrine events were found in all patients in a dose-dependent way, in responders and non-responders equally. It seems likely that these metabolic events are non-specific. On the other hand, blood levels of circulating hormones do not per se reflect the hormonal situation around the tumor. The patients of the present study were not pre-selected for hormonal treatment by presence of a receptor. Further investigations, in patients with known ER, PgR, AR and GR contents in tumor specimens may elucidate, whether these endocrine effects mentioned might have influenced tumor growth. It should be kept in mind that each additive endocrine therapy usually exceeds physiological hormone levels by far and consequently may produce a direct cytotoxic effect on both normal and tumor cells in addition to endocrine and metabolic changes.

It was the purpose of our study to find an optimal effect using the minimal dose of the drug to avoid the adverse effects. From the three dosages of MA used, the 180 mg dose seems to fulfil these aims.

4. In what sequence should hormonal treatment with megestrol acetate be used ? (Chapter VI)

From our retrospective study no clear answer could be given. The compared groups differ markedly with respect to median age, previous therapy such as ovariectomy and disease-free interval. The overall results, the

response rates and their duration and survival did not differ when using the sequence MA/TAM or TAM/MA.

Theoretically, if the action of both drugs on the receptors is taken into account, the sequence of TAM/MA should give favourable responses due to deplenishment of ER and replenishment of PgR by TAM to create an optimal situation for progestin action.

It should be considered, that the receptor status is changing[190] in patients on long-term treatment and therefore this phenomenon could be used in an alternating treatment regimen, which will be the matter of our future investigations.

5. **Does the combination of megestrol acetate and tamoxifen have an additional effect on endocrine events; especially can the MA-induced hyperprolactinaemia be suppressed by the administration of tamoxifen ? (Chapter VII)**

Treatment with this combination exerted an additional effect by lowering the circulating gonadotropins levels further than by MA alone. The elevated basal plasma PRL concentration remained unaffected by addition of TAM, while the hyperresponse of PRL to TRH became normalized. It needs to be established whether the lowering of PRL itself could be of importance as a primary goal for the treatment of metastatic breast cancer. Since this combination might become a new treatment approach it will be a matter of a next large study, directed not only to tumor response, but also to the possibility of dose reduction and diminishing of adverse effects.

CONCLUSIONS

1. Megestrol acetate is effective in the treatment of metastatic breast cancer; response rate and its duration is similar as could be expected from any hormonal treatment; serious side effects are negligible.

2. No clear correlation between clinical response to treatment with MA and ER and PgR has been found; for MA, it seems that only the androgen receptor may be useful as a marker for response to this therapy. All tumors high in androgen receptor were also ER+.

3. Megestrol acetate caused (dose dependently) an increase of the basal and TRH-stimulated plasma PRL levels and of basal insulin concentrations, whereas LH and FSH, estradiol, SHBG and the pituitary-adrenal axis were suppressed. The dose of 180 mg seems to be optimal. None of these metabolic effect showed a correlation with the clinical response.

4. Megestrol acetate is effective as the first as well as the second line in additive hormonal treatment.

5. The combination of megestrol + tamoxifen exerts an additional effect by further lowering the circulating gonadotropins levels and normalized the hyperresponse of PRL to TRH stimulation.

SAMENVATTING

In dit proefschrift wordt besproken de rol van het synthetische progestageen megestrolacetaat in de behandeling van gemetastaseerd borstkanker. Nadat het resultaat op het tumorproces vastgesteld was, kwamen vragen op omtrent het werkingsmechanisme, de optimale plasma concentratie, de invloed op endocriene en metabole functies, ongewenste bijwerkingen, indicaties voor deze therapie, en over eventuele verbetering van het therapeutisch effect door combineren met een ander geneesmiddel.

In hoofdstuk I zijn de historische aspecten behandeld. Ondanks de ontdekking van progesteron al in de dertiger jaren was het gebruik van dit hormoon in de behandeling van borstkanker naast andere hormonale therapie beperkt door de korte biologische beschikbaarheid na orale toediening. Dit probleem werd opgelost toen in de zestiger jaren synthetische progestagenen met langere levensduur ter beschikking waren gekomen. Deze stoffen waren niet alleen aktiever dan progesteron, maar waren ook werkzaam bij orale toediening.

Voor veel fysiologische werkingen van progesteron is een "priming" met oestrogenen noodzakelijk. Terwijl oestrogenen de groei stimuleren, bevordert progesteron in het algemeen de rijping en differentiatie van de cellen. Synthetische progestagenen kunnen op basis van de chemische structuur meerdere eigenschappen hebben, o.a. androgene, anti-androgene en glucocorticoide werkingen.

De plasmaspiegel van megestrolacetaat, gemeten na 60 mg orale dosis bereikte een maximum na 1-4 uur (43 ng/ml), was nog meetbaar na 24 uur (9,6 - 26 ng/ml) en nog aanwezig in zeer lage concentratie (0,7 - 3 ng/ml) na 7 dagen. De biologische halveringstijd van deze dosis was $3\frac{1}{2}$ dag.

Het is de veronderstelling, dat een steroid hormoon zijn functie in de cel pas kan uitoefenen als een daarvoor specifieke receptor aanwezig is in het cytoplasma. Tumor regressie na therapie met progestativa zou mogelijk

gezien moeten worden in relatie tot het endocriene milieu om de kankercellen heen en de therapie zou voornamelijk gericht moeten worden op interferentie met een van oestrogenen afhankelijk groeiproces. Hormonale manipulatie zou kunnen interfereren met de synthese en turnover van de oestrogeen receptor en ook met die van andere receptoren.

Verdere mogelijkheden zijn een directe cytotoxiciteit van hoge concentraties van een hormoon of een indirekte invloed op het hormonale milieu, zoals daling van plasma concentraties van LH, FSH, oestrogenen, androgenen, cortisol en ACTH en stijging van prolactine.

In hoofdstuk II werd in een klinische studie van 160 patienten het effect van megestrolacetaat op gemetastaseerd mammacarcinoom beschreven. De resultaten zijn beoordeeld volgens algemeen geaccepteerde UICC criteria. Remissie trad op in 30%, stabilisatie van de ziekte in 36% terwijl 34% van de patienten niet reageerden op de therapie. De remissies duurden gemiddeld 9 maanden.

De postmenopauzale status is een belangrijke prognostische faktor bij mammacarcinoom. Bij de verdeling van de hele groep naar een postmenopauzale status minder dan 5 en meer dan of gelijk aan 5 jaar bleek dat de groep van patienten meer dan of gelijk aan 5 jaar postmenopauze een significant beter resultaat had.

In hoofdstuk 1 - 8 en discussie is dieper ingegaan op de problemen met de juiste beoordeling van de resultaten. De indeling naar "dominant sites of metastases" is gebaseerd op een aantal studies, waaruit bleek dat patienten met viscerale metastasen de slechtste prognose hadden wat betreft overleving.

In onze studie hebben wij tegen de op deze verdeling gebaseerde verwachting een zeer goed resultaat gevonden bij viscerale metastasen. Deze bevindingen komen overeen met de opvatting van andere onderzoekers dat de begrippen "dominant" en "visceral sites" misleidend kunnen zijn en dat het classificeren van resultaten van therapie bij gemetastaseerd mammacarcinoom tevens naar gespecificeerde localisaties dient te gebeuren.

In hoofdstuk III en IV, werd het werkingsmechanisme van megestrolacetaat besproken. Er is geen duidelijke correlatie gevonden tussen de aanwezigheid van oestrogeenreceptoren en de reactie van de metastasen, echter, de duur van de remissie bij de patienten met ER+ tumoren was significant langer ($p < 0,01$) dan bij ER- tumoren.
De veronderstelling dat de activiteit van het enzym 17β-dehydrogenase verhoogd zou zijn als bewijs van betrokkenheid van de progestageenreceptor in het werkingsmechanisme kon in onze studie niet worden bevestigd.

Aan de andere kant is er een significante correlatie gevonden tussen het optreden van tumor respons en de gevonden hoeveelheid van androgeen-receptor en een net niet significante correlatie met die van glucocorticoid-receptor.

Gezien de androgene- en glucocorticoide eigenschappen van megestrol-acetaat was deze gevonden correlatie te verwachten.

In hoofdstuk V, werd de invloed van behandeling met megestrolacetaat op sommige endocriene parameters besproken evenals de relatie met de gegeven dosis.

Megestrolacetaat remt de functie van het hypothalamus-hypofyse-systeem hetgeen zich uit in een verminderde secretie van LH, FSH, ACTH en verhoogde secretie van prolactine met een daling in de concentratie van oestradiol en cortisol. De gevonden verhoogde plasma insulinespiegel, kunnen toegeschreven worden aan de progesteronachtige werking, waaraan de glucocorticoide werking van megestrolacetaat een additioneel effect geeft.

Al deze endocriene veranderingen werden gevonden bij alle patienten; het effect was dosis-afhankelijk bij responders zowel als bij non-responders. Het leek dus waarschijnlijk dat al deze effecten niet specifiek van invloed waren op de reactie van de tumor. Bovendien hoeft de plasmaspiegel van hormonen geen informatie te geven over de concentratie van deze hormonen in de (naaste omgeving van de) tumor.

Hoofdstuk VI behandelt de plaats van megestrolacetaat in de hormonale therapie bij mammacarcinoom. Er is een vergelijking gemaakt tussen de volgorde antioestrogeen gevolgd door megestrolacetaat en omgekeerd. De

resultaten van de ene en de andere volgorde waren hetzelfde wat betreft percentage- en duur van remissie en van overleving.

In hoofdstuk VII werd nagegaan of een combinatie van megestrol-acetaat met tamoxifen nog een additioneel effect kan hebben op de endocriene parameters, met name normalisatie van door de megestrolacetaat geinduceerde hyperprolactinaemie.

Door deze combinatie werd een nog diepere daling van gonadotropinen bereikt. De verhoogde basale plasma prolactine concentratie werd niet beinvloed door de bovengenoemde combinatie, terwijl de hyperreactie van door TRH gestimuleerde prolactine wel geëlimineerd werd.

Het is nog niet duidelijk of verlaging van de plasma prolactine concentratie een belangrijke factor is voor de succesvolle behandeling van gemetastaseerde borstkanker.

Deze beperkte endocriene studie moet in de toekomst uitgebreid worden om de invloed van deze combinatietherapie op de tumorgroei en regressie vast te leggen.

ACKNOWLEDGEMENTS

Especially, of all who helped me with preparation of this thesis, I would like to thank:

- all patients who cooperated kindly in these studies;
- medical, nursing, administrative staff and technical assistents of Rotterdamsch Radio-Therapeutisch Instituut / Dr. Daniel den Hoed Kliniek for delivering the patients, assistance in the studies and performing of the laboratory determinations;
- the board of directors of Rotterdamsch Radio-Therapeutisch Instituut / Dr. Daniel den Hoed Kliniek for given facilities;

- Mrs. A. Sugiarsi who typed the manuscripts;
- Mr. P. van Assendelft who drew the graphs;
- Mr. L. Ries and Mr. J. Vuik, audio-visual service of Rotterdamsch Radio-Therapeutisch Instituut / Dr. Daniel den Hoed Kliniek for all the photographs;
- Mrs. E.L.A. Vonk-Neele and Mrs. M. Westerhout-Kersten, librarians, for the literature data;
- Mrs. L.G. van der Meulen for her secretarial help;

- all my co-authors having an important deal and bringing their special knowledge in this work;
- NOVO Industri, Copenhagen, Denmark, especially to Mrs. M.S. Christensen, for their interest and financial support of laboratory investigations;
- F.A.G. Teulings in memoriam and Dr. M.A. Blankenstein, biochemists; only close cooperation with biochemistry makes clinical research possible;
- Dr. H.A. van Gilse, who guided me throughout the main part of these studies with her non-compromising criticism;
- Prof. Dr. S.W.J. Lamberts, promotor, for his constant interest in my work, his moral and scientifical support.

CURRICULUM VITAE

De schrijfster van dit proefschrift werd geboren op 22 maart 1940 te Bratislava, Tsjechoslowakije. Na beëindiging van de middelbare school in 1957 is zij in 1958 begonnen met de studie van de geneeskunde aan de Medische Faculteit in Bratislava, verder voortgezet in Praag, waar zij het artsexamen aflegde op 22 april 1964. In de jaren 1964-1968 was zij werkzaam als arts-assistente op de interne afdelingen van het Algemeen Ziekenhuis in Mlada Boleslav en in Praag. Sinds 1968 leeft zij in Nederland. De interne opleiding werd voortgezet onder leiding van Prof. Dr. J. Gerbrandy, Interne Kliniek I, Academisch Ziekenhuis Dijkzigt, Rotterdam, in de periode van februari 1969 - februari 1973.

Erkenning van het artsdiploma en beëdiging als arts geschiedde op 16 juli 1970 op het Ministerie van Volksgezondheid te Leidschendam. Sinds 1 maart 1973 is zij ingeschreven in het specialistenregister als interniste en werkzaam in het Rotterdamsch Radio-Therapeutisch Instituut / Dr. Daniel den Hoed Kliniek in Rotterdam (voormalig hoofd Interne Afdeling R.E. Treurniet, huidig waarnemend hoofd J.G.M. Klijn). Op 30 oktober 1974 heeft zij de nederlandse nationaliteit gekregen.