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Oestradiol and tamoxifen inhibit murine Colon 38 cancer growth and increase the cytotoxic effect of fluorouracil

Estradiol i tamoksyfen hamują wzrost mysiego raka jelita grubego Colon 38 oraz nasilają cytotoksyczne działanie fluorouracylu

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Abstract

The poor efficacy of reference chemotherapy (fluorouracil -FU) in colon cancer has resulted in a constant search for agents which could augment the action of FU. Epidemiological data, such as the decreased risk of colorectal cancer among menopausal women receiving hormonal replacement therapy, indicate the role of oestrogen in the pathogenesis of this disease. The differences between normal and neoplastic colon cells in the expression of oestrogen receptor β (ERβ) could confirm this association. However, the direct influence of oestrogen or tamoxifen (SERM, selective oestrogen receptor modulator) on colon cancer growth has rarely been studied.

The aim of the present study was to examine the direct effects of various concentrations of oestradiol and tamoxifen (10^{-4} to 10^{-12} M), applied alone or together with FU, on the growth of murine Colon 38 cancer *in vitro* as assessed by three colorimetric methods: Mosmann's method, incorporation of BrdU into cell nuclei and the TUNEL method.

At high concentrations oestradiol and tamoxifen decreased the cancer growth in a dose- and time-dependent manner (the Mosmann and BrdU methods) and at some concentrations augmented the cytotoxic action of FU (Mosmann's method). Tamoxifen exerted a very early and potent inhibitory effect, inducing even total cancer growth inhibition at the concentration of 10^{-4} M (the Mosmann and BrdU methods). All the substances studied at different concentrations and at different incubation time points increased the apoptosis of tumour cells (the TUNEL method).

The results indicate that oestradiol and tamoxifen inhibit Colon 38 cancer growth and increase the cytotoxic effect of FU, which confirms the role of sex steroids in colon carcinogenesis and even suggests new therapeutic schemes.

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Key words: *oestradiol, tamoxifen, fluorouracil, proliferation, apoptosis, colon cancer*

Streszczenie

Niezadowalająca skuteczność fluorouracylu (FU) w leczeniu uzupełniającym raka jelita grubego skłania do poszukiwania nowych leków, w tym substancji nasilających przeciwnowotworowe działanie tego cytostatyku. Protekcyjną rolę estrogenów w karcynogenezie jelita grubego sugerują wyniki badań epidemiologicznych wykazujące zmniejszenie ryzyka rozwoju tego nowotworu u kobiet przyjmujących hormonalną terapię zastępczą (HTZ), a także różnice w ekspresji receptora estrogenowego β (ERβ) w prawidłowych i nowotworowych kolonocytach. Jednak badania doświadczalne nad bezpośrednim wpływem estrogenów lub tamoksyfenu (selektywnego modulatora receptorów estrogenowych) na wzrost raka jelita grubego są nieliczne, a ich wyniki niejednoznaczne.

Celem pracy było zbadanie bezpośredniego wpływu estradiolu i tamoksyfenu w stężeniach od 10^{-4} do 10^{-12} M stosowanych osobno lub łącznie z FU na wzrost mysiej linii raka jelita grubego Colon 38 oceniany za pomocą 3 metod kolorymetrycznych: metody Mosmanna, metody opartej o wbudowywanie BrdU i metody TUNEL.

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Estradiol i tamoksyfen hamowały wzrost badanej linii w wąskim zakresie wysokich stężeń z siłą narastającą wraz ze stężeniem leku i czasem trwania inkubacji (metoda Mosmanna i BrdU), a w wybranych stężeniach potęgowały cytotoksyczne działanie FU (metoda Mosmanna). Tamoksyfen wywoływał bardzo wczesny i silny efekt hamujący, powodując nawet całkowite zahamowanie wzrostu tej linii w stężeniu 10^{-4} M (metoda Mosmanna i BrdU). Wszystkie badane substancje w wybranych stężeniach i w określonych punktach czasowych nasilały apoptozę komórek tego raka (metoda TUNEL). Uzyskane wyniki wskazują, że estradiol i tamoksyfen hamują wzrost raka Colon 38, a także nasilają cytotoksyczne działanie FU, co potwierdza udział hormonów płciowych w karcynogenezie jelita grubego, a potencjalne sugeruje nawet nowe schematy lecznicze.

(Endokrynol Pol 2007; 58 (5): 426–434)

***Słowa kluczowe**: estradiol, tamoksyfen, fluorouracyl, proliferacja, apoptoza, rak jelita grubego*

Introduction

In the developed countries colorectal cancer represents a major health problem [1]. In Poland too colorectal cancer is one of the most common cancers and its incidence and mortality rates are still growing. Approximately 30% of all patients with colon cancer have metastatic disease at diagnosis, and the other 50% of patients will eventually develop it [2]. For many years fluorouracil (FU) has remained the main chemotherapeutic agent in this cancer. Modulation of its anti-neoplastic effects by leucovorin became one of the standard treatment regimens for advanced stages of colon cancer. Combining it with other known agents has not improved patient survival [2]. However, during the last decade the USA Food and Drug Administration (FDA) has approved five new drugs for the treatment of advanced colon cancer. Among these are three cytotoxic agents: irinotecan (1996), oxaliplatin (2002), the oral formulation of fluorouracil — capecitabine (1998) and two monoclonal antibodies — bevacizumab and cetuximab (2004). The new agents doubled survival among patients but also led to a great increase in drug costs. The average survival is still little more than 20 months, and the treatment remains palliative [3].

The positive results of colon cancer treatment with bevacizumab (targeting vascular endothelial growth factor) and cetuximab (directed against epithelial growth factor receptor) confirm the effectiveness of biotherapy in cancer treatment [2, 4, 5].

The potential role of oestrogens in the pathogenesis of colon cancer has been discussed for many years. This association is suggested by epidemiological data such as sex differences in site-specific incidences of colorectal cancer, its increased occurrence in women with breast cancer and the protective effect of increasing parity [6]. The protective effects of hormonal replacement therapy (HRT) on colon cancer are supported by a number of observational studies [7]. A randomised primary prevention trial, the Women's Health Initiative (WHI), comprising 16 608 women aged 50–79 years, also confirmed the 37% reduction in colon cancer risk in users of combined oestrogen and progestin HRT [8]. However, in the parallel trial of oestrogen alone in women after hysterectomy the protective effect was not observed [9]. Interestingly, the colon cancers which occurred among women using HRT were characterised by a greater number of positive lymph nodes and were more advanced [10].

Differences in the expression of oestrogen receptors (ER) in normal colonocytes and colon cancer cells also suggest the role of oestrogen in colorectal carcinogenesis. ERα is reported to be minimally expressed in normal colon mucosa and colon cancer cells [11, 12], whereas the predominant ER subtype in the human colon is ERβ and its expression declines in colon cancer [11–13]. Therefore some authors even suggest a potential use for ERβ agonists in colon cancer treatment [14].

Furthermore, the role of local oestrogen metabolism seems to be pivotal in the pathogenesis of colon cancer. A change in 17β-hydroxysteroid dehydrogenase (17β-HSD) activity, which is responsible for the interconversion of oestradiol (E2) to oestrone (E1), has been revealed in colon tumours. A decreased oxidative activity of 17β-HSD in colon cancer lines *in vitro* [15] and a reduction in the expression of 17α-HSD isoform 2 in colon cancer tissues *in vivo* [16] correlated inversely with cell proliferation.

The number of studies examining the direct effect of oestrogen on colon cancer growth is limited and these have often given conflicting results. Studies *in vitro* and experiments with ovariectomised animals have shown that oestrogen can inhibit as well as stimulate colon cancer growth [17–20].

The positive effects of hormonal modulation in the treatment of prostate and breast cancers are well known. One of the most commonly used drugs in breast cancer adjuvant therapy is tamoxifen. This is one of the group of selective oestrogen receptor modulators (SERMs) and after binding to oestrogen receptors (ER) it exerts oestrogenic effects on certain genes while having anti-oestrogenic effects on others. This mixed action of tamoxifen is species-, tissue- and cell-dependent [21]. There are

also data showing other ER-independent anti-tumour effects of tamoxifen, such as inhibition of calmodulin, induction of TGF-α secretion, antioxidant activity and interaction with the multi-drug resistance protein P-glycoprotein [22].

Thus the aim of this study was to examine the direct effects of oestradiol and tamoxifen, applied alone or together with FU, on murine Colon 38 cancer growth *in vitro* as assessed by methods reflecting changes in proliferation and apoptosis.

Materials and methods

Murine Colon 38 cancer cells were used in the study. The cells were routinely grown in a humidified incubator at 37^0 C with 5% CO_2 in RPMI 1640 medium (Sigma), supplemented with the following: 25 nM Hepes buffer (Sigma), 4 mM L-glutamine (Sigma), 100 U/ml penicillin and 100 μg/ml streptomycin solution (Sigma), 2 g/l sodium bicarbonate (Sigma) and 5% foetal calf serum (FCS, Biochrom). The cells were passaged every 7 days with 0.05% trypsin/0.02% EDTA (Trypsin-EDTA, Sigma) and the medium was changed every 3–4 days.

After one of the trypsinisation procedures the cells were plated (depending on time and method: 20 000––30 000/well for the Mosmann and BrdU methods and 60 000–90 000/well for the TUNEL method) into 96-multiwell plates (Nunc). To avoid the influence of oestrogens and oestrogen-like substances the cells were cultured in phenol red-free RPMI 1640 medium (Sigma) supplemented with 5% charcoal-treated hormone-free FCS (Biochrom). After preincubation (24 h) the cells were cultured for a further 1, 4, 8, 12, 24 or 72 h in the presence of various concentrations of the substances examined (fluorouracil, oestradiol and tamoxifen), applied alone or in combination.

To assess the interaction with other substances, FU (Fluoro-uracil, Roche) was used at concentration of 1 μM. This was chosen from a wide range of examined concentrations (1–1024 μM; data not shown) as inducing minor cancer growth inhibition. The control group for FU received the medium. Oestradiol (17-β-oestradiol 17-hemisucinate, ICN Biomedicals Inc.) and tamoxifen (Tamoxifen, Sigma) were dissolved in absolute ethanol (in a proportion of 1 mg/1ml and 1mg/650 μl, respectively) and were examined in the range of concentrations from 10^{-4} to 10^{-12} M. Control cells received an adequate concentration of the ethanol vehicle without the test substance (maximum ethanol concentration: 3.7 vol%).

Cancer growth was assessed by the three colorimetric methods:

— the Mosmann method (Easy for You, The 4th Generation Non-Radioactive Cell Proliferation & Cytotoxity Assay, Biomedica Gruppe, Austria, Bellco Biomedica. Poland) based on the measurement of the total metabolic activity of the cultured cells, which reflects changes in proliferation and cell death;
— a method based on bromodeoxiuridin incorporation into the cell nuclei (Cell Proliferation ELISA, BrdU; Roche Applied Science), directly correlating with cell proliferation;
— the terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) method (Titer Tacs, R&D) of evaluating cell apoptosis.

In the BrdU incorporation method BrdU was added to each well 4 h prior to termination of the experiment.

The intensity of the reaction was estimated by means of measurement of the optical density (OD) using an ELISA reader (λ = 450 nm). The statistical significance was determined using one-way ANOVA with a post-hoc least significant difference (LSD) or non-parametric Mann Whitney test. $P < 0.05$ was considered to be a statistically significant difference. The correlation between cancer growth inhibition and concentrations of the examined substances or the duration of cultures was determined by Pearson's r coefficient, and the significance of differences was then analysed with Student's *t*-test.

The results obtained in the BrdU incorporation and Mosmann methods were presented as a percentage of the OD of the control group. The results of the cell apoptosis measurement were shown as an apoptotic index, calculated as previously described by other authors [23] as a ratio of the total amount of apoptosis, as measured by the TUNEL method, per cell number equivalent, as measured by the Mosmann method, in adequate samples.

Results

Oestradiol in the narrow range of high concentrations (10^{-5} to 10^{-4} M) inhibited Colon 38 cancer growth in a time- and dose-dependent manner (Fig. 1 and 2; Tab. I). The onset of its effect was observed in the 12 h culture in both methods (Mosmann and BrdU). However, after 12 h incubation the cancer growth inhibition of both examined oestradiol concentrations (4×10^{-5} and 10^{-4}M) was revealed only by the Mosmann method, while the BrdU incorporation method detected the inhibitory effect exclusively of the higher concentration of E2 (Fig. 2). Oestradiol at some concentrations (10^{-7} to 10^{-4}M) enhanced the cytotoxic action of FU used at the concentration of 1 μM (Fig. 3). Interestingly, this additive effect was also observed for oestradiol at concentrations of 10^{-7}, 10^{-6}, and 4×10^{-6} M, at which this hormone applied alone was ineffective. Oestradiol in 24 h culture induced cancer cell apoptosis at concentrations of 4×10^{-5} and 10^{-4} M and after 72 h incubation at concentrations of 10^{-4} M (Fig. 4).

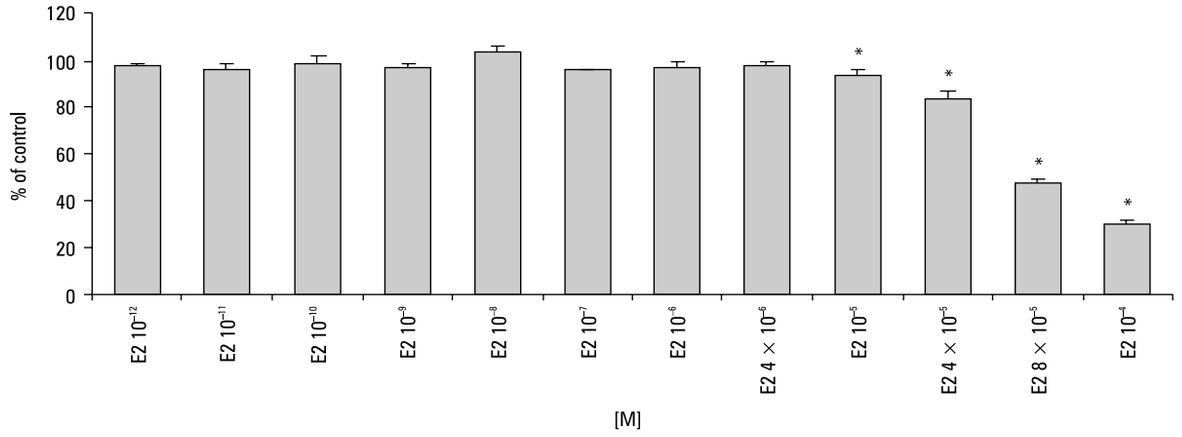

Figure 1. *The effect of oestradiol (E2) on the growth of Colon 38 cancer as assessed by the Mosmann method in 72 h culture. X ± SEM, *p < 0.05* vs. *control*

Rycina 1. *Wpływ estradiolu (E2) na wzrost raka Colon 38 oceniany metodą Mosmanna w hodowli 72 h. X ± SEM, *p < 0,05* vs. *kontrola*

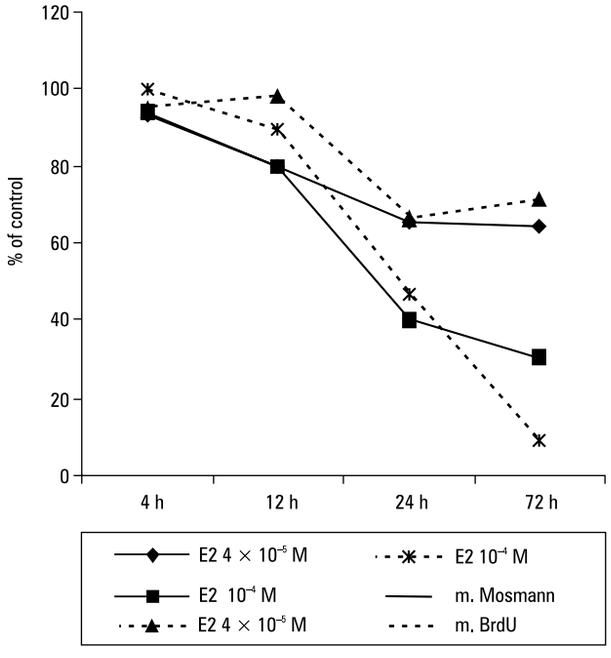

Figure 2. *The effect of oestradiol (E2) on the growth of Colon 38 cancer as assessed by the Mosmann method and BrdU incorporation method in 4, 12, 24 and 72 h cultures*

Rycina 2. *Wpływ estradiolu (E2) na wzrost raka Colon 38 oceniany metodą Mosmanna i metodą wbudowywania BrdU w hodowlach 4, 12, 24 i 72 h*

Tamoxifen in the narrow range of high concentrations (10^{-5} to 10^{-4} M) exerted a strong inhibitory effect on cancer growth (Fig. 5), which was revealed (for concentrations 10^{-4} and 4×10^{-5} M) as early as after 4 h of incubation (Mosmann and BrdU method) (Fig. 6). Using tamoxifen in this range of concentration we also observed a time- and dose-response effect (Fig. 6; Tab. I). Moreover, tamoxifen at concentrations of 8×10^{-5} and 10^{-4} M even evoked complete cell growth inhibition (Fig. 5). Like oestradiol, tamoxifen (8×10^{-6} and 10^{-5} M) intensified the cytotoxic action of FU (Fig. 7). The proapoptotic effect of tamoxifen (10^{-5} and 4×10^{-5} M) was stronger and observed earlier than the effect of oestradiol. Tamoxifen induced apoptosis after 8, 24 and 72 h (Fig. 4).

Discussion

In the present study we have shown that oestradiol and tamoxifen inhibited Colon 38 cancer growth by influencing proliferation and apoptosis and increased the cytotoxic action of FU. Our results are consistent with recent reports demonstrating that oestradiol evoked growth inhibition of human colon cancer cell lines [24, 26] and that its proapoptotic activity could be involved in this action [17]. However, some authors did not observe any such effect [27, 28] or even reported the opposite, that oestrogen had a stimulatory effect on cultured colon cancer cells [18, 25, 26]. These conflicting findings from different studies may be due to different types of cell lines, different oestrogen receptor patterns and variations in the concentration of oestradiol. It was revealed that most of the colon cancer lines (HCT8, HCT116, DLD-1, LoVo, HT29, Colo320, SW480, CACO-2, SW620, COLO205) expressed ERβ [17, 26–28], whereas ERα was rarely detected [17]. As noticed by some authors [27] the stimulatory effect of oestradiol was demonstrated only in two colon cancer lines (the human Caco-2 and mouse MC-26 lines) and both were characterised by expression of ERα [18, 25]. Moreover, oestrogens induced colon cancer cell apoptosis only in one (COLO205) of the four lines examined. All four lines expressed ERβ, while only COLO205 did not

Table I

The correlations between Colon 38 cancer growth inhibition as assessed by the Mosmann or BrdU incorporation methods and concentrations of the substances examined (E2 — oestradiol, T — tamoxifen) or duration of cultures, r — Pearson coefficient, "–" not determined, NS — not significant

Tabela I

Korelacja pomiędzy zahamowaniem wzrostu raka Colon 38 ocenianym metodą Mosmanna lub wbudowywaniem BrdU a stężeniem badanych substancji (E2 — estradiol, T — tamoksyfen) lub czasem trwania hodowli, r — współczynnik korelacji Pearsona, "–" nie oceniano, NS — statystycznie nieznamienne

			Mosmann method		BrdU method	
			r	p	r	p
E2	dose (10^{-5} to 10^{-4} M) — response		–0.9149	0.0000	–	–
	time (4–72 h) – response	4×10^{-5} M	–0.6915	0.0002	–0.6338	0.0010
		10^{-4} M	–0.8274	0.0000	–0.9358	0.0000
T	dose (10^{-5} to 10^{-4} M) — response		–0.9386	0.0000	–	–
	time (4–72 h) — response	10^{-5} M	–0.7433	0.0002	–0.7129	0.0003
		4×10^{-5} M	–0.5332	0.0069	–0.5612	0.0050
		10^{-4} M	–0.1283	NS	–0.5432	0.0084

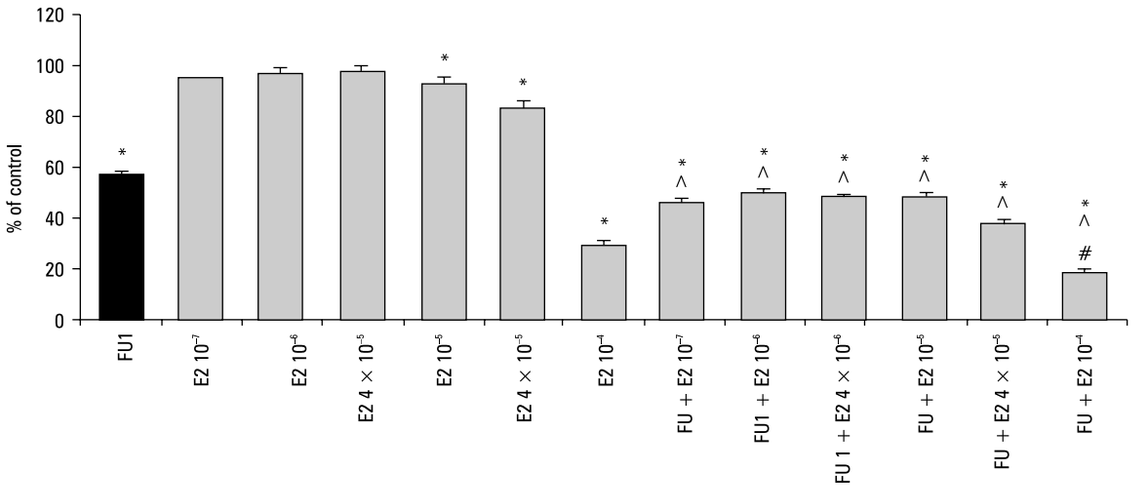

Figure 3. *The effect of oestradiol (E2) applied alone or jointly with fluorouracil (FU) on the growth of Colon 38 cancer as assessed by the Mosmann method in 72 h culture. X±SEM, *p < 0.05* vs. *control , ^ p < 0.05* vs. *FU1, #p < 0.05* vs. *E2 10^{-4}. FU1 — fluorouracil 1 μM; E2 10^{-7}, E2 10^{-4} — oestradiol 10^{-7} M, oestradiol 10^{-4} M*

Rycina 3. *Wpływ estradiolu (E2) zastosowanego oddzielnie lub w połączeniu z fluorouracylem (FU) na wzrost raka Colon 38 oceniany metodą Mosmanna w hodowli 72 h. X±SEM, *p < 0,05* vs. *kontrola, ^ p < 0,05* vs. *FU1, #p < 0,05* vs. *E2 10^{-4}. FU1 — fluorouracil 1 μM; E2 10^{-7}, E2 10^{-4} — estradiol 10^{-7} M, estradiol 10^{-4} M*

express ERα [17]. It is therefore suggested that ERα and ERβ may function in opposite ways in these cells. Interestingly, in this study LoVo colon cancer cells were shown to express ERα, which was not detected in these cells by other authors [27], although they used the same method (RT-PCR). However, from both subtypes of ER, the pivotal role in colon cancer growth regulation seems to be played not by ERα, but by ERβ. Over-expression of ERβ in an engineered human colon cancer line inhibited cell proliferation and increased adhesion in a ligand-independent manner [29]. In our study we revealed that Colon 38 cancer cells express ERβ (data not published). We therefore suppose that the inhibitory effect of oestradiol found in our experiments can be mediated by this receptor.

The diverse effect of oestrogens on colon cancer growth observed in various studies might also be linked to the use of different concentrations of oestradiol.

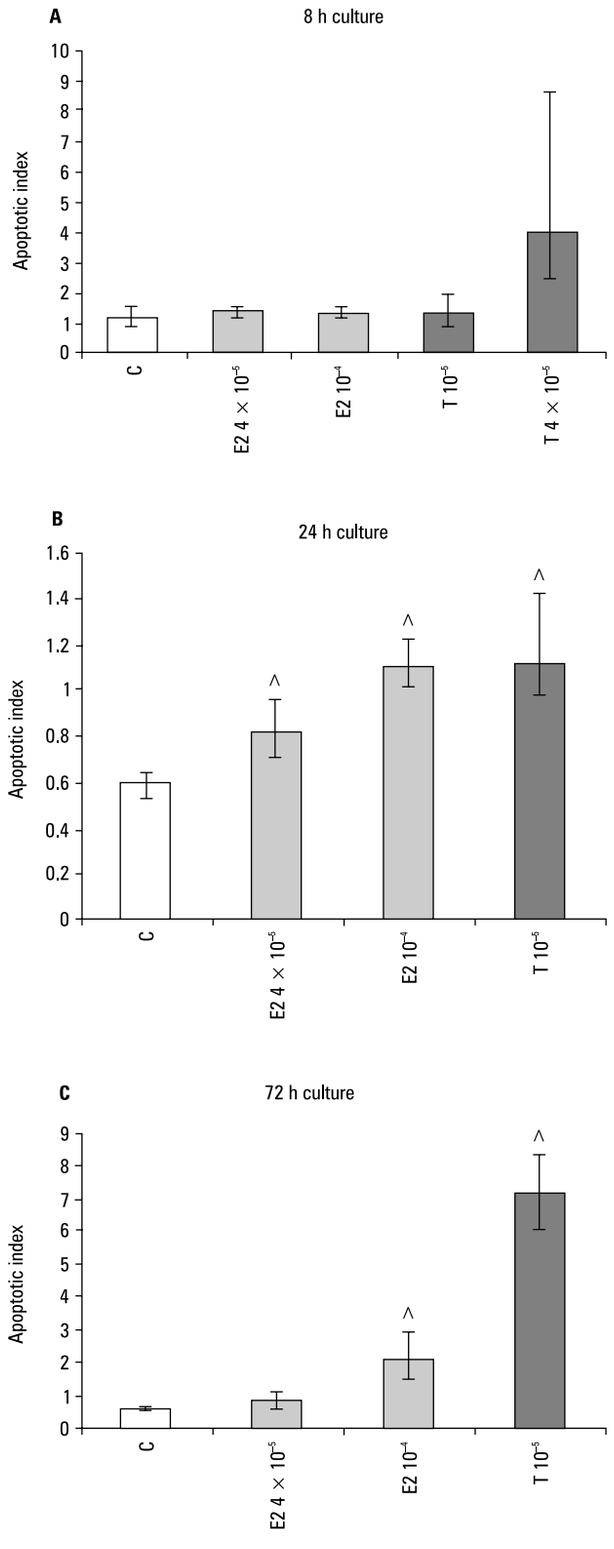

Figure 4. *Effect of oestradiol (E2) and tamoxifen (T) on apoptosis of Colon 38 cancer cells in 8, 24 and 72 h cultures. Me (Max--Min), ^p < 0.05* vs. *control (C)*

Rycina 4. *Wpływ estradiolu (E2) i tamoksyfenu (T) na nasilenie apoptozy komórek raka Colon 38 w hodowlach 8, 24 i 72 h. Me (Max-Min), *p < 0,05* vs. *kontrola medium, ^p < 0,05* vs. *kontrola (C)*

It has been suggested that oestradiol at low concentrations enhances cell proliferation, while reducing it at high concentrations [26]. Thus we examined the influence of oestradiol in a wide range of concentrations (from 10^{-12} to10^{-4}M). In our study we observed exclusively the inhibitory effect of oestradiol, which was evoked by the hormone at high concentrations (between 10^{-5} and 10^{-4} M). However, in one of the shorter (24 h) cultures oestradiol at low concentrations (10^{-6} to 10^{-12} M) seemed to stimulate cancer growth. This effect turned out to be an error connected with a badly matched control group. Unexpectedly, the highest ethanol concentration routinely used in 72 h experiments as the only control group for all oestradiol concentrations (10^{-4} to 10^{-12} M) was shown to inhibit control cell growth in cultures lasting 24 h or less. This unwanted control cell growth inhibition resulted in the apparent stimulatory effect of lower oestradiol concentrations containing a smaller amount of ethanol solvent. This experience persuaded us to use, in shorter experiments, multiple control groups with the full range of ethanol concentrations adequate for the ethanol concentrations present in the groups examined.

In this study tamoxifen at high concentrations (between 10^{-5} and 10^{-4} M) decreased Colon 38 cell proliferation with great potency, even causing total cancer growth inhibition at concentrations of 8 x 10^{-5} and 10^{-4}M. These results are compatible with other studies, in which tamoxifen at various concentrations inhibited colon cancer growth [24, 27, 28, 30, 31]. Moreover, some authors have demonstrated that tamoxifen at a concentration of 10^{-5} M were lethal to colon cancer cells [28, 30]. It is known that tamoxifen can exert anti-oestrogenic and oestrogenic effects, which are tissue- , cell- and gene-dependent [21]. In our study tamoxifen and oestradiol alike inhibited colon cancer growth. In agreement with our study, other authors have observed the same inhibitory effect of these two compounds at a similar range of concentrations [24].

To our knowledge, we have revealed for the first time that oestradiol and tamoxifen can enhance the cytotoxic effect of FU on colon cancer cells. However, it has been shown *in vitro* that oestradiol, which did not inhibit colon cancer growth when given alone, intensified the inhibiting effect of tamoxifen [28]. Furthermore, the compounds applied together increased cellular sensitivity to FU [28]. The interaction of tamoxifen and FU was examined using two gastric cancer lines. The combination therapy of these two drugs resulted in a synergistic anti-proliferative activity on one line and an antagonistic effect on another line, in which tamoxifen attenuated the cytotoxic effect of FU [32]. Moreover, it was reported that tamoxifen can reverse drug (doxo-

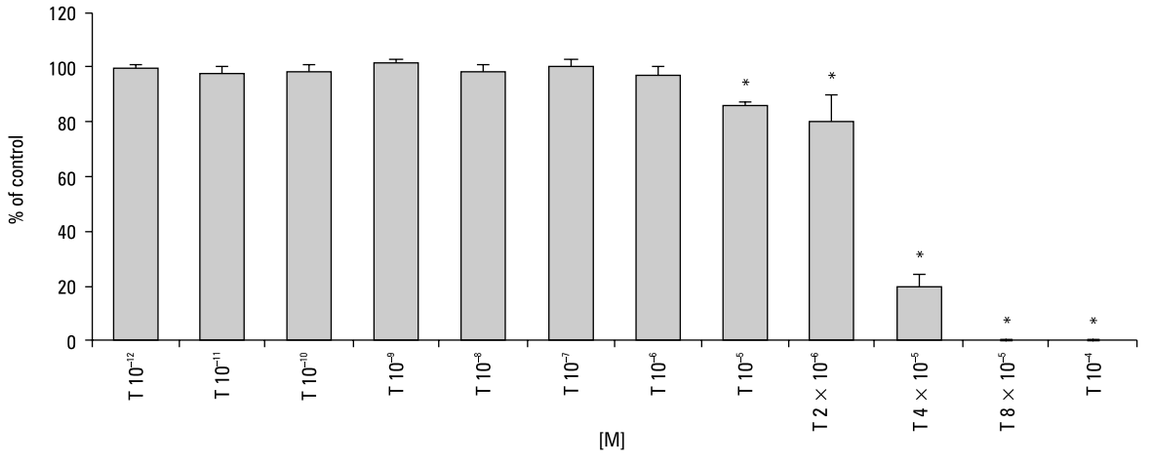

Figure 5. *The effect of tamoxifen (T) on the growth of Colon 38 cancer as assessed by the Mosmann method in 72 h culture. X ± SEM, *p < 0.05* vs. *control*

Rycina 5. *Wpływ tamoksyfenu (T) na wzrost raka Colon 38 oceniany metodą Mosmanna w hodowli 72 h. X ± SEM, *p < 0,05* vs. *kontrola*

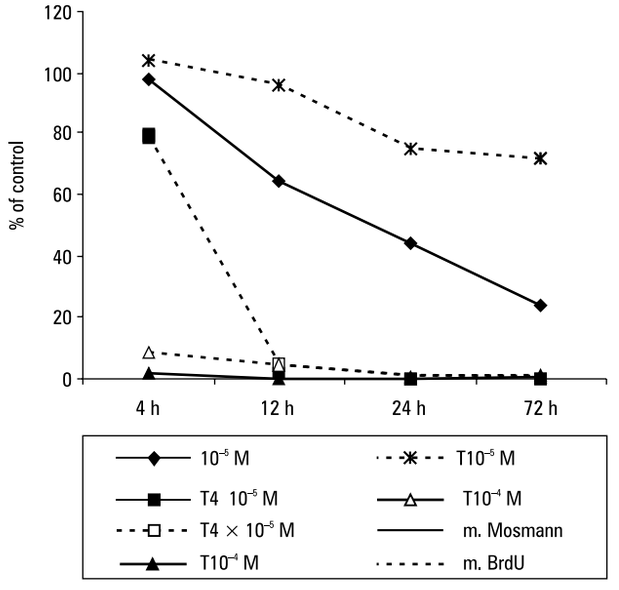

Figure 6. *The effect of tamoxifen (T) on the growth of Colon 38 cancer as assessed by the Mosmann method and BrdU incorporation method in 4, 12, 24 and 72 h cultures*

Rycina 6. *Wpływ tamoksyfenu (E2) na wzrost raka Colon 38 oceniany metodą Mosmanna i metodą wbudowywania BrdU w hodowlach 4, 12, 24 i 72 h*

rubicin) resistance in different tumours including colon cancer [33, 34].

It was demonstrated *in vitro* [35, 37] and *in vivo* [36] that tamoxifen can activate the apoptosis pathway in various cancers. An *in vitro* study on HeLa cells showed that tamoxifen at concentrations of 10–20 μM induced apoptosis in an ER-independent way, while at submicromolar concentrations it did so in an ER-dependent way [37]. In our apoptosis assay we examined the effect of tamoxifen only at high concentrations (from 10^{-5} to 10^{-4} M), demonstrating its proapoptotic effect at a concentration of 10^{-5} and 4×10^{-5} M. This is in agreement with our earlier studies, where tamoxifen only at higher doses induced apoptosis in transplantable Colon 38 cancer [36], while lower doses were ineffective [38]. On the other hand, tamoxifen at the concentrations similar to the concentrations used by us did not induce apoptosis in two other colon cancer lines (HCT8, HCT116) [30].

Conclusions

To summarise, we have shown in this article that oestradiol and tamoxifen inhibit Colon 38 cancer growth through their anti-proliferative and proapoptotic effects. Moreover, both substances enhanced the cytotoxic effect of FU. Our results suggest that sex steroids are involved in colon carcinogenesis. We also hypothesise that oestrogens and an oestrogen receptor modulator (tamoxifen) may potentially be useful in colon cancer treatment by enhancing the efficacy of FU. Further studies are needed to elucidate whether this suggestion is true in human colon cancer and whether it merits clinical application.

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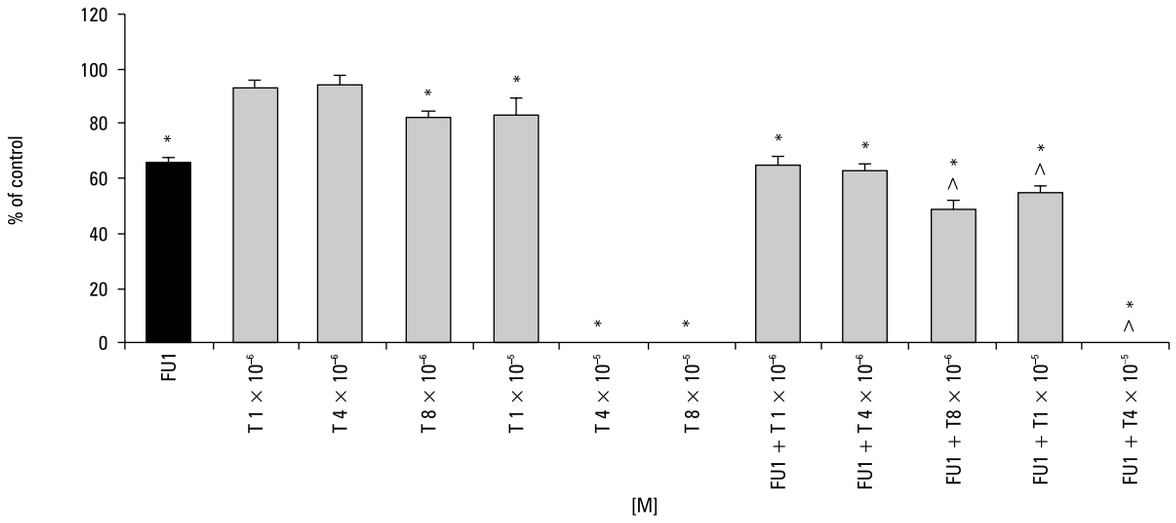

Figure 7. *The effect of tamoxifen (T) applied alone or jointly with fluorouracil (FU) on the growth of Colon 38 cancer as assessed by the Mosmann method in 72 h culture. X±SEM, *p < 0.05* vs. *control, ^ p < 0.05* vs. *FU1.*

FU1 — fluorouracil 1 μM; T 1 × 10–6, T 8 × 10–5 — tamoxifen 1 × 10⁻⁶ M, tamoxifen 8 × 10⁻⁵ M

Rycina 7. *Wpływ tamoksyfenu (T) zastosowanego oddzielnie lub w połączeniu z fluorouracylem (FU) na wzrost raka Colon 38 oceniany metodą Mosmanna w hodowli 72 h. X±SEM, *p < 0,05* vs. *kontrola, ^ p < 0,05* vs. *FU1.*

FU1 — fluorouracil 1 μM; T 1 × 10–6, T 8 × 10–5 — tamoksyfen 1 × 10⁻⁶ M, tamoksyfen 8 × 10⁻⁵ M

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