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Effects of Ramipril on the Hormone Concentrations in Serum of Hypertensive Patients

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Summary: The effects of the angiotensin-converting enzyme inhibitor ramipril on thirteen endocrinological tests were evaluated. These tests comprised serum follitropin, lutropin, prolactin, thyrotropin, free thyroxine, total thyroxine, free triiodothyronine, parathyrin, cortisol, testosterone, sex hormone binding globulin, androstenedione and dehydroepiandrosterone sulphate.

Eleven hypertensive outpatients, 9 men and 2 women, treated at the department of internal medicine in Turku University Central Hospital, received 5 mg of ramipril once a day for the study period of four weeks. The above mentioned endocrinological tests were performed before and at the end of the ramipril treatment. Ramipril decreased the value of free thyroxine statistically significantly, $p = 0.011$, from the mean value of 17.1 pmol/l to the mean value of 16.0 pmol/l when measured with Amerlex-MAB* free thyroxine kit. The mean within-subject difference was −1.10 pmol/l with a 95% confidence interval of −1.87 – −0.33 pmol/l. With the AutoDELFIA™ free thyroxine kit and with the reference method dialysis+RIA no effect was detected. Other endocrinological tests examined were not affected by ramipril. Since the decreasing effect of ramipril on free thyroxine was detected only with Amerlex-MAB* but neither with AutoDELFIA™ nor with dialysis+RIA, the effect was concluded to be analytical. The underlying mechanism and the component ultimately interfering with the analysis is unknown.

Introduction

Drugs affect laboratory test results (1, 2). Drug effects on laboratory tests are either biological or analytical. For instance, amiodarone is known to increase the value of thyrotropin biologically (3–5) whereas several cephalosporines are known to interfere with current methods for measuring creatinine (6, 7).

The interfering drug effects should be known and they should also be recognized by clinicians in order to interpret laboratory test results correctly. Since new drugs and new laboratory methods are introduced frequently, laboratory staff should be alert and eager to actively search for drug effects on laboratory tests. For this purpose, it is not ethically suitable to treat healthy volunteers with drugs. However, related to other drug investigations, it is reasonable to also examine the unknown effects of investigational drugs on laboratory tests. This applies especially to endocrinological tests, which usually are not included in the safety tests of pharmaceutical industry but often, however, play an important role in clinical diagnostics.

Ramipril is a long-acting angiotensin-converting enzyme inhibitor, which is converted to its active metabolite, ramiprilat, in the liver (8). Even though ramipril was introduced into clinical use several years ago, little is known about its effects on endocrinological tests. Only the effects on the renin-angiotensin-aldosterone system (9–11), cortisol, catecholamines, vasopressin (9) and insulin (12) have been studied. The present study was carried out to examine whether ramipril has other endocrinological effects.

Materials and Methods

Subjects

The study population consisted of 11 patients with essential hypertension, 9 men and 2 women, mean age 41.3 years (range 36–49 years). All subjects gave their informed consent to the study. One subject used low-dose budesonide and beclomethasone inhalations, others had no constant medication. All subjects had diastolic blood pressure between 95 and 110 mm Hg repeatedly and the mean 24 h-blood pressure in the ambulatory recording above 140/85 mm Hg. The patients were examined by an internist to exclude secondary hypertension. Despite hypertension, the patients were healthy.

Study protocol

All subjects received the angiotensin-converting enzyme inhibitor ramipril 5 mg (Ramace® 5 mg tabl, Suomen Astra Oy, Finland) once a day for the study period of four weeks. Subjects were tested for serum follitropin, lutropin, prolactin, thyrotropin, free thyrox-

ine, total thyroxine, free triiodothyronine, parathyrin, cortisol, testosterone, sex hormone binding globulin, androstenedione and dehydroepiandrosterone sulphate before medication and at the end of the study period. Blood samples were taken between 7 a.m. and 9 a.m. after fasting from 10 p.m. the previous evening. Methods, manufacturers and coefficients of inter-assay variation (CV%) of the assays are displayed in table 1. The study protocol was approved by the Ethics Committee of the Turku University Central Hospital and the study followed the recommendations for biomedical research involving human subjects according to the current version of the Declaration of Helsinki.

Statistics

The 95% confidence interval for the within-subject difference in a paired case was used as a measure of statistical significance. *Student*'s t-test (paired, two-sided) was used to find out the exact p-value (13).

Results

The effects of ramipril on the hormonal status of study patients are shown in table 2. Ramipril decreased the value of free thyroxine when measured with Amerlex-MAB* (Amersham, UK): The mean value of free thyroxine before medication was 17.1 pmol/l and at the end of medication 16.0 pmol/l. The mean within-subject difference was −1.10 pmol/l with a 95% confidence interval of −1.87 pmol/l − −0.33 pmol/l and $p = 0.011$ (*Student*'s t-test). When measured with AutoDELFIA™ (Wallac, Finland) the value of free thyroxine did not change statistically significantly, $p = 0.268$. Neither did the value of free thyroxine change statistically signifi-

Tab. 1 Hormone assays: methods, manufacturers and coefficients of inter-assay variation (CV%) at the given concentration.

Assay	Method	Manufacturer	CV%	at concentration
Follitropin	DELFIA®	Wallac	3.3	0.8 U/l
Lutropin	DELFIA®	Wallac	4.5	0.4 U/l
Prolactin	AutoDELFIA™	Wallac	2.8	5.9 µg/l
Thyrotropin	AutoDELFIA™	Wallac	5.0	3.4 mU/l
Free thyroxine	Amerlex-MAB*	Amersham	5.4	18.7 pmol/l
Free thyroxine	AutoDELFIA™	Wallac	4.1	27.3 pmol/l
Free thyroxine	Dialysis+RIA[a]	Medix Clinical Laboratories	7.7	16 pmol/l
Total thyroxine	DELFIA®	Wallac	3.6	92.2 pmol/l
Free triiodothyronine	AutoDELFIA™	Wallac	8.7	4.1 pmol/l
Parathyrin	IRMA	Incstar	13.1	45 ng/l
Cortisol	Spectria RIA	Orion Diagnostica	6.5	836 nmol/l
Testosterone	Spectria RIA	Orion Diagnostica	6.9	21.6 nmol/l
Sex hormone binding globulin	Spectria IRMA	Orion Diagnostica	7.0	10 nmol/l
Androstenedione	Extraction+RIA	Steranti Research (antiserum) Amersham ([^{3}H]androstenedione)	11.0	3.8 nmol/l
Dehydroepiandrosterone sulphate	RIA	Sorin Biomedica	8.6	5.3 µmol/l

DELFIA: time-resolved fluoroimmunoassay; IRMA: immunoradiometric assay.
RIA: radioimmunoassay; [a] In-house method (see l. c. (14)).

Tab. 2 Effects of ramipril on the serum hormonal status of study patients. In follitropin, lutropin, testosterone, sex hormone binding globulin, androstenedione and dehydroepiandrosterone sulphate values, only males are included.

Hormone		Before ramipril treatment		After ramipril treatment		Δ[a]
		Mean	Range	Mean	Range	Mean
Follitropin	U/l	4.4	1.7 −6.7	4.4	2.2 −6.4	−0.04
Lutropin	U/l	3.6	1.6 −5.9	3.0	2.2 −4.2	−0.55
Prolactin	µg/l	7.1	4.0 −11.0	6.6	3.6 −11.0	−0.45
Thyrotropin	mU/l	1.9	0.8 −3.2	1.6	1.1 −2.4	−0.31
Free thyroxine[b]	pmol/l	17.1	12.3−22.5	16.0	13.5−21.9	−1.10[e]
Free thyroxine[c]	pmol/l	12.2	9.4 −14.6	12.6	9.3 −15.5	0.35[f]
Free thyroxine[d]	pmol/l	13.5	10 −17	13.6	11 −16	0.10[f]
Total thyroxine	pmol/l	113	74 −150	110	98 −130	−3.0
Free triiodothyronine	pmol/l	5.9	4.9 −7.7	6.3	4.8 −7.7	0.38
Parathyrin	ng/l	36	17 −57	35	16 −59	−0.5
Cortisol	nmol/l	465	232 −635	472	415 −635	6.9
Testosterone	nmol/l	18	9 −28	18	6 −27	0.3
Sex hormone binding globulin	nmol/l	36	14 −56	36	12 −58	0.1
Androstenedione	nmol/l	5.8	3.2 −8.6	5.6	4.1 −9.3	−0.16
Dehydroepiandrosterone sulphate	µmol/l	6.8	2.8 −11.1	6.8	2.4 −13.8	−0.09

[a] mean within-subject difference;
[b] Amerlex-MAB*;
[c] AutoDELFIA™;
[d] dialysis+RIA (see l. c. (14));
[e] $p < 0.05$;
[f] non-significant.

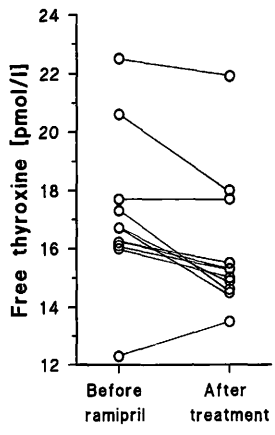

Fig. 1 Effects of ramipril on the free thyroxine values of individual patients measured with Amerlex-MAB*.

cantly, $p = 0.858$, when measured with the reference method dialysis+RIA (Medix Clinical Laboratories, Finland) (14). Ramipril did not affect other endocrinological tests. The effects of ramipril on free thyroxine of individual patients measured with Amerlex-MAB* are described in figure 1.

Discussion and Conclusion

The effects of angiotensin-converting enzyme inhibitors on endocrinological tests are not well known (2). Generally, angiotensin-converting enzyme inhibitors are shown to decrease serum aldosterone concentration and increase serum renin concentration (2), which also applies to ramipril (9, 11). Serum insulin has been found to decrease, increase or remain unaffected depending on the angiotensin-converting enzyme inhibitor (2). According to earlier studies, angiotensin-converting enzyme inhibitors do not affect the concentration of serum cortisol, catecholamines and antidiuretic hormone (2). Similarly, during ramipril treatment, the level of insulin secretion (12), cortisol, catecholamines and vasopressin (9) have been reported to remain unaffected.

In the present study, the effects of ramipril on 13 endocrinological tests were examined. No major endocrinological effects were found. Ramipril did not affect serum cortisol concentration, which is in line with the findings of *Crozier* and co-workers (9). Neither did ramipril have statistically significant effects on serum follitropin, lutropin, prolactin, thyrotropin, total thyroxine, free triiodothyronine, parathyrin, testosterone, sex hormone binding globulin, androstenedione or dehydroepiandrosterone sulphate concentrations.

However, serum free thyroxine decreased from the mean concentration of 17.1 pmol/l to the mean concentration of 16.0 pmol/l during ramipril treatment when measured with Amerlex-MAB*, which was the standard free thyroxine assay in our laboratory during the study. As the specimens were reanalyzed with the new AutoDELFIA™ assay, no effect of ramipril was detected. Whenever an unexpected drug effect on a laboratory test is found, it is essential to assess the nature of the effect as analytical or biological. A biological effect causes a real increase or decrease of a biochemical component whereas an analytical effect is associated only with the method used in measuring. The minimal evidence needed for this classification requires at least two assays based on different methodological principles. To be certain about the nature of the effect, the specimens were reanalyzed using the reference method for free thyroxine, dialysis+RIA (14). The results of the dialysis+RIA were parallel with the AutoDELFIA™, i. e., no effect of ramipril on free thyroxine concentration was detected. Accordingly, the decreasing effect of ramipril on free thyroxine was associated with the Amerlex-MAB* but neither with the AutoDELFIA™ nor with dialysis+RIA. The decreasing effect of ramipril on free thyroxine was therefore concluded to be analytical.

Free thyroxine immunoassays have been strongly criticized because of the misleading diagnostic results they often yield due to abnormal serum concentrations of albumin or other proteins, antibodies or inhibitors of hormone binding (15, 16). Recently, *Van Blerk* and coworkers (17) compared four radioisotope immunoassays of free thyroxine and they evaluated the performance of Amerlex-MAB* free thyroxine as excellent. Endogenous albumin, however, was found to interfere with the method and have weak positive correlation with free thyroxine results. According to our measurements, ramipril did not affect the concentration of serum albumin (data not shown) and therefore albumin could not be the component interfering with the Amerlex-MAB* free thyroxine in this study. The mechanism of the effect is still unknown. All the same, the AutoDELFIA™ free thyroxine assay based on the back-titration principle was less sensitive to the interfering components in the specimens.

In Turku University Central Hospital we use a computerized system for managing patient medication data, laboratory data and drug-laboratory interference data (18–20). Utilizing this system, we found that 20% of the patients tested for free thyroxine in our hospital were treated with an angiotensin-converting enzyme inhibitor. In the light of this finding and the fact that the Amerlex-MAB* assay is widely and commonly used, the effect of ramipril or possibly all angiotensin-converting enzyme inhibitors on free thyroxine is probably quite a common problem in laboratories. Even though the decrease in free thyroxine was slight and none of the patients in this study was misclassified as hypothyroid, attention should be paid to this problem, because misclassification of hyperthyroid patients as normal or normal patients as hypothyroid may occur in borderline cases.

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