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Metabolic effects of the combination of furosemide and captopril in rat

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Diuretics typically provoke increased serum lipid levels and may provoke increased serum uric acid levels and/or glucose intolerance. Furosemide is widely used as an antihypertensive, but in patients for whom furosemide treatment alone proves insufficient to reduce hypertension, a common procedure is to co-administer the angiotensin-converting-enzyme (ACE) inhibitor captopril. The metabolic effects in rats of joint administration of furosemide (15 mg/kg/day) and captopril (2 mg/kg/day) are evaluated over a two-week period. At the end of this period, the serum levels of lipids, uric acid, bilirubin, proteins and various enzymes were determined as well as the effect of the treatment on intestinal absorption of glucose and calcium. Furosemide/captopril led to an increase in serum albumin and alkaline phosphatase levels, and a decrease in triglyceride levels. The results of this work support the view that this drug combination is suitable for hypertension treatment.

Key words: Furosemide, Captopril, Glucose, Lipids, Calcium.

The long-term use of diuretics for the treatment of hypertension (one of the principal risk factors for cardiac insufficiency) is frequent among middle-aged and elderly people. However, while the efficacy of diuretics for reduction of hypertension is widely accepted, numerous authors have reported that their use by patients with moderate/severe hypertension tends to provoke increased serum levels of cholesterol and other lipids (2, 4, 12, 16, 21, 24, 30), and often leads to glucose intolerance, thus increasing the risk of diabetes in susceptible patients (17).

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These negative effects may partially explain the lack of any appreciable effects of diuretic treatment on the risk of myocardiac infarct (15).

Furthermore, loop diuretics such as furosemide have potent natriuretic activity, and thus tend to provoke increased renal elimination of calcium. This may have a hypocalcemic effect, particularly important in patients at risk for renal calculi or osteoporosis (23, 28).

In addition to these clearly negative effects, diuretics may affect serum levels of creatinine, urea, albumin, total protein, transaminases and other metabolites, though for many such metabolites conflicting results have been obtained as regards the effects of diuretic treatment.

In some patients, the administration of a diuretic alone proves to be insufficient to reduce hypertension, and in such cases there is an increasing tendency to apply combined therapies. A number of studies (6, 8, 10, 19, 20, 32) have indicated that the most effective combination is that of a diuretic plus an angiotensin-converting-enzyme inhibitor (ACEI), which acts synergistically to reduce arterial tension (1, 25). Diuretics tend to stimulate both the secretion of renin and the release of catecholamines, whereas ACEI counteracts both effects by blocking the angiotensinogen conversion pathway and inhibiting catecholamine release (11, 27). Furthermore, ACEI appears to ameliorate the negative effects of diuretics on glucose metabolism (9).

There are relatively few studies on the metabolic effects of combined administration of loop diuretics and ACEI, and furthermore, their results are discordant.

In the present work, we aimed at evaluating the effects of combined administration of the loop diuretic furosemide and the ACEI captopril on various aspects of metabolism in rats. Following a two-week treatment period, intestinal absorption of glucose and calcium was evaluated, and serum levels of total cholesterol, triglycerides, urea, albumin, total protein, calcium, total bilirubin, creatinine, alkaline phosphatase, lactate dehydrogenase (LDH) and transaminases (GOT and GPT) were determined. Results for treated rats were compared with those obtained for sham-treated control groups.

Materials and Methods

Animals.– Male Sprague-Dawley rats weighing about 200 g were used in all experiments. They were kept in plastic cages at 20 ± 2 °C under a 14:10 h light-to-dark cycle, and received a commercial feed and water *ad libitum*.

Drugs.– Furosemide (Claudio Barcia, Madrid, Spain) and captopril (Sigma) were used. In all the experiments both products were administered jointly in a 1 % hydroxymethylcellulose (HMC) solution, since furosemide is scarcely soluble in water. HMC solution was made by slowly adding the polymer powder to boiling water with continuous stirring. The solution was allowed to cool at room temperature, then kept at 4 °C for 24 h. Furosemide was then slowly added, with continuous stirring, and when it had dissolved, captopril was added. The mixture was left shaking until it became fully homogeneous. Amounts of furosemide and captopril were such that the required daily dose (see below) was present in 1 ml of mixture. The mixture (hereinafter "furosemide/captopril/HMC") was stored in a dark cold room.

Furosemide/captopril/HMC or HMC alone, was administered intragastrically via an oral catheter. Both furosemide and captopril doses were 3 and 0.4 mg/day, respectively in both cases as a single dose at the same time each day. These doses

correspond to the maximum recommended dosage rates for human therapy (15 mg/kg/day and 2 mg/kg/day, respectively).

Experimental design.– The rats were divided into four groups of 10 rats each: treated groups A1 and B1 and control groups A2 and B2. Treated rats received furosemide/captopril/HMC, daily for two weeks; and control animals received HMC alone, likewise daily for two weeks. Groups A1 and A2 were used to investigate the effects of combined administration of furosemide and captopril on blood parameters. Groups B1 and B2 were used to assess the effects of the combined therapy on intestinal absorption of glucose and calcium.

Serum analysis.– After the two-week treatment period, rats in groups A1 and A2 were fasted but with water *ad libitum* for 24 h. The jugular vein was then cut, and blood samples were collected in graduated tubes containing 100 IU of heparin per ml of blood. Serum was separated by centrifugation (3500 rpm for 15 min) and stored at –20 °C until analysis.

Analyses were performed with a Spotchem SP-4410 automatic analyser (Menarini Diagnostics, Barcelona). The following serum components were determined: total cholesterol, triglycerides, urea (blood urea nitrogen, BUN), albumin, total protein, calcium, total bilirubin, creatinine, alkaline phosphatase, lactate dehydrogenase (LDH) and transaminases (GOT and GPT).

Glucose/calcium absorption.– After the two-week treatment period, rats from groups B1 and B2 underwent assays of *in vivo* intestinal absorption of glucose and calcium (22). After anaesthesia (12.5 % urethane, at 1.2 ml/100 g, for 10 min), the body cavity was opened and tubes were inserted just below the stomach and above the end of the ileum. Intestinal absorption rate (mol min^{-1}) was estimated by pumping a solution containing 2 mmol/l glucose or 1.25 mmol/l $CaCl_2$ (pH 7.4) through the intubated section. The absorption rate recorded for each rat was the mean of three consecutive determinations. Outflow samples were stored at 4 °C until analysis.

Glucose content in outflow samples was determined with the GOD-PAP kit (Spinreact, Gerona, Spain) in a DU-50 spectrophotometer (Beckman Instruments). Calcium was determined with a colorimetry kit from Sigma.

Statistical analysis.– Between-group comparisons of means were by Student's *t* test, means are cited ± SD, and $p < 0.05$ is taken to indicate statistical significance.

Results

The results of serum biochemistry determinations in the furosemide/captopril-treated group A1 and the sham-treated control group A2 are summarized in table I. Mean triglyceride level in the treated group was significantly lower than that of control group.

In the treated group albumin and alkaline phosphatase levels were significantly higher, the mean creatinine level being also higher, although not statistically significant. None of the other serum components determined differed significantly between the two groups.

The results of the intestinal glucose absorption for the treated group (4.44 ± 0.66) and control group (4.46 ± 0.57) and the values of the calcium absorption for the treated (3.95 ± 0.44) and the control animals (3.70 ± 0.49) did not show any significant differences.

Table I. *Serum analysis of of rats treated for two weeks with furosemide/captopril (treated group) or sham-treat-ed over the same period (control group).*
Values are means ± SD. BUN = blood urea nitrogen; ALP = alkaline phosphatase; LDH = lactate dehydrogenase (n = 10).

Parameter	Control	Treated
Cholesterol (mg/dl)	57.25 ±11.13	63.28 ± 7.47
Triglycerides (mg/dl)	72.42 ±15.3	48.00 ± 14.21[a]
BUN (mg/dl)	25.40 ± 3.64	28.33 ± 3.66
Albumin (g/dl)	2.76 ± 0.18	3.01 ± 0.24[b]
Tot. protein (g/dl)	4.75 ± 0.29	4.85 ± 0.32
Calcium (mg/dl)	10.22 ± 0.56	10.24 ± 0.60
Bilirubin (mg/dl)	0.34 ± 0.14	0.32 ± 0.09
Creatinine (mg/dl)	0.82 ± 0.23	0.98 ± 0.16
ALP (IU/l)	399.16 ± 44.82	468.80 ± 33.54[a]
LDH (IU/l)	1870.5 ± 230.8	1878.1 ± 422.1
GOT (IU/l)	472.25 ± 15.50	480.21 ± 45.63
GPT (IU/l)	143.33 ± 29.36	126.50 ± 23.33

[a]$p < 0.01$ and [b]$p < 0.05$ versus control group.

Discussion

In the present study, joint administration of furosemide and captopril to rats, over a two-week period, had no significant effect on serum cholesterol level, in accordance with results in humans (19). These authors found that joint administration of hydrochlorothiazide and enalapril had no significant effect on serum cholesterol level. In a recent review on the effects of the combination hydrochlorothiazide/captopril (1), administration of the former alone has been reported to lead to a marked increase in serum cholesterol level, whereas co-administration of captopril counteracts this effect; indeed, long-term administration of this combination typically leads to statistically significant reductions in serum cholesterol. Similarly, captopril was dose-dependently found to attenuate or prevent the rise in cholesterol level induced by diuretics as well as in triglycerides (31).

Furosemide/captopril treatment led to a significant decline in serum triglyceride levels, in acordance with other results (5, 31) that found that captopril dose-dependently attenuated the rise in triglyceride level induced by diuretics.

The attenuation of the negative effects on serum lipid profile is of particular interest in the context of long-term antihypertensive treatment, given the strong evidence that hyperlipidaemia increases the risk of atherosclerosis and thus of cardiac disease (3, 14).

The negative effects on serum lipid profile may be attributable to effects either on lipid synthesis in the liver, or on subsequent lipid metabolism pathways. Other authors have investigated whether diuretics inhibit phosphodiesterases or stimulate lipoprotein lipases (12): to date, however, no evidence has risen to support either mechanism. The observed effects of diuretics on serum lipid profile may be due to effects on the nervous system (29). Large doses of diuretics stimulate sympathetic activity by stimulating cate-

cholamine (particularly norepinephrine) release, as a result of the effects of these drugs on blood volume and blood pressure (29). Raised catecholamine levels may promote lipolysis, thus stimulating cholesterol synthesis and leading to increased serum cholesterol levels (13); indeed, norepinephrine levels have been found to be positively correlated with serum cholesterol level (14).

The hypothesis that diuretics affect lipid metabolism as an indirect result of their stimulation of catecholamine release is consistent with the observed attenuatory effects of ACEI , since these drugs are known to block sympathetic activity.

No significant differences in serum urea level between the furosemide/captopril-treated and control groups were detected. By contrast, diuretic/ACEI combinations have been reported to lead to increased urea levels (19, 31). MALINI *et al.* (18) reported increased urea levels in hypertensive patients treated with a diuretic/ACE inhibitor combination, though these authors did not detect any significant increase in uric acid levels after one year of treatment. Previous studies have shown that treatment with diuretics alone tends to lead to increased uric acid levels, sometimes causing gout (26), attributable to the reduction in blood volume, leading to increased blood urate concentration and thus increased reabsorption of uric acid in the proximal tubule, and/or to competition for excretion between uric acid and the drug.

Serum creatinine level was not statistically different in either group. However BARBA *et al.* (5) did not detect any significant effect of diuretic/ACE inhibitor treatment on creatinine level, though levels showed a tendency to decline in the long term and similar results were obtained with enalapril alone (20).

Serum albumin level in the furosemide/captopril-treated group was significantly higher. This is of interest in antihypertensive treatment of patients at risk for diabetes. Diuretics lead to a reduction in blood volume, and consequent haemoconcentration and renal stress. ACEI protect against renal insufficiency by reducing intraglomerular pressure in hypertensive patients, in whom renal blood flow is typically reduced and the flow resistance of both veins and efferent glomerular arterioles is typically high (6, 7).

Previous studies have indicated that furosemide induces increased urinary excretion of calcium, by inhibiting reabsorption of NaCl in Henle's loop and thus may lead to a compensatory increase in intestinal calcium absorption (7, 28). It has also been suggested that hypercalciuria induced by furosemide may be partially attributable to the increased secretion of aldosterone and vasopresin induced by this drug (28). ACEI counteracts these effects on hormonal secretion, and may thus counteract diuretic-induced hypercalciuria.

In the present study, we did not observe any significant differences between the treated and the control groups either in serum calcium levels or in intestinal calcium absorption. The use of diuretics for hypertension treatment is very frequent among elderly people and often causes hyperglycemia. We did not detect any significant differences in glucose absorption between treated and control groups. It seems, therefore, that the combination of furosemide and captopril does not interfere on glucose absorption.

In conclusion, the results of this study suggest that the furosemide/captopril combination is a rational choice for hypertension treatment.

R. LÓPEZ, C. TABOADA, C. RIVAS y A. SAN MIGUEL. *Efectos metabólicos de la combinación de captopril y furosemida en rata.*

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La utilización de diuréticos incrementa los niveles de colesterol y de triglicéridos y puede provocar intolerancia a la glucosa y aumento en los niveles de ácido úrico. La furosemida, agente natriurético que incrementa la eliminación renal de calcio, puede afectar a la homeostasis cálcica. Administrada sóla no es suficiente para controlar la hipertensión y se suele asociar con captopril, un inhibidor de la enzima de conversión de la angiotensina que carece de efectos metabólicos adversos. El presente estudio evalúa el efecto de la combinación de furosemida (15 mg/kg/día) y captopril (2 mg/kg/día) sobre el metabolismo de lípidos, ácido úrico, bilirrubina, proteínas y diversas enzimas (GOT, GPT, LDH, ALP, etc) tras un período de tratamiento de dos semanas. También se valoran los efectos sobre la absorción intestinal *in vivo* de glucosa y calcio. No se observan variaciones en la absorción de glucosa y de calcio y sí aumento estadísticamente significativo de la albúmina plasmática y de la fosfatasa alcalina, y descenso de los triglicéridos. De los resultados obtenidos se puede concluir que la combinación de furosemida y captopril presenta una buena tolerancia bioquímica sin alteraciones importantes en los parámetros metabólicos.

Palabras clave: Furosemida, Captopril, Glucosa, Lípidos, Calcio.

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