HPLC Determination and Antihypertensive Effect of Metipamide

J. Klíma*[1] / J. Grafnetterová[1] / Z. Kubelka[2] / Jin. Klíma[2]

[1] Institute for Clinical and Experimental Medicine, [2] Faculty Thomayer's Hospital, Videňská 800, 140 00 Prague 4, CSSR

Key Words

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Summary

Metipamide was monitored during a two month cap period of treatment to determine whether the whole-blood levels estimated by HPLC provide a relevant indicator of the possible accumulation of the drug. We also analysed antihypertensive activity and biochemical changes in blood of twenty hypertonic patients. Results showed that metipamide is an effective, first-line antihypertensive agent, combining satisfactory blood pressure reduction with low frequency of side effects and a simple once-daily dosage regime.

Introduction

Metipamide (4-chloro-N-(2-methyl-1-anilino-)3-sulfamoylbenzamide) is a new Czechoslovak sulfonamide diuretic with an antihypertensive effect developed by the Research Institute of Pharmacy and Biochemistry in Prague. Metipamide differs from indapamide by substitution of aniline in place of inuline in the molecule. Indapamide is used in Europe, the United States, Canada, Australia and Japan in the treatment of essential arterial hypertension [1, 2]. Metipamide was selected from a series of indoline and isoindoline derivates of chlorosulfamoylbenzamide as an agent with minimal natriuretic and substantial antihypertensive properties. Pharmacological preclinical studies of metipamide showed very good antihypertensive activity. The antihypertensive effect of metipamide is explained by a dual mechanism of action: a limited diuretic activity combined with antivasoconstrictive effects, resulting in decreased peripheral vascular resistance.

The aim of our study was to monitor metipamide during a two-month period of treatment and to determine whether the whole-blood levels estimated by HPLC, provide a relevant indicator of the possible cumulation of drug. During a two-month period of treatment we also analysed antihypertensive activity and biochemical changes in blood of hypertonic patients.

Patients and Treatment

Twenty hypertonic patients were studied. Age of patients ranged from 31 to 73 years and body weight from 51 to 128 kg. 13 patients had a hypertension stage I (10 male, 3 female) and 7 patients had hypertension stage II (3 male, 4 female). Patients with hypertension stage I were defined as patients with systolic blood pressure higher than 160 mmHg (21.3 kPa) or diastolic blood pressure higher than 95 mmHg (12.6 kPa) and patients with hypertension stage II as patients with higher systolic or diastolic blood pressure than those above and with presence of other clinical symptoms, such as left ventricule hyperthrophy, local or generalized occlusion of retinal arterias, presence of proteinuria or of hypercreatinemia. Patients were treated with metipamide for two months. Metipamide was given orally, in one daily dose ranging from 1.25 mg to 5 mg before the morning meal. The total daily dose was controlled according to blood pressure. In addition to metipamide four hypertonic patients received a β-adrenoreceptor antagonist (Trimepranol, SPOFA, Prague, CSSR), introduced after four weeks of clinical trial. Every two weeks patients were checked by a physician and blood for metipamide assay was drawn into tubes before the morning dose of metipamide and stored at 4 °C.

Determination of Metipamide

Metipamide levels in whole blood were measured by HPLC. We adapted the specific isocratic HPLC procedure developed by V. Miller and E. Kraus from the Czech Research Institute of Pharmacy and Biochemistry.

Metipamide was isolated from heparinized whole blood by repeated extraction with acetronitrile containing benzanilide as internal standard. 2 ml of whole blood were added to 2 ml of acetonitrile containing benzanilide (200 μg/l). This

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mixture was vortexed 1 min and then 50 µl of saturated $ZnSO_4$ were added.

This mixture was vortexed 1 min and then centrifuged at 3000 r.p.m. for 3 min. The supernatant was transfered into a dry tube containing 200 mg NaCl and vortexed for 2 min. After centrifugation (3000 r.p.m. for 3 min) the supernatant was transfered into a conical tube and the residue was re-extracted with 2 ml of acetonitrile without benzanilide. Collected extracts were evaporated at 40 °C under N_2 to dryness and the residue was dissolved in 400 µl of acidified 50 % methanol and a 50 µl aliquot of this solution was injected into the analytical column.

A SP 8000B liquid chromatograph, equipped with a column oven, a Model SP 8400 variable-wavelength detector and a Data System SP 4000 (all from Spectra-Physics, San Jose, CA, USA), was employed. The samples were injected using a Valco valve with a 50 µl sample loop, mounted on the chromatograph.

Acetonitrile of HPLC grade were obtained from Fluka (Ulm, FRG). Isopropanol, methanol, benzanilide, zinc sulfate heptahydrate acetic acid and sodium acetate were obtained from Lachema (Brno, Czechoslovakia). Metipamide standard and metipamide tablets were obtained from the Research Institute for Pharmacy and Biochemistry (Prague, Czechoslovakia). Trimepranol tablets were obtained from Spofa (Prague, CSSR).

The stainless steel analytical column (30 cm x 4.6 mm) packed with Separon SGX C 18, 10-µm was obtained from Tessek (Aarhus, Denmark).

Water was redestilled in glass and filtered through 0.45-µm poly (tetrafluoroethylene) membrane filters (Sartorius, Göttingen, FRG).

For the chromatographic separation, a mixture of 200 ml acetonitrile, 70 ml of isopropanol and 730 ml of acetate buffer (pH 3.5) was used as the mobile-phase at a flow-rate of 0.5 ml/min. 200 µg per L of benzanilide in acetonitrile was used as internal standard. For calibration, standards of 200 and 400 µg/l of metipamide in whole blood were used.

Detection was at 254 nm with a sensitivity of 0.01 a.u.f.s.; the chart speed was 0.25 cm/min. The separation on the C-18 reversed phase column was carried out at 40 °C. The quantitative analysis of metipamide was based on the peak-area ratio of metipamide to benzanilide.

Results

The sensitivity of the HPLC method was better than 30 µg/l, and the recovery of metipamide, added to whole blood, ranged from 85 to 95 %. The calibration curve was linear to 2000 µg/l. Chromatograms of metipamide-free whole blood, of a 400 µg/l metipamide calibration standard and of a patients blood containing 320 µg/l are shown in Fig. 1.

Blood levels of metipamide were measured by HPLC in 80 specimens during a 2-months follow-up period. The means ± standard deviations (S.D.) of twenty hypertonic patients after treatment of metipamide by different daily doses are shown in Table I. The analysis of metipamide blood levels showed no cumulation effect during two months follow-up period of metipamide treatment.

Therapeutic efficiency of metipamide was calculated as a increase of number of patients with normal blood pressure during therapy. Table II shows the number of

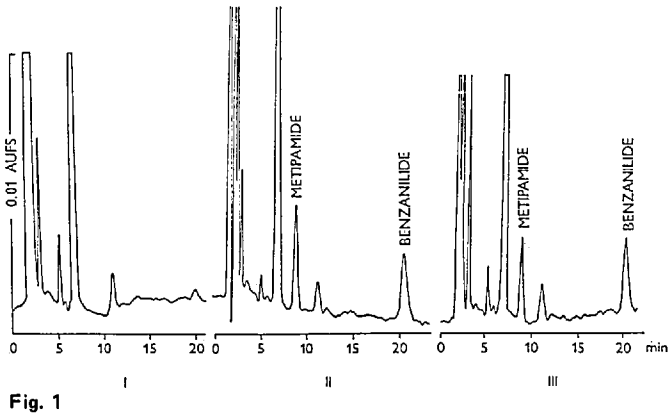

Fig. 1

Chromatograms of: metipamide-free whole blood (I), a 400 µg/l metipamide calibration standard (II), and of a patient's blood containing 320 µg/l of metipamide (III).

Table I. Blood concentrations of metipamide measured by HPLC during a 2-month follow-up period

Daily doses mg	Number of blood samples	Concentrations means ± S.D. µg/l
1.25	4	109 ± 51
2.50	45	297 ± 137
5.00	31	561 ± 233

Table II. Number of patients with normal blood pressure during metipamide therapy. (16 of 20 hypertonic patients were treated with metipamide and 4 patients with metipamide and trimepranol)

Week of therapy	Number of patients with normal blood pressure	
	Recumbent blood pressure	Postural blood pressure
0	0	0
2	11+	10+
4	12+	12+
6	18+	17+
8	18+	18+

($p < 0.005$)

Table III. Number of patients with normal blood pressure treated with metipamide only. Patients group consisted of 16 hypertonic patients.

Week of therapy	Number of patients with normal blood pressure	
	Recumbent blood pressure	Postural blood pressure
0	0	0
2	9+	8+
4	11+	11+
6	15+	14+
8	15+	15+

($p < 0.005$)

patients with normal blood pressure during a two-months follow-up period. 16 of 20 hypertonic patients were treated with metipamide and 4 patients with metipamide and trimepranol.

Table III shows the number of patients with normal blood pressure treated only with metipamide. In all cases described above, the statistical analysis consisted of a x^2 test. In all clinical situations, there were statistically significant differences between the number of patients with normal blood pressure during follow-up period of metipamide therapy and number of patients without therapy.

Hypokalemia was the most common biochemical adverse effect. After long-term treatment with metipamide, the mean decrease in serum potassium concentration was limited (0.3—0.4 mmol/L); potassium supplementation was rarely required and only in patients with low initial values.

During long-term treatment serum uric acid and urea levels have been found to increase, but decreased again after 6 weeks of metipamide therapy.

Glucose and lipid metabolism do not seem to be influenced to a significant extent by metipamide, as judged from the stability of blood glucose and serum lipid concentrations during long-term treatment.

The acceptability of metipamide once daily was good. Side effects were rare and mild. The asthenia, headache and gastrointestinal disturbances which occured were within the first month of treatment.

In four patients in which metipamide was associated with a beta blocker (trimepranol), the antihypertensive effect of the two drugs were consistently additive. In general, this combination was well tolerated because side effects were not additive.

Discussion

Treating hypertension in the elderly presents a number of problems. Drug treatment, if deemed necessary, should not be too aggressive and should be free of serious side effects. Therefore a modest reduction in blood pressure with relatively few side effects is an acceptable compromise.

Results of our clinical trial showed that metipamide once daily effectively reduces arterial blood pressure in more than two thirds of patients. Blood pressure-reducing effect was rapid within 1 or 2 weeks.

The drug is well tolerated and was not accumulated, the therapeutic window was wide and treatment was succesfully combined with beta blockers.

Metipamide is an effective, first-line antihypertensive agent, in that it combines satisfactory blood pressure reduction with low frequency of side effects and a simple once-daily dosage regime.

References

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