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THERAPEUTIC MONITORING OF METIPAMIDE DURING ANTIHYPERTENSIVE THERAPY

J. KLÍMA* and J. GRAFNETTEROVÁ

Institute for Clinical and Experimental Medicine, Vídeňská 800, 140 00 Prague 4 (Czechoslovakia)

and

Z. KUBELKA and JIN. KLÍMA

Faculty Thomayer's Hospital, Vídeňská 800, 140 00 Prague 4 (Czechoslovakia)

SUMMARY

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 high-performance liquid chromatography provide a relevant indicator of possible accumulation of the drug. We also analysed antihypertensive activity and biochemical changes in the blood of twenty hypertonic patients. The results of our clinical trial showed that metipamide is an effective first-line antihypertensive agent, in that it combines satisfactory reduction of blood pressure with a low frequency of side-effects and a simple once-daily dosage regime.

INTRODUCTION

Metipamide is a new Czechoslovak sulphonamide diuretic with antihypertensive effect and was developed by the Research Institute of Pharmacy and Biochemistry in Prague. Chemically it is a 4-chloro-N-(2-methyl-1-anilino)-3-sulphamoylbenzamide. Metipamide differs from indapamide by substitution of aniline instead of inuline in the indapamide molecule. Indapamide is used in Europe, the U.S.A., Canada, Australia and Japan in the treatment of essential arterial hypertension [1,2]. The metipamide molecule was selected from a series of indoline and isoindoline derivatives of chlorosulphamoylbenzamide as an agent with minimal natriuretic and substantial antihypertensive properties. Pharmacological preclinical studies of metipamide indicated very good antihypertensive activity, and these findings led to the introduction of this drug into the therapy of hypertonic patients. The antihypertensive effect of metipamide is explained by a dual mechanism of action: limited diuretic activity combined with antiva-

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soconstrictive effects, resulting in decreased peripheral vascular resistance. The mechanism of action was proved in several animal models of hypertension, including renal and spontaneously hypertensive rat and dog. As with other diuretics, metipamide has no hypotensive effect in normotensive animals or healthy humans [3,4].

Results from clinical trials stage I showed that 2.5 mg of metipamide once daily effectively reduced arterial blood pressure. After single oral doses of 2.5 and 5 mg, as well as after repeated administration of 2.5 mg daily for 14 days, the plasma elimination half-life of unchanged metipamide is ca. 31–55 h, indicating that once-daily dosing is possible. Results from an acute study in healthy volunteers as well as from short-term studies in hypertensive patients showed that metipamide has no significant effect on either the glomerular filtration rate or renal blood flow [5].

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 high-performance liquid chromatography (HPLC) provide a relevant indicator of the possible accumulation of the drug. During this period we also analysed antihypertensive activity and biochemical changes in blood of hypertonic patients.

EXPERIMENTAL

Patients and treatment

Twenty hypertonic patients were studied. Their ages ranged from 31 to 73 years, and their body mass from 51 to 128 kg. Thirteen patients had a hypertension stage I (ten males, three females) and seven had hypertension stage II (three males, four females). 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 the higher systolic or diastolic blood pressure described above and also with other clinical symptoms, such as left ventricular hypertrophy, local or generalized occlusion of retinal arterias, presence of proteinuria or of hypercreatinemia. Patients were treated with metipamide for 2 months. Metipamide was given orally, in one daily dose ranging from 1.25 mg to 5 mg. The metipamide tablets were taken before the morning meal. The total daily dose was controlled according to blood pressure. In addition to metipamide, four hypertonic patients received β-adrenoreceptor antagonist (Trimepranol; SPOFA, Prague, Czechoslovakia), introduced after 4 weeks of the clinical trial. Every 2 weeks patients were checked by a physician, and blood samples for metipamide assay were 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 Miller and Kraus [6] as follows: Metipamide was isolated from heparinized whole blood by repeated extraction

with acetonitrile containing the internal standard benzanilide. Then 2 ml of whole blood were added to 2 ml of acetonitrile containing the internal standard (benzanilide, 200 μg/l). This mixture was vortexed for 1 min and then 50 μl of saturated zinc sulphate solution were added. This mixture was vortexed for 1 min and then centrifuged at 10 000 *g* for 3 min. The supernatant was transferred to a dry tube containing 200 mg of sodium chloride and vortexed for 2 min. After centrifugation (10 000 *g* for 3 min) the supernatant was transferred to a conical tube, and the residue was reextracted with 2 ml of acetonitrile without benzanilide. Collected extracts were evaporated to dryness at 40 °C under nitrogen. 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.

An 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, U.S.A.), was employed. The samples were injected into a Valco valve with a 50-μl sample loop, mounted on the chromatograph.

Acetonitrile (HPLC grade) was obtained from Fluka (Ulm, F.R.G.). 2-Propanol, methanol, benzanilide, zinc sulphate 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 the product of SPOFA.

A stainless-steel analytical column (30 cm × 4.6 mm I.D.) 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, F.R.G.).

For the chromatographic separation, a mixture of 200 ml of acetonitrile, 70 ml of 2-propanol and 730 ml of 0.1 *M* acetate buffer (pH 3.5) was used. The mobile phase flow-rate was 0.5 ml/min. Benzanilide (200 μg in acetonitrile) was used as internal standard. For calibration, standards of 200 and 400 μg/l metipamide in whole blood were used.

The wavelength for analysis was set at 254 nm and the sensitivity at 0.01 absorbance units full scale; the chart speed was 0.25 cm/min. The separation on the C_{18} reversed-phase column was achieved at a temperature of 40 °C. The quantitative analysis of metipamide was based on the peak-area ratio of metipamide to benzanilide.

RESULTS

The limit of detection 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 patient's blood containing 320 μg/l are shown in Fig. 1.

Blood levels of metipamide were measured by HPLC in 80 specimens during a two-month follow-up period. The mean ± standard deviation levels for twenty hypertonic patients after treatment with metipamide by different daily doses are

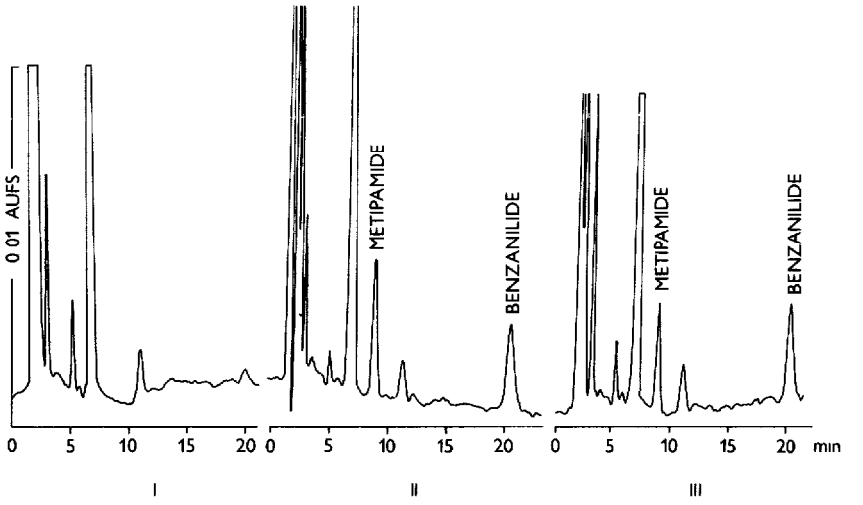

Fig. 1. Chromatograms of (I) metipamide-free whole blood, (II) a 400 μg/l metipamide calibration standard, and (III) a patient's blood containing 320 μg/l metipamide.

TABLE I

BLOOD CONCENTRATIONS OF METIPAMIDE MEASURED BY HPLC DURING A 2-MONTH FOLLOW-UP PERIOD

Daily dose (mg)	Number of blood samples	Concentration (mean ± S.D.) (μg/l)
1.25	4	109 ± 51
2.50	45	297 ± 137
5.00	31	561 ± 233

shown in Table I. The analysis of metipamide blood levels showed no cumulative effect during this time.

The therapeutic efficiency of metipamide was calculated as the increase in the number of patients with normal blood pressure during therapy. Table II shows the number of patients with normal blood pressure during the two-month follow-up period. Sixteen of the twenty patients were treated with metipamide, and four with metipamide and trimepranol.

Table III shows the number of patients with normal blood pressure treated with metipamide only. In all cases, the statistical analysis was based on the x^2 test. In all clinical situations, there were statistically significant differences between the number of patients with normal blood pressure during the follow-up period and the 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.

TABLE II

NUMBER OF PATIENTS WITH NORMAL BLOOD PRESSURE DURING METIPAMIDE THERAPY

Sixteen hypertonic patients were treated with metipamide, and four 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[a]	10[a]
4	12[a]	12[a]
6	18[a]	17[a]
8	18[a]	18[a]

[a] $p < 0.005$.

TABLE III

NUMBER OF PATIENTS WITH NORMAL BLOOD PRESSURE TREATED WITH METIPAMIDE ONLY

The group contained sixteen patients.

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

[a] $p < 0.005$.

During long-term treatment, serum uric acid and urea levels were 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 occurred within the first month of treatment.

In the four patients who took metipamide with a β-blocker (trimepranol), the antihypertensive effect of the two drugs was consistently additive. This combination was well tolerated because side-effects were, on the contrary, not additive.

DISCUSSION

The treatment of hypertension, particularly in the elderly, presents numerous problems. Drug treatment, if deemed necessary, should not be too aggresive and

should be free from serious side-effects. Therefore a modest reduction in blood pressure with relatively few side-effects is an acceptable compromise. The results of our clinical trial show that metipamide taken once daily effectively reduces arterial blood pressure in roughly two-thirds of patients. The reduction was rapid, occurring within 1 or 2 weeks. The drug is well tolerated, does not accumulate in the body, and can be successfully combined with a β-blocker. Moreover, it can be easily monitored by HPLC, if necessary, during long-term treatment.

REFERENCES

1 R.J. Noveck, F.G. McMahon, A. Quiroz and T. Giles, Am. Heart J., 106 (1983) 221–228.
2 J.H. Morledge, Am. Heart J., 106 (1983) 229–232.
3 V. Fedelešová, K. Sedláková, G. Brimichová and R. Dzúrik, Vnitřní Lék., 31 (1985) 55–59.
4 R. Dzúrik, V. Fedelešová, K. Sedláková, G. Brimichová and Š. Moricová, Bratisl. Lék. Listy, 85 (1986) 672–679.
5 L. Bachárová, Š. Moricová, N. Plevová, K. Sedláková, V. Fedelešová and R. Dzúrik, Bratisl. Lék. Listy, 86 (1986) 315–323.
6 V. Miller and E. Kraus, Research Institute of Pharmacy and Biochemistry, Prague, personal communication.