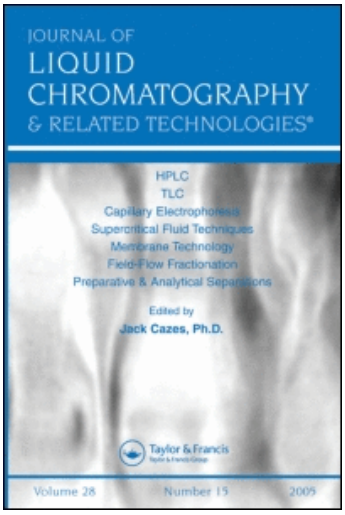

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EPININE KINETICS AND PLASMA CATECHOLAMINE CHANGES FOLLOWING ORAL ADMINISTRATION OF THE PRODRUG IBOPAMINE IN PATIENTS WITH CHRONIC HEART FAILURE

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ABSTRACT

Epinine plasma levels, and Epinine - induced plasma Catecholamine changes were investigated in nine patients with chronic heart failure (NYHA class I and II), following oral administration of Ibopamine 100 mg. Norepinephrine, Epinephrine, Dopamine, and Epinine plasma levels were monitored for up to four hours after the drug administration. Arterial pressure and heart rate were recorded, too. Epinine plasma levels were lower than previously estimated, the maximal individual level ranging from 2792 to 7228 pg/mL. Both Norepinephrine and Epinephrine showed a very small decrease (less than 18%) after Ibopamine administration. No changes were shown in arterial pressure and heart rate. Previous assessment of Epinine pharmacokinetics is likely to be reviewed extensively owing to possible overestimation by assay methods used in former studies. Epinine - induced plasma Catecholamine changes may be mediated by (i) the claimed hemodynamic effect of the drug, and/or (ii) by the complex interrelationships between Epinine itself and both adrenergic and dopaminergic receptors.

INTRODUCTION

N-methyl dopamine (Epinine) is the active moiety of N-methyl dopamine O, O'- diisobutyrol ester (Ibopamine) a cardiovascular orally active prodrug which shows alpha - and beta - adrenergic properties (1). This drug is claimed to have both inotropic and vasodilator effects. The dopaminergic nature of Epinine implies endocrine effects, such as decrease in prolactin and Aldosterone plasma levels (1). Moreover the drug seems to decrease plasma Norepinephrine and to increase plasma Epinephrine in patients with chronic heart failure (1). In this field, as well as in pharmacokinetic investigation, the employed methods were unable to assess simultaneously both Epinine and Catecholamine circulating levels. Further, some possible pitfalls in previous assay methods were not considered, some degree of overestimation being introduced in the evaluation of both the pharmacokinetics and the therapeutic dose range.

In fact previous methods seem not to take into account the large circulating amount of conjugated Epinine following ibopamine administration per os. Deproteinization steps were employed in some assay methods, by means of $HClO_4$ addition to plasma samples (2). This heavy acidification may easily hydrolize weak sulfate-esters such as conjugated Epinine. This in vitro produced free Epinine is obviously determined together with

circulating free Epinine by some methods. The present study was undertaken to verify, by an improved HPLC method, the pharmacokinetic of plasma Epinine following a single oral dose of Ibopamine (100 mg, i.e. the usual therapeutic regimen) in patients with chronic heart failure.

Moreover, it would be of interest to measure plasma levels of Catecholamines (Norepinephrine, Epinephrine and Dopamine), in order to verify indirectly the hemodynamic effects of plasma Epinine. Norepinephrine is generally considered as an index of cardiac function and the expected decrease of Norepinephrine concentration would support the claimed inotropic effect of Epinine.

SUBJECTS

We studied six men and three women with N.Y.H.A. functional class I - II congestive heart failure; they gave the informed consent to participate in this study. The age of these patients averaged 63 $\pm$ 5 years (mean $\pm$ SD), range 52 to 74 yrs. No patient had a previous history of drug treatment.

Subjects were studied supine in a quiet, semi-dark, temperature - controlled room.

With the patient recumbent after an overnight fast, a 21 gauge needle was inserted in a forearm vein (08:00

AM) and patency was maintained by a very slow (0.3 mL/min) infusion of normal saline. After 30 min of supine rest a basal sample was drawn, thereafter Epinine was administered as Ibopamine (100 mg per os). The other samples were drawn at +30, +60, +90, +120, and +240 min.

Blood samples were drawn in chilled tubes containing EDTA and Sodium metabisulfite, and centrifuged immediately at 2000 x g at + 4·C; then plasma was separated and stored in liquid nitrogen, until assayed within one week, by HPLC-ECD (3).

Arterial pressure was recorded by mercury manometer following each sampling procedure. Mean Arterial Pressure (MAP) were obtained as dyastolic blood pressure + 1/3 pulse pressure.

MATERIALS

Norepinephrine, Epinephrine, Dopamine, Dihydroxybenzylamine, Epinine, Alumina type WA-4, Sodium Metabisulfite, Sodium Phosphate, Sodium Acetate, Sodium dodecilsulfate, TRIS, and microfilters, were from Sigma Chemical Co., St.Louis, MO. Acetronitrile LiChrosolv, Phosphoric, Hydrochloric, and Perchloric acids, and EDTA, were Merck products (Darmstadt, Germany). Water was purified in a Milli-Q apparatus (15 - 18 Mohm, 0.22 µm pore size, Millipore Corp., Bedford, MA). All solutions were filtered in a solvent clarification appa-

ratus (0.22 µm pore size, Millipore Corp.). pH was determined at room temperature.

METHODS

Instrumentation: HPLC apparatus consisted of Model 510 constant flow pump (Waters, Milford, MA), Model 7125 valve fitted with a 50 µL sample loop (Rheodyne, Cotati, CA), Model 5100A electrochemical detector (ESA, Bedford, MA) consisting in a series of three electrodes (Model 5011 and 5021 cells) working in ox-red mode. The analytical column was a stainless steel Supelcosil LC-18-DB 7.5 cm x 4.6 mm ID prepacked with 3µm ODS (Supelco Inc., Bellefonte, PA), protected by a zero-dead volume inlet filter (Rheodyne). Mobile phase consisted of an 84:16 mixture of 50 mMol/L sodium phosphate, 50 mMol/L sodium acetate, and acetonitrile, and also contained 0.6 mMol/L of sodium dodecilsulfate and 0.5 mMol/L of EDTA. The final pH was adjusted to 3.00 with 85% phosphoric acid. The column was equilibrated with the mobile phase at least 6 h before use.

The elution profiles were integrated by an LCD CI-10B (Milton Roy, Riviera Beach, FL) and displayed on a Sekonics plotter.

Extraction: for assay of free Catecholamines and free Epinine, 2 mL of freshly thawed plasma were added to 25

mg of activated alumina in a 12 mL plastic tube. To this were added 1 mL of filtered TRIS HCl buffer (1.5 Mol/L, pH 8.6, containing 0.5 mMol/L of EDTA and 0.4 mMol/L sodium metabisulfite per liter) and 500 pg of Dihydroxybenzylamine (DHBA, internal standard). The sample was spun on a vortex-mixer for 10 minutes. The supernatant was discarded and the alumina was washed three times with 3 mL portions of 50 fold dilutions of TRIS HCl buffer in chilled water. The alumina slurry was transferred by disposable pipette in microfilters and centrifuged. Catecholamines and Epinine were desorbed with 100 µL of filtered 0.1 Mol/L perchloric acid. The mixture was vortexed for at least 10 seconds. Finally the supernatant was separated by centrifuge.

Analysis: the plasma extracts were analyzed by injecting 50 µL aliquots into the column. The effluent was monitored at the following potentials: +300 mV (1st electrode), +60 mV (2nd, screen electrode), and -300 mV (3rd, quantifying electrode). Full scale sensitivity was 20 nA. Pump flow was 1.2 mL/min, pressure on the column was 1750 psi.

Sensitivity of the method is below 5 pg/mL for all Catecholamines. Intra- and inter-assay CVs are below 9%. Recovery rate is: Norepinephrine 0.86; Epinephrine 0.95; Dopamine 0.76; Epinine 0.82.

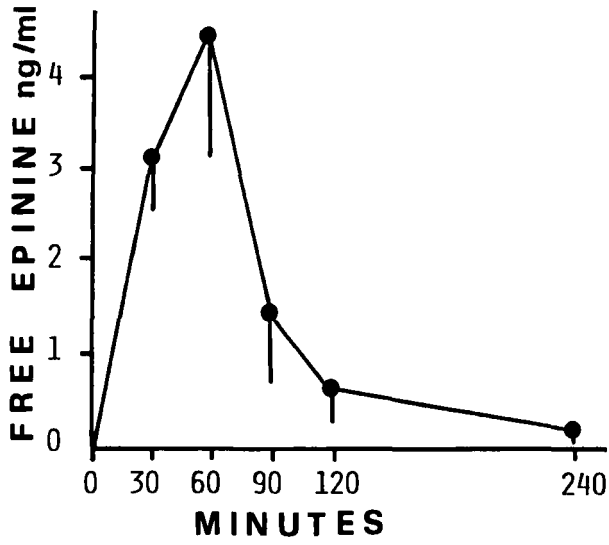

Fig. 1. Plasma Epinine concentrations following Ibopamine administration per os (time 0). Ibopamine was administered as a single 100 mg tablet (equivalent to 54.5 mg of Epinine).

Table 1. Mean and SD values of Norepinephrine (NE), Epinephrine (Epi), Dopamine (DA), and Epinine (NMD) in nine patients with chronic heart failure, following Ibopamine administration (100 mg per os). All plasma values are expressed in pg/mL.

Time	Basal	+30min	+60min	+90min	+120min	+240min
NE mean	279	243	243	239	257	242*
SD	80	60	69	73	67	66
Epi mean	33	27#	28*	25*	26	25*
SD	12	9	8	8	8	8
DA mean	38	36	35	33	34	34
SD	18	16	19	19	22	24
NMD mean	--	3284	4380	1468	641	203
SD	--	672	1678	748	310	74

Statistics: * $p<0.04$; # $p<0.01$ (t test for paired data)

RESULTS

Plasma levels of Epinine (Fig. 1 and Table 1).

After Ibopamine administration (100 mg per os, equivalent to Epinine 54.5 mg) (4), free Epinine levels averaged 3284 pg/mL at 30 min, 4380 at 60 min, 1468 at 90 min, 641 at 120 min, and 203 at 240 min.

Maximal individual concentrations ranged between 2792 and 7228 pg/mL. Mean time to plasma peak level was 60 min.

Plasma levels of Norepinephrine (Table 1).

After Ibopamine administration Norepinephrine mean values decreased significantly from 279 to 241 pg/mL ($p < 0.04$).

Plasma levels of Epinephrine and Dopamine (Table 1).

Epinephrine decreased significantly after Ibopamine administration from 33 to 28 - 25 pg/mL ($p < 0.04$ and $p < 0.01$). Dopamine plasma levels were unaffected by Ibopamine administration.

Mean Arterial Pressure (MAP) and Heart Rate (HR).

MAP and HR were not significantly affected. MAP decreased from 87.8 $\pm$ 6.4 (basal, mean $\pm$ SD), to 85.4 $\pm$ 5.6 (+240 min, not significant); HR decreased from 92.6 $\pm$ 4.0 (basal) to 91.2 $\pm$ 3.8 (+240 min, not significant).

DISCUSSION

Previous studies about plasma free Epinine kinetics following Ibopamine administration per os in man,

reported free Epinine plasma concentrations higher than in the present paper (1). These discrepancies may be due to a possible overestimation of free Epinine circulating values.

Previously reported methods for the assay of Epinine plasma levels seem to be affected by some pitfalls.

Gifford (4) described a method by HPLC with electrochemical detection, where the elution time of the Epinine peak is delayed to about 20 minutes. This slow separation shows broad and tailed peaks. These factors, together with a recovery ratio below 60%, and with a detection limit of 500 pg/mL, can affect the sensitivity and the validity of the method, when routinely employed.

Other Authors (2) reported HPLC analysis where the first step was an acidic deproteinization of plasma samples. Deproteinization by HClO4 is at a very high risk of overestimation of free Epinine, because of possible hydrolysis of the weak sulfate - ester bond of the very large amount of plasma conjugated Epinine.

Other Authors did not report validation data (5).

Sensitivity limits are higher and recovery ratios lower in previous methods respect to the method employed in the present paper.

Our data are in agreement with previous studies about the Epinine - induced decrease in Norepinephrine plasma

levels (5), although the decrement shown by us is very smooth (below 18 %), and p value is weak.

Moreover, in our patients we demonstrated a similar decrease in Epinephrine plasma values. The discrepancies with previous literature (6) are quite noticeable, and may be explained by the choice of a quite selected group of patients and by the use of a sensitive and reliable method in the present paper.

Plasma Dopamine seems unaltered by the administration of Epinine. MAP and HR were unaltered, too.

The decrease of both Norepinephrine and Epinephrine in our patients may be viewed as: (i) the result of the claimed hemodynamic effects of Epinine (i.e. an improvement of cardiac function) (1); (ii) the activation of alpha-2 presynaptic adrenoceptors (inhibitory on catecholamine release) (7); (iii) the result of the interaction between Epinine and DA-2 receptors (Dopamine is known to exert an inhibitory influence on catecholamine release through DA2 receptors when the sympathetic system is activated) (8); (iv) the complex result of the effects of Epinine on both alpha and beta adrenoceptors.

In fact, Epinine shows alpha, beta, and DA receptor affinity (1), owing to the structural similarity with Dopamine and Epinephrine, and it is possible that the net effect shown by us on catecholamine plasma levels

may be the result of the complex interaction of Epinine with these receptors.

Finally, the therapeutic drug dosage is likely to be reviewed following a careful series of studies on the pharmacokinetic and pharmacodynamic properties of Epinine in man, by the use of fully validated methods.

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