ELSEVIER

Il Farmaco 53 (1998) 690–692

IL FARMACO

Determination of bezafibrate concentration by high performance liquid-chromatography in serum of rats treated with lead nitrate☆

Carlo Anchisi [a,*], Anna Maria Fadda [a], Anna Maria Maccioni [a], Sandra Dessì [b]

[a] *Dipartimento Farmaco Chimico Tecnologico, Facoltà di Farmacia, Via Ospedale 72, 09124 Cagliari, Italy*
[b] *Istituto di Patologia Sperimentale, Facoltà di Medicina, Via Porcell 4, 09124 Cagliari, Italy*

Abstract

In the present study, the bezafibrate levels were measured in serum of rats treated with lead nitrate using a high performance liquid chromatography (HPLC) method. The results have shown that the peak corresponding to bezafibrate in the chromatogram is reduced in serum of rats treated with bezafibrate plus lead, indicating that lead treatment accelerates the metabolism of bezafibrate in rats. © 1998 Elsevier Science S.A. All rights reserved.

Keywords: Bezafibrate metabolism; Lead nitrate; Liver hyperplasia; HPLC determination

1. Introduction

Our previous studies [1] have shown that bezafibrate, a lipid-lowering drug of the clofibrate type administered to rats, is able to prevent the alterations of cholesterol metabolism normally found during hyperplasia induced by a potent mitogen (lead nitrate), as well as during proliferative processes occurring in other tissues [2–6].

In the present study, the bezafibrate levels were measured in serum of rats treated with lead nitrate after 12 days of a diet containing 0.1% of bezafibrate using a high performance liquid chromatography (HPLC) method [7]. Our aim was to determine whether the proliferative process is able to alter the pharmacokinetic of the drug.

2. Experimental

2.1. Equipment

The analyses were carried out on a Series 4 Perkin–Elmer liquid chromatograph equipped with a LC-85B Perkin–Elmer variable wavelength UV detector and a 3396 Series II Hewlett–Packard integrator. Analytical separations were carried out on a Spherisorb C-18 ODS2 (25 cm × 4.6 mm i.d.; 5 μm particle size) column (Merck) using a Rheodyne 7125 injection valve with a 20 μl injection volume.

2.2. Reagents for analysis

Analitycal grade bezafibrate, clofibric acid (I.S.) and lead nitrate were purchased from Aldrich (Milan). Reagent grade sodium dihydrogen phosphate, hydrochloric acid, methanol for HPLC and diethyl ether were obtained from Carlo Erba (Milan). Distilled water was further purified with a Milli-Q system (Millipore, Bedford, USA).

2.3. Material for animal experiments

Male Wistar rats, weighing 200–250 g, were used in these experiments. The animals were maintained on a 12 h dark and light cycle and free access to food and water.

The animals were divided in two groups: group 1 received bezafibrate (0.1% in the diet, corresponding to about 10 mg die/rat) alone, group 2 lead nitrate and bezafibrate.

Lead nitrate, dissolved in saline, was injected intravenously under light ether anaesthesia at a dose of 100 μmol/kg body wt. The rats were killed at different time intervals after the administration of the heavy metal salt (0, 36, 72 h).

☆ Dedicated to Professor Antonio Maccioni.

* Corresponding author.

PII: S0014-827X(98)00088-3

The treatment with bezafibrate started 12 days before lead nitrate injection and was continued until sacrifice.

2.4. Procedure

Sera obtained from rats fed with a diet containing 0.1% of bezafibrate and treated or not with lead nitrate were used for this study.

The internal standard solution was added to 1 ml of plasma samples (1 μg/10 μl). Bezafibrate was extracted from samples, acidified with 10 μl of concentrated HCl, with 8 ml of diethyl ether. The ethereal layer, after centrifugation (1500 × *g* for 10 min), was dried under nitrogen stream at room temperature. The residue was dissolved with 2 ml of the mobile phase and aliquots of 20 μl were injected into the chromatograph.

The mobile phase was 0.01 M phosphate buffer (pH 3.5)–methanol (35:65).

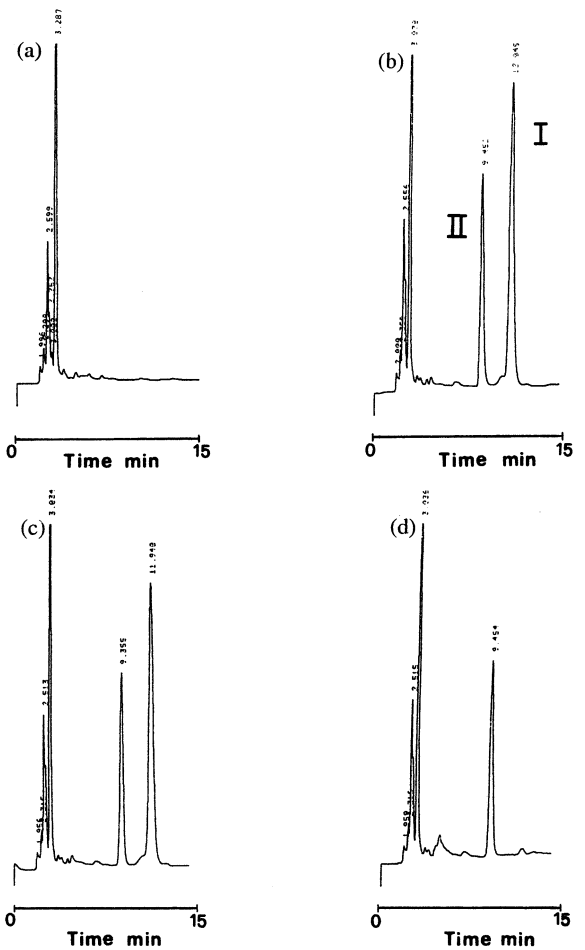

Fig. 1. Chromatograms of rat plasma. (a) Specimen from rat treated with lead nitrate; (b) specimen from rat treated with bezafibrate (I) and clofibric acid (II); (c) specimen from rat treated with lead nitrate and bezafibrate (36 h); (d) specimen from rat treated with lead nitrate and bezafibrate (72 h).

Table 1
Concentration of bezafibrate (Bz) in plasma of rats treated with bezafibrate and with bezafibrate plus lead nitrate (36 and 72 h)[a]

No. analyses	Bz (μg/ml)	Bz+lead (μg/ml) (36 h)	Bz+lead (μg/ml) (72 h)
3	3.38 ± 0.016	3.35 ± 0.020	n.d.[b]

[a] The values are expressed as the mean ± S.E.
[b] n.d. = not detectable.

The chromatography was carried out at a flow rate of 0.9 ml/min at room temperature. Bezafibrate was detected spectrophotometrically at 230 nm.

3. Results and discussion

The calibration curve (correlation coefficient $r = 0.9996$) was obtained by using appropriate amount of bezafibrate and clofibric acid (I.S.) stock solution.

Fig. 1 shows chromatograms of plasma obtained from rats treated with bezafibrate and with bezafibrate plus lead nitrate (36 and 72 h).

Reported results (Table 1) indicated that the peak corresponding to bezafibrate in the chromatogram was strongly reduced in serum of rats treated with bezafibrate plus lead (72 h) compared with that observed in serum of rats treated with bezafibrate alone or with bezafibrate plus lead (36 h).

These results indicated that lead treatment accelerated the metabolism of bezafibrate in rats. Although the interpretation of these data was not easy, we suggested that the metabolism of bezafibrate may be accelerated by the proliferative process induced by lead. This may be related to an increased amount of drug needed to normalise the alterations of cholesterol metabolism due to the liver hyperplasia induced by lead nitrate [1].

However, a possible role of lead in drug metabolising enzyme system (DMES) inhibition could not be excluded.

Acknowledgements

This work was supported by grants from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST) and Consiglio Nazionale delle Ricerche (CNR), Rome.

References

[1] S. Dessì, B. Batetta, A. Carrucciu, D. Pulisci, S. Laconi, A.M. Fadda, C. Anchisi, P. Pani, Variations of serum lipoproteins during cell proliferation induced by lead nitrate, Exp. Mol. Pathol. 51 (1989) 97–102.

[2] C. Anchisi, S. Dessì, C. Chiodino, B. Batetta, A.M. Fadda, P. Pani, Hepathic glucose-6-phosphate dehydrogenase, cholesterogenesis and serum lipoproteins in liver regeneration after partial hepactetomy, Exp. Mol. Pathol. 44 (1986) 169–176.
[3] S. Dessì, B. Batetta, C. Anchisi, P. Pani, G. Broccia, L. Tessitore, P. Costelli, F.M. Baccino, Cholesterol metabolism in normal and neoplastic cell proliferation, Arch. Gerontal. Geriatr. Suppl. 2 (1991) 563–568.
[4] S. Dessì, B. Batetta, D. Pulisci, O. Spano, R. Cherchi, G. Lanfranco, L. Tessitore, P. Costelli, F.M. Baccino, C. Anchisi, P. Pani, Altered pattern of lipid metabolism in patients with lung cancer, Oncology 49 (1992) 436–441.
[5] S. Dessì, B. Batetta, D. Pulisci, O. Spano, C. Anchisi, L. Tessitore, P. Costelli, F.M. Baccino, E. Aroasio, P. Pani, Cholesterol content in tumor tissues is inversely associated with HDL cholesterol in serum in patients with gastrointestinal cancer, Cancer 73 (1994) 253–258.
[6] C. Anchisi, B. Batetta, F. Sanna, A.M. Fadda, A.M. Maccioni, S. Dessì, HDL subfractions as altered in cancer patients, Pharm. Biom. Anal. 13 (1995) 65–71.
[7] D. Castoldi, V. Monzani, O. Tofanetti, Determination of bezafibrate in human plasma and urine by high-performance liquid chromatography, J. Chromatogr. 344 (1985) 259–265.