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Investigation of the Effects of Concomitant Caffeine Administration on the Metabolic Disposition of Pyrazinamide in Rats

Aida Mehmedagic[a], Philippe Verite[b], Sabine Menager[b], Christine Tharasse[b], Christiane Chabenat[b], Dominique Andre[b] and Olivier Lafont[b,*]

[a] *Department of Pharmacokinetics, University of Sarajevo-Faculty of Pharmacy, 71000 Sarajevo, Bosnia and Herzegovina*

[b] *Laboratorie de Pharmacochimie, Faculte de Medecine et de Pharmacie de Rouen, 22, boulevard Gambetta, 76183 Rouen cedex, France*

ABSTRACT: The utility of pyrazinamide (PZA) in the short-course antituberculous treatment is well established. All available data support the idea that the PZA metabolite pyrazinoic acid (PA) is the active compound against *M. tuberculosis*. This situation warranted a deeper investigation of possible interactions with respect to its metabolic disposition. Caffeine, which is widely used as a drug and is a common constituent of most diets, shares with PZA the same metabolic enzyme, xanthine oxidase (XO). This study investigated if, and in what manner, concomitant administration of caffeine affects PZA metabolism. PZA and caffeine, in various doses (PZA = 50 or 100 mg kg^{-1} and caffeine = 0, 50, 100, and 150 mg kg^{-1}), were administered to female Sprague-Dawley rats. PZA and its three main metabolites were quantified in 24 h urine samples by reversed phase-HPLC Concomitant administration of 100 mg kg^{-1} caffeine and 50 mg kg^{-1} PZA increased from the excretion ($p < 0.05$) of the most water-soluble and the least toxic PZA metabolite 5-hydroxypyrazinoic acid (5-OH-PA) from 66.18 $\pm$ 10.87 to 94.56 $\pm$ 8.65 μmol/24 h. This effect was more pronounced when 100 mg kg^{-1} of PZA was administered increasing excretion of 5-OH-PA from 113.28 $\pm$ 70 to 173.23 $\pm$ 17.82 μmol/24 h. These results show that the metabolic disposition of PZA is affected by concomitant caffeine intake. Copyright © 2002 John Wiley & Sons, Ltd.

Key words: pyrazinamide; caffeine; HPLC; metabolic interactions

Introduction

The undoubted efficacy of pyrazinamide (PZA), led to its reintroduction in the short-course antituberculous therapeutic schedule [1]. Recommended diminution of 50–70 mg kg^{-1} daily doses to 25–30 mg kg^{-1}, reduced the incidence of side effects [2]. However this did not mean a complete eradication of the most serious side effect, hepatotoxicity, which seems to be dose related. Furthermore the hepatotoxicity of the two main drugs in the same therapeutic protocol, isoniazid and rifampicin remained unchanged [3,4].

Metabolic pathways of PZA have been well investigated [5–7] (Figure 1). The main steps of PZA metabolism, which take place in liver, involve enzymatic deamidation, followed by formation of the main active metabolite, pyrazinoic acid (PA) [8]. This acid is further oxidized by the action of xanthine oxidase (XO) to 5-hydroxypyrazinoic acid (5-OH-PA), the main excretory metabolite of PZA. Direct oxidation of PZA by XO leads to 5-hydroxypyrazinamide (5-OH-PZA). The three above-mentioned metabolites are mainly excreted in urine, after PZA administration, together with small quantities of

*Correspondence to: Laboratoire de Pharmacochimie, Faculte de Medicine et de Pharmacie de Rouen, 22, boulevard Gambetta, 76183 Rouen Cedex, France.

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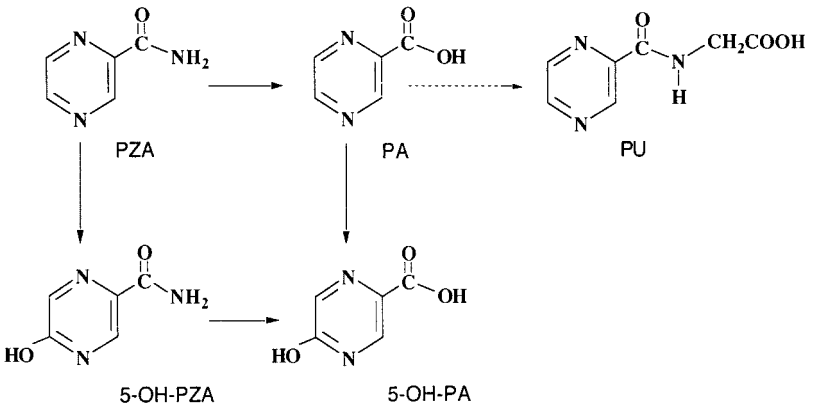

Figure 1. Metabolic pathways of pyrazinamide (PZA)

pyrazinuric acid (PU) produced by conjugation of PA with glycine.

Quantitative aspects of PZA metabolism have been investigated in humans and rats [9–11]. However, possible effects of other drugs or of food constituents on this metabolism have not been reported, although PZA pharmacokinetics has been explored in reference to concomitant antituberculous therapy [12,13], and concomitant food intake [14]. Drugs affecting XO activity such as allopurinol, significantly affected PZA metabolism [15,16].

The metabolism of caffeine, a compound which is present in most diets, is well known to reflect the *in vivo* XO activity [17], caffeine, which is extensively metabolized [18,19], can be employed in estimation of the *N*-acetyltransferase. Cytochrome P 450 (CYP 1A2), and XO activities in healthy subjects [20–22] as well as in some pathological conditions [23,24]. Despite the widespread presence of caffeine in the diet of many individuals few studies investigating the influence of this compound on drug disposition have been published. Some reports indicate the induction of acetanilide hydroxylation [25] and inhibition of *N*-demethylation [26] with caffeine in rats. More recent studies revealed the inductive effect of caffeine on CYP 1A2 in rats [27] which was the first evidence of the influence of caffeine on its own metabolizing enzyme, apart from a report of *in vitro* and *in vivo* inhibition of XO [28] by caffeine and other xanthines. The results concerning the effect of 1-methylxanthine (1-MX, a metabolite of caffeine) on XO activity showed the induction of XO activity under low concentrations of 1-MX, and above these values, the inhibition of the activity [29]. The possible effects of caffeine on XO activity can influence the metabolism of xenobiotics also metabolized by this enzyme. In the case of PZA this should be particularly important since more than 50% PZA metabolism is mediated by XO.

In view of the data presented above, the aim of the present work was to explore quantitative ratios of the main PZA metabolites (PA, 5-OH-PA and 5-OH-PZA) in 24 h rats urine samples after the administration of different doses of PZA, either with or without concomitant caffeine intake.

Materials and Methods

Two groups of nine Sprague-Dawley female rats were included in a trial. Each group received a single dose of PZA (50 mg kg^{-1}: experiment A or 100 mg kg^{-1}: experiment B). The influence of concomitantly administered caffeine was investigated on both PZA dosage levels. In this purpose, increasing amounts of caffeine were given to rats: 0, 50, 100, and 150 mg kg^{-1}, respectively, for experiments A1, A2, A3, and A4 or 0, 50 and 100 mg kg^{-1}, respectively, for experiments B1, B2, and B3. Caffeine was administered at 9 a.m. and PZA 30 min later. Water solutions (10 mg ml^{-1}) of both compounds were administered, *p.o.* using a curved intragastric cannula 60/10 (Carrier®). The rats were placed in metabolic cages (Pajon®). In order to

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avoid discrepancies caused by intra-species variability, the same group of rats was used for all A-type experiments and another group for all B-type experiments. Successive experiments were separated by a period of two weeks to avoid possible effects of enzyme induction. For each rat, urine samples were collected for 24 h after PZA administration. PZA and its three main metabolites were quantified in each sample of urine, using the HPLC method previously described [30]. PZA and PA standards were purchased from Aldrich (Belgium). 5-OH-PA and 5-OH-PZA were prepared *in vitro* by buttermilk XO (from Aldrich, Belgium) action, respectively, on PA and PZA and were purified by preparative TLC. The structures of 5-OH-PA and 5-OH-PZA standards were verified by GC-MS analysis of their sylilated derivatives. The same procedure was applied to the compounds present in rats urine after administration of PZA. Quantification of PZA and its metabolites by HPLC was performed using appropriately diluted and ultrafiltered urine samples. The mobile phase (0.01 mol l^{-1} KH_2PO_4 in water, adjusted to pH 5.2 by K_2HPO_4), was pumped at a flow-rate 0.9 ml min^{-1} through C18 silica column, protected with a guard cartridge of similar characteristics, at 22°C. The method was successfully validated: low detection limits were verified for PZA, PA, 5-OH-PA, and 5-OH-PZA at 0.300, 0.125, 0.070 and 0.092 μg ml^{-1}, respectively, and low quantification limits at 0.998, 0.416, 0.233 and 0.306 μg ml^{-1}, respectively.

In order to avoid discrepancies caused by intra-species variability, the results are given for each group of rats and for each experiment. The amounts of compounds are expressed in μmol/24 h, to obtain clear proportions of metabolites in urine in respect to the differences in molecular mass.

For statistical evaluation the paired Student's *t*-test was employed. A p value <0.05 was accepted as significant. When the death of an animal occurred during the experiment, the results concerning the dead rat were eliminated from the statistical analysis but have been retained for calculation of data in Table 1.

Results and Discussion

PZA administration significantly ($p<0.01$) increased urine volumes from 5.9 ± 1.5 ml (pH = 9.7 ± 0.6), in the control group ($n=9$), to 16.5 ± 5.7 ml (pH = 8.2 ± 0.7) in experiment A1 ($n=7$), and to 16.9 ± 14.41 ml (pH = 7.2 ± 0.7) in experiment B1 ($n=8$). Slight decrease of pH could be explained by the presence of acidic PZA metabolites. Concomitant caffeine administration did not result in any further significant increases in urine volume.

Table 1. Drugs administered and average amounts of PZA main metabolites, recovered in rat urine

Administered		A1 $n=7$	A2 $n=9$	A3 $n=8$	A4 $n=8$	B1 $n=8$	B2 $n=9$	B3 $n=9$
PZA	mg kg^{-1}	50	50	50	50	100	100	100
	μmol kg^{-1}	162.6	162.6	162.6	162.6	325.2	325.2	325.2
Caffeine	mg kg^{-1}	0	50	100	150	0	50	100
	μmol kg^{-1}	0	260	520	780	0	260	520
Recovered		A1	A2	A3	A4	B1	B2	B3
5-OH-PA	Avg	66.18	89.19	94.56	86.77	113.28	144.47	173.23
(μmol/24 h)	st dev	10.87	11.18	16.05	8.65	8.70	21.03	17.82
PA	Avg	23.19	16.67	18.47	17.41	45.82	34.40	33.39
(μmol/24 h)	St dev	3.78	1.26	4.16	3.30	4.51	3.83	7.83
5-OH-PZA	Avg	41.63	36.26	37.63	40.41	75.92	74.87	82.46
(μmol/24 h)	st dev	6.60	4.26	13.06	11.16	16.19	14.85	13.05
TOTAL	Avg	130.99	142.11	150.66	142.70	235.05	255.60	289.08
(μmol/24 h)	St dev	19.04	12.05	15.20	14.07	20.44	27.40	13.10

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When 50 mg kg^{-1} PZA was administered with caffeine (Table 1), the total amount of metabolites excreted did not change significantly ($p > 0.05$), with any caffeine dose. When 100 mg kg^{-1} PZA was administered with caffeine a significant increase ($p < 0.01$), in total metabolites excreted was observed after 50 and 100 mg kg^{-1} dose of caffeine ($p < 0.05$).

At both PZA doses (50 mg kg^{-1}–A_1, and 100 mg kg^{-1}–B_1), caffeine administration (50 mg kg^{-1}–A_2, B_2) significantly increased the quantities of 5-OH-PA recovered in rats urine ($p = 0.036$ and 0.014, respectively), and decreased those of PA ($p = 0.004$ and 0.001, respectively). With the dose of 50 mg kg^{-1} PZA increasing amounts of caffeine (100 mg kg^{-1}–A_3, and 150 mg kg^{-1}–A_4) did not produce any additional increase in excreted 5-OH-PA, or decrease in PA. On the contrary, at 100 mg kg^{-1} PZA, increasing doses of caffeine (50 mg kg^{-1}–B_2 and 100 mg kg^{-1}–B_3) additionally promoted 5-OH-PA excretion ($p = 0.004$), which was not accompanied by any further significant ($p > 0.05$) diminution of PA excretion.

No influence on 5-OH-PZA excretion was observed, at either PZA dose, regardless of the dose of caffeine ($p > 0.05$). This observation suggests that PZA hydroxylation may be not mediated by the same enzymatic system as caffeine.

It can be concluded that metabolite excretion was similar and showed the same quantitative ratios for the three main metabolites recovered in 24 h urine, after the two different doses of PZA studied. However, concomitant administration of caffeine promoted excretion of the most water-soluble and the least toxic PZA metabolite, 5-OH-PA. This effect was more prominent when toxic amounts of PZA (100 mg kg^{-1}) were administered indicating that caffeine administration could facilitate elimination of PZA. The explanation given for the activity of PZA against *Mycobacterium tuberculosis* involves the convertion of PZA into PA *in situ*, while direct PA administration *per os* was less significant than that of PZA, because of poor absorption and high protein binding. PA, when generated *in situ*, from PZA accumulates sufficiently to lower pH to toxic levels for *Mycobacterium tuberculosis* [8,31–37].

Bearing in mind the importance of PA in the PZA mechanism of action, the effect of caffeine in promoting conversion of PA to 5-OH-PA may need to be considered when managing antituberculous therapy.

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