Interaction between allopurinol and pyrazinamide

C. Lacroix*, C. Guyonnaud*, M. Chaou*, H. Duwoos*, O. Lafont


ABSTRACT: Pyrazinamide (PZA) is increasingly used with isoniazid and rifampicin, in short-course antituberculous chemotherapy in service programmes conditions. Complicating arthralgias occur due to hyperuricaemia induced by the inhibition of renal tubular secretion of uric acid by pyrazinoic acid, the main PZA metabolite. Allopurinol (Al), a hypouricaemic agent, provides no substantial clinical improvement. Pharmacokinetics of PZA and its metabolites were studied in six healthy volunteers, in a cross-over design, after a single oral dose of PZA alone and, in a second trial, after the same dose together with Al. Plasma and urinary concentrations were measured by high pressure liquid chromatography with a column of cation exchange resin. Analysis of the pharmacokinetic parameters showed that Al induced marked changes in levels of PZA metabolites and accumulation of pyrazinoic acid. Despite decreasing uric acid synthesis, allopurinol increased plasma concentrations of pyrazinoic acid, which is directly responsible for the inhibition of renal urate secretion. Other drugs, which do not involve xanthine oxidase inhibition, should be used in the treatment of this side effect of chemotherapy. Eur Respir J, 1998, 1, 807-811.

The association of pyrazinamide (PZA) to isoniazid, rifampicin and, in many cases, to streptomycin during the first two months of a six-month chemotherapy regimen [1–5] yields significantly lower relapse rates [1, 3, 5] and more rapid sputum conversions [2]. These results are consistent with experimental data showing that PZA is a potent drug against bacilli in an acid environment, especially in macrophages [1, 6–8]. Therefore, PZA is recommended in short-course regimens in programme conditions [3, 9].

Apart from hepatic toxicity, PZA induces a consistent hyperuricaemia in most cases [4, 10]. The frequency of arthralgias after 15–30 day chemotherapies is variable [2, 10, 11] but far less important than the prevalence of hyperuricaemia itself [10]. The latter is ascribed to inhibition of tubular secretion of uric acid by the main PZA metabolite, i.e. 2-pyrazinonic acid (PA) [12–14].

In a clinical study, HORSFALL et al. [15] compared the evolution of arthralgias in two groups of patients receiving either aspirin or allopurinol (a uric acid synthesis inhibitor) and in a control group receiving placebo. The improvement of symptoms was less frequent in the allopurinol group than in the other groups. Moreover, the hypothesis of a slightly unfavourable effect of allopurinol (Al) was not excluded.

As described by WsNER and TINKER [13], the main stages of PZA metabolism involve a microsomal deamidase at first which induces PA formation, and secondly xanthine oxidase which induces 5-OH-pyrazinoic acid (5-OH-PA) formation. They found another metabolite not very different from 5-OH-PA. This finding was confirmed later by AUSC~ et al. [16]. The nature of this component, as defined in rat liver cells by PIRKE et al. [17] and, thereafter, in man by YAMAMOTO et al. [18] and BERETTA et al. [19], is 5-hydroxy-pyrazinamide (5-OH-PZA) which results from direct action of xanthine oxidase on PZA. However, another oxidizing enzyme seems to be involved in the process [20].

Hence, 5-OH-PA has a double origin: PA and 5-OH-PZA. Through a minor process, PA is combined with glycine to form pyrazinuric acid (PZU). These considerations enable us to propose the metabolic scheme as illustrated in figure 1.

The aims of the present study were to evaluate the pharmacokinetic alterations of PZA as a result of its association with allopurinol and to define the qualitative and quantitative effects of xanthine oxidase inhibition. For this purpose we had to evaluate PA, 5-OH-PA, 5-OH-PZA and PZU. Pharmacokinetics are well known as regards PA [13, 21–23], less well known as regards 5-OH-PA [13] and are yet to be defined for 5-OH-PZA and PZU.

Materials and methods

Six healthy male volunteers, aged 28–38 yrs, gave informed consent to participate in this trial according to the advice of the local Ethics Committee. Each subject

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underwent two successive pharmacokinetic studies, in a cross-over design, at least seven days apart: one trial with PZA alone (four tablets of 500 mg each, thus 28±4 mg·kg⁻¹, mean±so) and the other trial with the same dose of PZA associated with A1 (200 mg daily) taken over two days before the trial, on the day of PZA ingestion and on the following day. The tablets were taken at 7 am in fasting conditions.

Sampling

Venous blood sampling, in heparinized vacutainers, was performed 0, 1, 2, 4, 8, 12, 24 and 36 h after PZA ingestion. Urine fractional sampling was performed at 0-2, 2-4, 4-6, 6-8, 8-24, 12-24 and 24-36 h. Samples were frozen at -20°C.

Dosage of PZA and of metabolites

Plasma and urinary concentrations were measured by high pressure liquid chromatography: 50 µl of plasma were deproteinized by 50 µl perchloric acid 1.5 M. After centrifugation, 20 µl of supernatant were introduced into the chromatograph. Urinary samples were diluted 1/4 in distilled water before introduction. The column contained a cation exchange resin of the styrene-divinyl-benzene type, sulphonated under form H⁺ (Aminex HPX-87 H, 300×7.8 mm Biorad), preceded by a similar guard cartridge. Both units were maintained by thermostat at 62°C. The elution solvent was a sulphuric acid solution (0.005 M) with a flow of 0.8 ml·min⁻¹. Detection was performed by fluorimetry (excitation: 265 nm; emission: 410 nm).
PZA and PA are of commercial origin (Aldrich), 5-OH-PA was obligingly provided by WEINER and TINKER and 5-OH-PZA was obtained from the action of xanthine oxidase on PZA [18]. Pyrazinuric acid or N-pyrazinylglycine [24] was synthesized (O. Lafont) from PA and glycine by a mixed anhydride reaction with ethylchloroformate in the presence of triethylamine according to the method described by WEINER and TINKER [13].

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated assuming an open monocompartment model. The apparent elimination constant ($\beta$ ($h^{-1}$)) was the slope of the monoexponential segment of plasma concentration-time curve after adjustment according to the least squares method. Half-lives ($T_{0.5}$ = 0.693-0.8) were expressed in hours and calculated using concentrations from the 2nd–36th h for PZA and from the 8th–36th h for 5-OH-PZA, 5-OH-PA and PA.

Areas under the curve (AUC) were computed from 0–36 h by the trapezoidal method and extrapolated to infinity from the elimination constant:

$$\text{AUC}_{(0\rightarrow\infty)} = \frac{C_{36}}{\beta}$$

The total plasma clearance ($Cl_p$ ml·min$^{-1}$) was derived from the equation:

$$Cl_p = \frac{F \cdot \text{Dose}}{AUC_{(0\rightarrow\infty)}}$$

where $F$ is the absolute bioavailability.

The renal clearance ($Cl_r$ ml·min$^{-1}$) was determined from the ratio between the amount eliminated from 0–36 h in urine and AUC during the same time:

$$Cl_r = \frac{U_{(0\rightarrow36)}}{AUC_{(0\rightarrow36)}}$$

The apparent volume of distribution ($V_d$ kg$^{-1}$) is:

$$V_d = \frac{Cl_p}{\beta}$$

The bioavailability of PZA per os (F) is unknown:

therefore only $Cl_r$ and $V_d$ are reported.

Results

Pharmacokinetics of PZA alone

PZA per os was rapidly absorbed and plasma peak concentration was never delayed beyond 1 h (fig. 2). Half-life was 9.8±1.8 h (mean±sd). The low urinary elimination of unchanged PZA (3.5±1.2% over 36 h) with a renal clearance of 2.2±0.5 ml·min$^{-1}$ demonstrated a marked reabsorption.

PA, 5-OH-PA and 5-OH-PZA had similar plasma characteristics as regards $T_{0.5}$ and $Cl_{ur}$ (table 1). In urine, the quantities of estimated 5-OH-PA and 5-OH-PZA were also similar (13 and 14% of the ingested dose, respectively) with a marked active secretion of 5-OH-PA ($Cl_r=478±541$ ml·min$^{-1}$) but a far less obvious secretion of 5-OH-PZA ($Cl_r=190±37$ ml·min$^{-1}$).

PA was the main form of elimination (31% of

<table>
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<tr>
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<th>PZA</th>
<th>PA</th>
<th>5-OH-PA</th>
<th>5-OH-PZA</th>
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<tbody>
<tr>
<td>T$_{0.5}$h</td>
<td>9.8±1.8</td>
<td>10.2±1.6</td>
<td>12.9±2.5</td>
<td>0.05</td>
</tr>
<tr>
<td>C$_{max}$mg·l$^{-1}$</td>
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<td>39.8±6.7</td>
<td>5.8±1.9</td>
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<tr>
<td>T$_{max}$h</td>
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<td>5.3±2.1</td>
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<td>4.3±2.3</td>
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<tr>
<td>AUCmg·l$^{-1}$h</td>
<td>574±77</td>
<td>596±126</td>
<td>124±14</td>
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<tr>
<td>V/Flkg$^{-1}$</td>
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<td>0.69±0.06</td>
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</tr>
<tr>
<td>Cl/Fml·min$^{-1}$</td>
<td>59±18</td>
<td>58±12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cl$_r$ml·min$^{-1}$</td>
<td>2.2±0.6</td>
<td>2.3±0.7</td>
<td>10±16</td>
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**Table 1.** Pharmacokinetic parameters (in plasma and urine) of PZA and of its metabolites (mean±sd) measured during the two trials

PZA: pyrazinamide; PA: 2-pyrazinoic acid; 5-OH-PA: 5-hydroxy-pyrazinoic acid; 5-OH-PZA: 5-hydroxy-pyrazinamide; $F$: below plasma level of sensitivity of the analysis method; I: PZA; II: PZA with allopurinol; $T_{0.5}$: half-life; $C_{max}$: plasma peak concentration; $T_{max}$: time to plasma peak; AUC: area under curve; $V/F$ and $Cl/F$: ratio of apparent volume of distribution and total clearance, respectively, to absolute bioavailability (F); $Cl_r$: renal clearance; NS: not significant.
concentrations in plasma and urine exhibit a highly significant decreased elimination, over 36 percentages are distributed as follows: PZA=3.8%; olites over 36 h (table 1) represents nearly 62% of the found by Ellardcreted and its renal clearance are (respectively).

The pyrazinoic acid AUC was clearly increased (73%) and T0.5 was slightly, but not significantly, augmented. Urinary elimination was increased from 633±68 mg over 36 h (control data) to 860±109 mg, but renal clearance was strictly constant.

The 5-OH-PA plasma concentrations were drastically decreased under the detection limit of our analytical method (i.e. 0.07 mg·L⁻¹). Therefore, the relevant elimination parameters could not be computed but AUC was consistently decreased and the quantity eliminated over 36 h decreased from 293 to 36 mg.

The AUC of 5-OH-PZA was decreased by 34% without any significant alteration of T0.5. The cumulated urinary excretion was decreased but renal clearance was unaltered. PZU could not be quantified in plasma but its elimination remained minute (0.33±0.14%).

Pharmacokinetics of PZA with allopurinol

PZA kinetics were unaltered by associated AI as regards either plasma parameters (\(T_{0.5}, CL, C_{max}, V_{sys}, AUC\)) or urinary data (\(\frac{U_{0-36h}}{Cl}\)). In contrast, parameters relevant to the metabolites were altered.

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Discussion

The PZA half-life (9.8±1.8 h) is consistent with the data of Ellard [21] in a healthy man (9 h with a dose of 1.5 g and 9.8 h with 3 g), but clearly higher than the mean value (6.1 h) in African tuberculous patients reported in the same study. In a study concerning Chinese patients, Ellard et al. [25] found a half-life of nearly 9 h with doses of 1.5 or 2.25 g. The estimates for percentage of unchanged PZA eliminated in the urine and for its renal clearance are very similar to those originally found by Ellard [21] (3.3–4.4%, and 1.7 ml·min⁻¹, respectively).

As regards PA, the estimates of percentage dose excreted and its renal clearance are also similar to the data found by Ellard et al. (30% and 111 ml·min⁻¹, respectively).

The urinary cumulative excretion of PZA and of metabolites over 36 h (table 1) represents nearly 62% of the ingested dose. After extrapolation to infinity, the percentages are distributed as follows: PZA=3.8%; PA=36%; 5-OH-PA=13.8%; 5-OH-PZA=15.4%. The total amount of 70% suggests a rather good bioavailability.

The study of Horsfall et al. [15] has clearly shown the ineffectiveness of AI on arthralgias and hyperuricaemia in patients with PZA. In the present study, uric acid concentrations in plasma and urine exhibit a highly significant decreased elimination, over 36 h, along with a slight decrease in renal clearance (with PZA alone: elimination=4.67 mmol, \(CL_c=6.7\) ml·min⁻¹; with PZA and AI: elimination=2.86 mmol, \(CL_c=5.0\) ml·min⁻¹). However, variations of uric acid AUC are similar (1.27 mmol·l⁻¹·h⁻¹ on PZA alone and 1.13 mmol·l⁻¹·h⁻¹ with PZA and AI). This suggests that the slight decrease in urate renal clearance, due to PA accumulation induced by AI, is sufficient to offset the hyperuricaemic effect of this drug. Therefore, any chronic administration of allopurinol will be ineffective.

Comprehensively, xanthine oxidase inhibition induces PA accumulation along with a slight increase of synthesized PZU, a drastic decrease of 5-OH-PZA due to inhibition of both its paths of synthesis and a diminution of 5-OH-PA formation. It is worth noting that the 5-OH-PZA synthesis persists but its AUC is decreased. This fact is consistent with the study of Yamamoto et al. [20] which suggests the existence of another oxidizing enzyme involved in 5-OH-PZA formation. Moreover, Auscher et al. [16] found this metabolite in xanthuric patients who were thought, therefore, to be deprived of xanthine oxidase.

Hence, as a result of allopurinol action, 5-OH-PA seems to be derived from the sequence: PZA → 5-OH-PZA → 5-OH-PA.

In contrast to expectations, on account of the known site of action of allopurinol, pharmacokinetics of PZA itself are unaltered since hydrolysis of its amide function is not involved and its oxidation to 5-OH-PZA remains possible.

The renal clearances are unaltered and it is the hepatic metabolism which is involved instead of a variation of renal function.

In conclusion, it is unlikely that increasing the doses of allopurinol, as suggested by Horsfall et al., would succeed in reducing hyperuricaemia induced by PZA, since this would result in an increased accumulation of PA. The treatment of complicating arthralgias should include either uricosuric drugs (e.g. benzofurane group) or perhaps synthesis-inhibiting substances which do not involve xanthine oxidase (such as thiopurinol).

References

5. Hong-Kong Chest Service/British Medical Research Council. – Five-year follow-up of a controlled trial of five six-months regimens of chemotherapy for pulmonary tuberculosis. Am Rev
**RÉSUMÉ**: La pyrazinamide (PZA) est utilisée de plus en plus souvent en association avec l’isoniazide et la rifampicine, ainsi qu’il est recommandé, dans les chimiothérapies courtes antituberculeuses, à titre systématique. Les arthralgies qui compliquent parfois ces traitements résultent de l’hyperuricémie induite par l’inhibition de la sécrétion tubulaire d’urate par l’acide pyrazinoïque, principal métabolite de la PZA. L’allopurinol (AU), agent hypouriciématant, n’apporte pas d’effet clinique appreciable. La pharmacocinétique de la PZA et de ses métabolites a été étudiée, en cross-over, chez six volontaires sains, après une dose orale unique de PZA seule puis, dans un second essai, après la même dose associée à l’allopurinol. Les concentrations plasmatiques et urinaires ont été mesurées par chromatographie liquide à haute pression sur une colonne de résine échangeuse de cations. L’analyse des différents paramètres pharmacocinétiques montre que l’AU induit d’importantes modifications de concentrations des métabolites de la PZA et notamment une accumulation d’acide pyrazinoïque. L’allopurinol, bien qu’il réduise la synthèse d’acide urique, accroît les concentrations plasmatiques d’acide pyrazinoïque qui est directement responsable de l’inhibition de la sécrétion rénale d’urate. D’autres médicaments, qui ne mettent pas en jeu l’inhibition de la xanthine oxydase, doivent être utilisés pour traiter cet effet secondaire de la chimiothérapie.