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SHORT COMMUNICATION

Xanthine oxidase inhibition by allopurinol increases *in vitro* pyrazinamide-induced hepatotoxicity in HepG2 cells

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Abstract

Despite the important role of pyrazinamide in tuberculosis treatment, little is known about the mechanism of pyrazinamide-induced hepatotoxicity. We inhibited xanthine oxidase in HepG2 cells by using a nontoxic concentration of allopurinol, a well-known xanthine-oxidase inhibitor. This increased *in vitro* pyrazinamide toxicity in HepG2 cells, which suggests that the hydroxy metabolites of pyrazinamide are probably not fully responsible for pyrazinamide-induced toxicity, and that pyrazinoic acid and pyrazinamide are involved in pyrazinamide toxicity.

Keywords: Tuberculosis; adverse effects; liver toxicity; antituberculosis treatment

Introduction

Despite the fact that the current standard tuberculosis (TB) treatment, which is given to more than 8 million people each year, is a product of the best scientific advance of the 1960s, little is known about the mechanism of hepatotoxicity, one of the most serious adverse effects of TB treatment. Hepatotoxicity can be fatal when not recognized in time. Between 2 and 28% of TB patients develop hepatotoxicity during TB treatment. Adverse effects have a negative impact on therapy adherence and may, therefore, have serious consequences for treatment success rates, relapse, and the emergence of drug resistance (Tostmann et al., 2008).

Recent studies suggest that pyrazinamide causes more hepatotoxicity than previously perceived (Chang et al., 2008; Yee et al., 2003). Further, the high rates of hepatotoxicity observed in pyrazinamide-containing prophylactic regimens for treatment of

latent TB have reassessed the hepatotoxic potential of this drug (Jasmer and Daley, 2003; Younossian et al., 2005). Despite the important role of pyrazinamide in TB treatment, little is known about the mechanism of pyrazinamide-induced hepatotoxicity. Reactive metabolites, rather than the parent drug itself, are suggested to be responsible for most idiosyncratic drug reactions (Knowles et al., 2000). Pyrazinamide may exhibit both dose-dependent and idiosyncratic hepatotoxicity (Chang et al., 2008).

Pyrazinamide (pyrazinoic acid amide) is converted to pyrazinoic acid by hepatic microsomal deamidase and is further hydroxylated to 5-hydroxy-pyrazinoic acid by xanthine oxidase (see Figure 1). A parallel metabolic pathway is the direct oxidation of pyrazinamide to 5-hydroxy-pyrazinamide by xanthine oxidase, which can be converted to 5-hydroxy-pyrazinoic acid by pyrazinamide deamidase. The metabolites are excreted in the urine, as is about 4% of the unchanged

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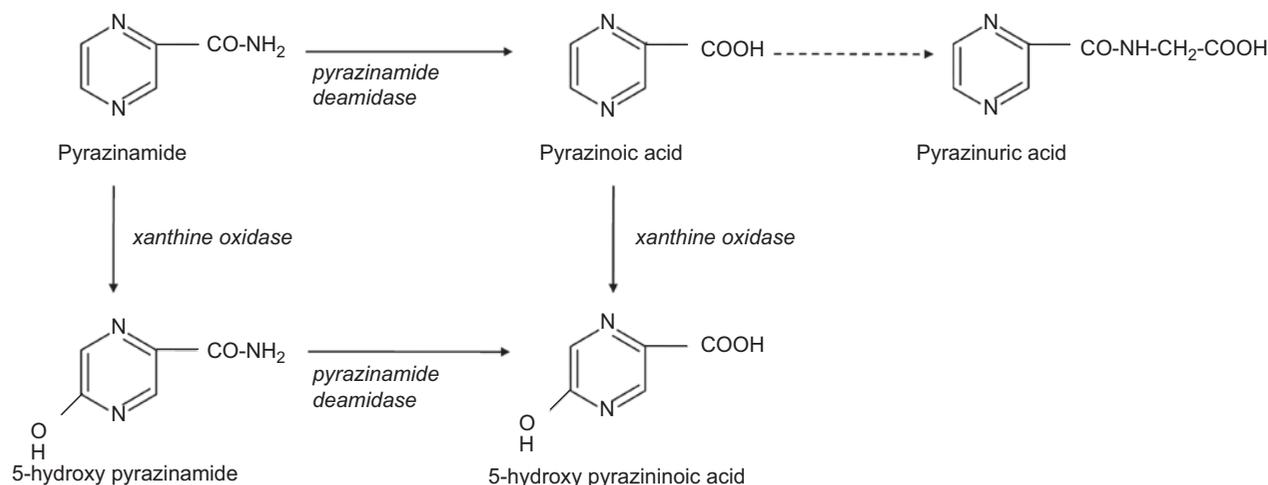


Figure 1. Metabolism of pyrazinamide (Liacroix et al., 1989).

pyrazinamide (Lacroix et al., 1989; Weiner and Tinker, 1972).

Allopurinol is a well-known xanthine-oxidase inhibitor. A study showing the interaction between allopurinol and pyrazinamide revealed that allopurinol increased the plasma concentrations of pyrazinoic acid and decreased those of 5-hydroxy-pyrazinoic acid and 5-hydroxy-pyrazinamide (Lacroix et al., 1989).

More knowledge on the mechanism of pyrazinamide-induced hepatotoxicity could be helpful in the search for hepatoprotective compounds. Whether pyrazinamide-induced hepatotoxicity is caused by pyrazinamide itself, pyrazinoic acid, or hydroxy metabolites could be studied by the selective inhibition of the metabolic pathways. We investigated the effect of xanthine-oxidase inhibition by allopurinol on *in vitro* pyrazinamide toxicity.

Methods

Cell culture

Human HepG2 hepatocellular carcinoma cells were grown in serum-free PC-1 medium (Cambrex, Verviers, Belgium), supplemented with 2 mM of L-glutamine at 37°C in a humidified atmosphere with 5% CO₂. The medium was renewed every 3 days, and when confluence was reached, cells were harvested with EDTA/trypsin (Cambrex), washed with phosphate-buffered saline (PBS), and used for cytotoxicity assays.

Effect of xanthine-oxidase inhibition on *in vitro* pyrazinamide toxicity

The effect of allopurinol (Sigma-Aldrich, Zwijndrecht, The Netherlands) on *in vitro* pyrazinamide toxicity was

determined by the WST-1 cytotoxicity assay (Roche Diagnostics GmbH, Penzberg, Germany). HepG2 cells were seeded in flat-bottomed 96-well microtiterplates (Costar, Corning Inc., Corning, New York, USA) at 60,000 cells/well and cultured for 24 hours. On day 2, the cells were exposed to a nontoxic concentration of 0.1 mM of allopurinol (for xanthine-oxidase inhibition) (Lee et al., 2000) or culture medium only (control cells) for 24 hours. On day 3, the cells were exposed to six increasing concentrations of pyrazinamide for 24 hours. At day 4, drug toxicity was assessed by the WST-1 assay, according to the supplier's instructions. This assay is based on the reduction of the tetrazolium salt, WST-1, into a colored, water-soluble formazan salt by viable cells only. The experiment was performed in triplicate and was repeated five times.

Statistical analyses

We calculated the area under the curve (AUC) of pyrazinamide-dependent cell survival to determine the effect of xanthine-oxidase inhibition on pyrazinamide toxicity. Changes in the AUC were compared by using the Wilcoxon signed ranks test. Statistical analyses were done by using SPSS for Windows (version 16.0; SPSS, Inc., Chicago, Illinois, USA). A *P*-value <0.05 was considered statistically significant.

Results

Pretreatment with 0.1 mM of allopurinol significantly decreased the area under the pyrazinamide concentration–cell survival by 13% (95% confidence interval; 2–24%; *P*=0.04). This means that xanthine-oxidase inhibition increased the *in vitro* pyrazinamide toxicity. Figure 2 shows the HepG2 cell survival at

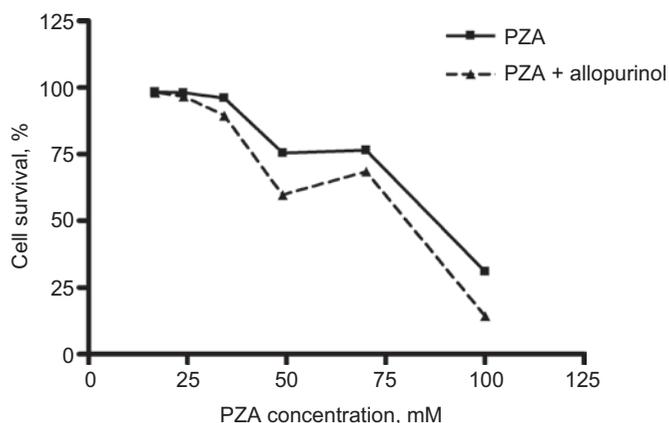


Figure 2. Effect of allopurinol on pyrazinamide toxicity in HepG2 cells. This figure shows the mean cell survival (\pm standard error of the mean) of HepG2 cells at different pyrazinamide concentrations with and without pretreatment with a nontoxic concentration of allopurinol (0.1 mM), obtained with the WST-1 cytotoxicity assay. PZA, pyrazinamide.

different pyrazinamide concentrations with and without allopurinol pretreatment.

Discussion

A study in human volunteers showed that xanthine-oxidase inhibition by allopurinol resulted in increased levels of pyrazinoic acid (mean AUC increased by 73%) and decreased levels of 5-hydroxy-pyrazinamide (mean AUC decreased by 34%) and 5-hydroxy-pyrazinoic acid (below detection level) (Lacroix et al., 1988). Considering these shifts in metabolic routes upon allopurinol administration, our finding that xanthine-oxidase inhibition increased the *in vitro* pyrazinamide toxicity suggests two things. First, it is unlikely that the hydroxy metabolites are solely responsible for pyrazinamide-induced hepatotoxicity, because if they were, *in vitro* pyrazinamide toxicity should have decreased by xanthine-oxidase inhibition and not increased. Second, pyrazinoic acid and, possibly, also pyrazinamide are at least partially responsible for pyrazinamide toxicity, since pyrazinoic-acid concentrations are probably increased by allopurinol pretreatment.

Arthralgia is another common adverse effect of pyrazinamide. This arthralgia is caused by the inhibition of the renal tubular secretion of uric acid by pyrazinoic acid that results in hyperuricemia (Lacroix et al., 1988). The study of Lacroix et al. showed that allopurinol causes a slight decrease in urate renal clearance due to pyrazinoic-acid accumulation, which is counterbalancing the hypouricemic effect of allopurinol. Therefore, allopurinol is ineffective for treating pyrazinamide-induced arthralgia (Horsfall et al., 1979; Lacroix et al., 1988). The finding that allopurinol may increase the risk on hepatotoxicity

is another reason not to use allopurinol as treatment of pyrazinamide-induced arthralgia.

Results of *in vitro* studies may not be directly translated into *in vivo* toxicity. However, allopurinol inhibits xanthine oxidase in HepG2 cells, and it is likely that *in vitro* xanthine-oxidase inhibition causes similar shifts in *in vitro* pyrazinamide metabolism, as seen in humans (Lee et al., 2000; Roy et al., 1995).

Conclusions

In conclusion, we observed that inhibition of xanthine oxidase by a nontoxic concentration of allopurinol increases *in vitro* pyrazinamide toxicity in HepG2 cells. This suggests that the hydroxy metabolites of pyrazinamide are probably not fully responsible for pyrazinamide-induced toxicity, and that pyrazinoic acid and pyrazinamide are involved in pyrazinamide toxicity.

Declaration of interest

This research was financially supported by the KNCV Tuberculosis Foundation. The authors declare no conflicts of interest.

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