IS THE USE OF 1-AMINOBENZOTRIAZOLE AS P450 INACTIVATOR IN RAT STUDIES APPROPRIATE?

Marc-Olivier Boily[1,2], Nathalie Chauret[2], Julie Laterreur[2], François A. Leblond[1], Chantal Boudreau[2], Marie-Claude Duquet[2], Jean-François Lévesque[2], Line Ste-Marie[2], Vincent Pichette[1]

1 : Service de Néphrologie and Centre de Recherche, Hôpital Maisonneuve-Rosemont, Faculté de Médicine, Université de Montréal, Québec, Canada

2 : DMPK, Vertex Pharmaceuticals Canada Incorporated, Laval, Quebec, Canada

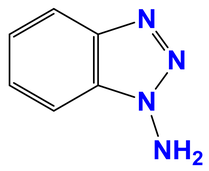
ABSTRACT

1-Aminobenzotriazole (ABT) is a well-known irreversible and non-specific cytochrome P450 inhibitor. Its pharmacokinetics properties, along with its good safety profile in rats, made it a perfect *in vivo* tool to understand the role of metabolism in the bioavailability or the toxicity of xenobiotics. Moreover, it was proposed that pre-treatments of rats with ABT administered orally versus intravenously could allow discrimination between intestinal and hepatic first pass metabolism[1]. However, recent findings challenged the ABT rat model to address P450 metabolism effect by suggesting that ABT may affect gastric emptying and consequently the absorption process[2]. Since no standard protocol is reported in the literature regarding the administration of ABT in drug-drug interaction (DDI) studies in rats, there is a need to understand the impact of varying the route of administration and the moment of administration of ABT on its effect on the metabolism and absorption of xenobiotics. To assess this question, we performed pharmacokinetic studies in untreated rats or rats pretreated with ABT given either orally 16 hours or intravenously (i.v.) 1 hour before oral dosing of metoprolol, a known P450 substrate. To evaluate the impact of ABT on hepatic and intestinal metabolism, we studied the metabolism of metoprolol in gut and liver microsomes obtained from untreated and ABT-treated rats and showed that ABT given orally or i.v inhibits similarly metoprolol intestinal and hepatic metabolism by 5-fold and 3-fold respectively. Finally, transit time studies under different ABT pre-treatments in rats were conducted to distinguish between an effect on drug absorption and metabolism. Our results indicate that ABT affects gastric emptying as we observed a 7-fold increase ($p<0.001$) in stomach weights when the rats were pretreated with ABT for a short period of time. This effect probably contributes significantly to the 5.5 hours delay in absorption of metoprolol and its metabolites in rats pretreated with ABT i.v. 1 hour before metoprolol dosing. Such delay in absorption was not seen in rats pretreated with ABT 16 hours prior to metoprolol administration or in untreated rats. In conclusion, these findings indicate that, depending on the ABT pre-treatment conditions, there could be a misinterpretation of the importance of P450 metabolism effect on the pharmacokinetic profiles of a drug in rat ABT DDI studies. Based on our results, we recommend pretreating rats with ABT 16 hours before the administration of a test compound to preserve the inhibitory effect on metabolism and avoid the effect on the gastric emptying and drug absorption. Finally, our results refute the assertion that administered in different routes, ABT could differentiate between intestinal and hepatic metabolism of drugs.

INTRODUCTION

- 1-Aminobenzotriazole (ABT) is a well-known irreversible and non-specific CYP450 inhibitor.
- It is used *in vivo* to understand the impact of phase I metabolism on pharmacokinetics and toxicity.

Figure 1. Structure of ABT

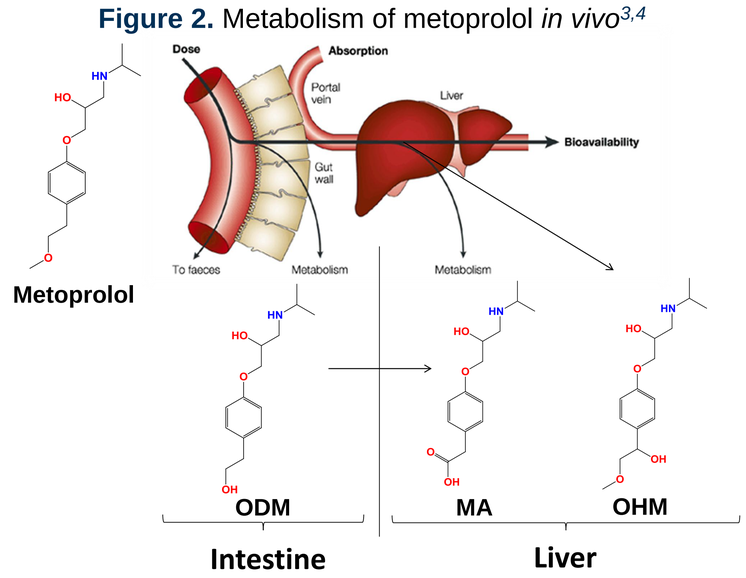


- It has been reported that PO and IV pre-treatments with ABT may distinguish between intestinal and hepatic metabolism.[1]
- Recent findings suggest that ABT may affect gastric emptying.[2]
- There are no consistent ABT pre-treatment protocols reported in the literature.

STUDY DESIGN

Objective : Characterize the effect of ABT on intestinal and hepatic metabolism and evaluate its effect on absorption in rats using metoprolol as a CYP substrate.

Figure 2. Metabolism of metoprolol *in vivo*[3,4]



Pretreatments evaluated :

-No pretreatment (Control)

-ABT 50mg/kg intravenous 1h prior to metoprolol dosing (ABT i.v.)

-ABT 50mg/kg oral 16h prior to metoprolol dosing (ABT p.o.)

-ABT 50mg/kg oral 1h prior to metoprolol dosing

Experiment performed :

-Pharmacokinetics of metoprolol upon oral administration 10mg/kg (n=5)

-Preparation of intestinal and hepatic microsomes from ABT-treated rats to evaluate impact on metabolism (n=3)

-Measure of gastric emptying and intestinal transit time. (n=4)

Methods details :

-Plasma and in vitro incubation analysis were performed by LC-MS-MS

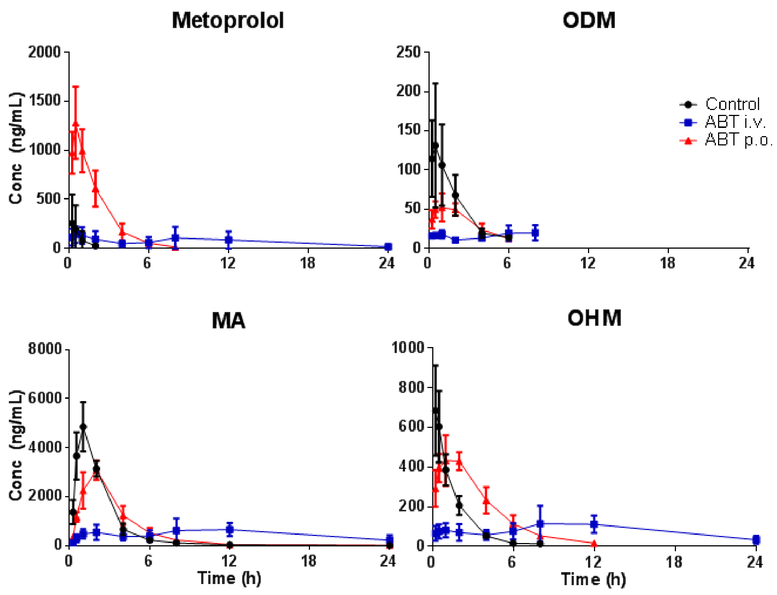
-Clearance measurement in microsomes were determined with the Km and Vmax method.

-Gastric and intestinal transit were determined after oral administration of 2 mL activated charcoal in ABT-treated rats.

RESULTS

Effect of ABT on the Pharmacokinetics of Metoprolol and its metabolites

Plasmatic concentrations



Pharmacokinetics parameters

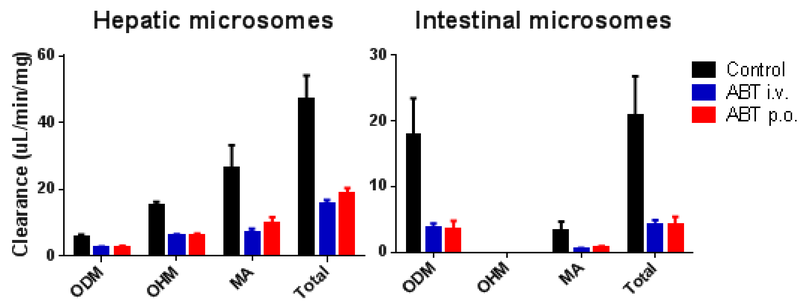
Parameters	Control	ABT i.v.	ABT p.o.
AUC (µg*h/mL)	0.166 ± 0.215	1.08 ± 0.37*	2.69 ± 0.77***
Tmax (h)	0.250	5.90 ± 4.98*	0.500
Cmax (µg/mL)	0.255 ± 0.296	0.233 ± 0.028	1.29 ± 0.37***

*Statistically significant differences: * P<0.05, ** P<0.01, *** P<0.001 versus control*

Key findings :

- ABT pretreatment significantly increased the AUC of metoprolol compare to control
- The effect is stronger in the group pretreated with ABT p.o. (16-fold compare to 6.5-fold in the group ABT i.v.)
- In the group pretreated with ABT i.v., there is a significant delay of the Tmax (5.9 h versus 0.25 h).
- ABT i.v. and ABT p.o. pretreatments affect differently the pharmacokinetic of metoprolol and its metabolites

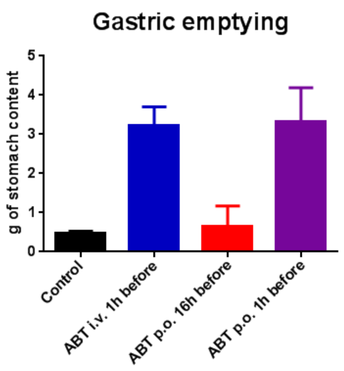
Effect of ABT on the intestinal and hepatic metabolic enzymes

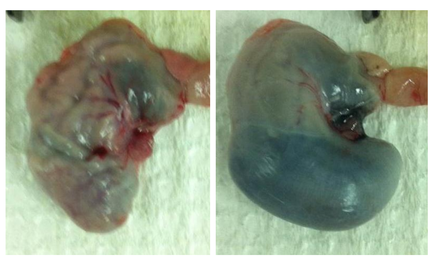


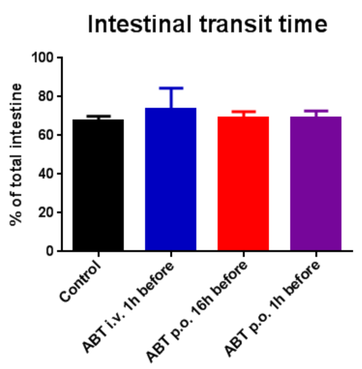
Key findings:

- ABT i.v. and p.o. inhibit similarly the metabolism of metoprolol
- The inhibition seems to be stronger in the intestine (80% inhibition) than in the liver (60% inhibition).
- Metabolite proportions are different in intestinal versus hepatic microsomes (intestine produce mainly ODM). This may explain the importance of the ODM and MA pathway *in vivo.*

Effect of ABT on gastric emptying and intestinal transit time







Key findings :

- ABT i.v. alters the absorption through inhibition of gastric emptying.
- The difference between ABT p.o. and ABT i.v. is due to the difference in pretreatment time as the absorption is also altered in the group ABT p.o. 1h before.
- No effect on the intestinal transit time is observed

ACKNOWLEDGMENTS

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CONCLUSION

- Varying the route of administration of ABT doesn't lead to a differential inhibition of intestinal and hepatic first pass metabolism
- ABT pretreatment affects the absorption when dosed for a short period of time through inhibition of gastric emptying.
- **Recommendation :** For future studies, ABT should be dosed 16h prior dosing of test compound to maintain CYP inactivation while avoiding the confounding effect on gastric emptying.

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