

## Experimental Goettingen Minipig and Beagle Dog as Two Species Used in Bioequivalence Studies for Clinical Pharmacology (5-Aminosalicylic Acid and Atenolol as Model Drugs)

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**Abstract.** Due to proven similarities in biotransformation between man and minipig, minipig seems to be the experimental animal of choice for preclinical pharmacokinetic studies when an experiment with a drug exhibiting a great first pass bioelimination (like 5-aminosalicylic acid) is to be realised. On the other hand, both minipig and dog may be suitable species for a pharmacokinetic study with a drug characterized by a small extent of first pass biotransformation (like atenolol).

**Key words:** Pharmacokinetics — Atenolol — 5-aminosalicylic acid — Man — Dog — Minipig

### Introduction

One of the limiting factors in the application of preclinical pharmacokinetic data to clinical studies with human volunteers is the choice of an adequate experimental animal species. For the solid p.o. dosage pharmaceutical forms, the choice is rather narrow as only the omnivorous animals as dogs, pigs and occasionally primates (macaca species) are available. There is evidence (Lázníček and Květina 1988) that the inter-species differences are based:

- on quantitative aspects such as the extent of drug binding, transport across the biological membranes,
- on qualitative differences, e.g. drug biotransformation.

The aim of this study is to compare experimental animals according to the character of the pharmacokinetic data for studied drugs. The postulate was tested experimentally with two model drugs:

- atenolol: a beta-blocking agent (Wood 1984) with a small extent of first pass biotransformation of the parent drug in man (less than 15 %, Lennard et al. 1986)

with the following kinetic parameters (Riddell et al. 1987):  $t_{\max} = 90 (\pm 30)$  min,  $t_{1/2} = 6-9$  h,

- 5-aminosalicylic acid: an antiphlogistic agent used in the treatment of ulcerative colitis (Greenfield et al. 1993) which is rapidly biotransformed in man to N-acetyl-metabolite (Klotz 1985):  $t_{\max}$  of the parent drug = 105 ( $\pm 35$ ) min, urinary excretion of the parent drug = 80%;  $t_{\max}$  of N-acetyl-5-ASA = 39 ( $\pm 12$ ) min, urinary excretion of the metabolite = 20%.

**Abbreviations:** ATE, atenolol, 5-ASA, 5-aminosalicylic acid; N-acetyl-5-ASA, N-acetyl-metabolite of 5-aminosalicylic acid; AUC, area under the curve,  $c_{\max}$ , maximal plasma concentration,  $t_{\max}$ , time at which maximal plasma concentration was reached;  $t_{1/2}$ , half-time of elimination.

## Materials and Methods

### Materials

Both compounds tested, ATE and 5-ASA, were applied as commercially available solid dosage forms ATE as Atenormal tab 100 mg (PRO MED CS Praha a s , Czech Republic) and 5-ASA as Salozinal tab 500 mg (PRO MED CS Praha a s , Czech Republic) Both dosage forms were administered orally as a single dose All chemicals used to prepare solutions were obtained from Sigma Aldrich (Praha, Czech Republic) and were of reagent grade

### Experimental design

Experimental animals fasted overnight and for 2 h after oral drug administration Blood samples were obtained before drug intake and at various intervals after each administration (at 15, 30, 60 min, 2, 4, 8, 12, 16, 20, 24 h, in the case of man also 48 h) Five Goettingen minipigs (average weight  $24.5 \pm 1.5$  kg, males) were used, blood samples were obtained from incannulated jugular vein From five Beagle dogs (average weight  $16.5 \pm 2.3$  kg, males), blood samples were obtained from veins at the surface of the legs (v *cephalica antebrachii* and v *metatarsa dorsalis*) Human subjects in the study were healthy middle-aged volunteers (12 in the case of ATE, 24 for 5-ASA) All experiments were approved by the corresponding ethical committees

### Assays

ATE HPLC determination using a  $150 \times 4$  mm column packed with Lichrospher CN and with an acetonitrile-phosphate buffer as the mobile phase was performed using metoprolol as internal standard The accuracy and precision of atenolol determination were found to range from 88.6 % to 96.8 % and from 1.0 % to 7.9 %, respectively

5-ASA Plasma levels of both the parent compound and the metabolite were determined by the reverse-phase HPLC method with a  $250 \times 4$  mm column packed with Lichrospher RP-18e stationary phase The analytes were eluted with acetonitrile / octanesulphonic acid buffer pH 2.8 with simultaneous UV and fluorescence detection N-acetyl-4-ASA was the internal standard The accuracy and precision of the N-acetyl-5-ASA determination were found to range from 92.9 % to 107.4 % and from 0.7 % to 8.6 %, respectively In the case of 5-ASA, the accuracy and precision were found to range from 87.3 % to 100.9 % and from 1.1 % to 3.2 %, respectively

In both cases an HPLC system (ThermoSeparation Products, USA) was used consisting of a quaternary pump, an autosampler and a SpectraFocus UV/VIS detector

## Results

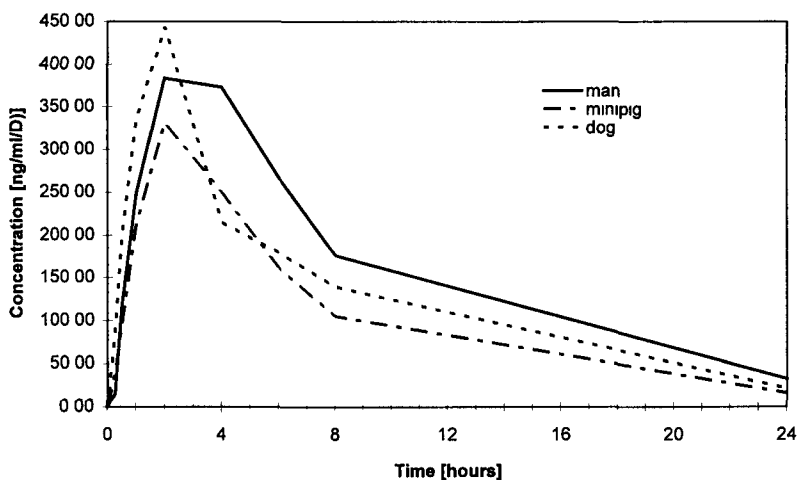
Plasma levels of both compounds (ATE and 5-ASA) as well as of the 5-ASA metabolite (N-acetyl-5-ASA) were determined. From the plasma concentration-time curves, three pharmacokinetic parameters were determined: AUC,  $c_{max}$ ,  $t_{max}$ . The parameters were calculated according to standard mathematical procedures (AUC, trapezoidal method;  $c_{max}$  and  $t_{max}$  were taken from experimental data).

For ATE the pharmacokinetic parameters were similar in all the three species examined (man, minipig, dog) (Table 1). This follows also from the course of the experimental curves (Fig. 1) when the dose is expressed relative to the dose and body weight.

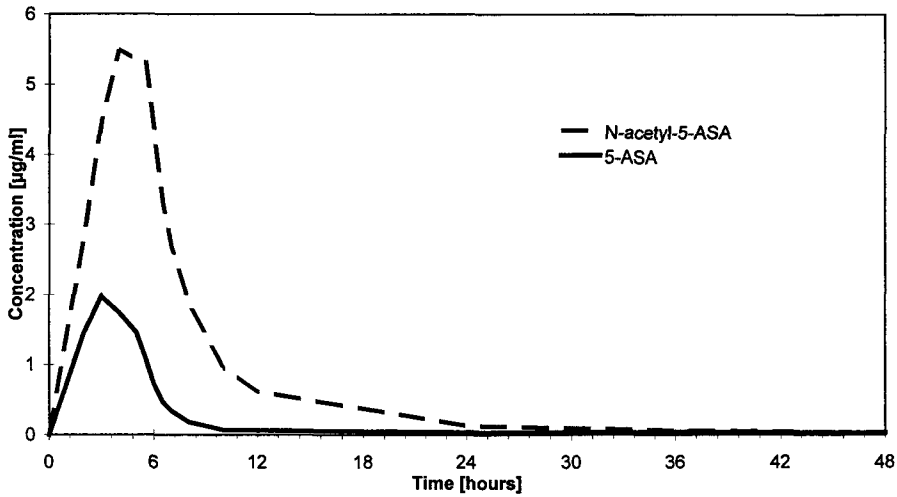
In the case of 5-ASA, the plasma levels in man (Fig. 2) and minipig (Fig. 3) were lower than those of N-acetyl-5-ASA. In the dog, no formation of N-acetyl-5-ASA was observed (Fig. 4). Pharmacokinetic parameters of both the parent compound 5-ASA and the metabolite were similar in man and minipig when the dose was expressed relative to the dose and body weight (Table 2).

**Table 1.** Pharmacokinetic parameters of atenolol in man, minipig and dog (expressed relatively dose to body weight)

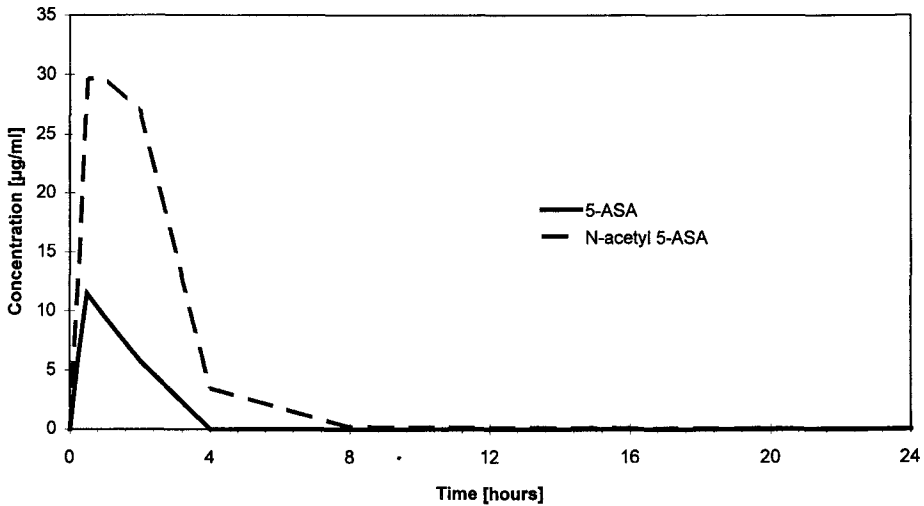
	man	minipig	dog
AUC [ng h/ml/D]	3934 3±1376 1	2601 1±509 2	3226 4±623 9
$c_{max}$ [ng/ml/D]	384 8±168 4	331 1±89 9	443 5±74 2
$t_{max}$ [h]	2 6±1 1	1 5±0 6	1 5±0 7



**Figure 1.** Plasma levels of atenolol after oral administration (100mg) to man, minipig and dog



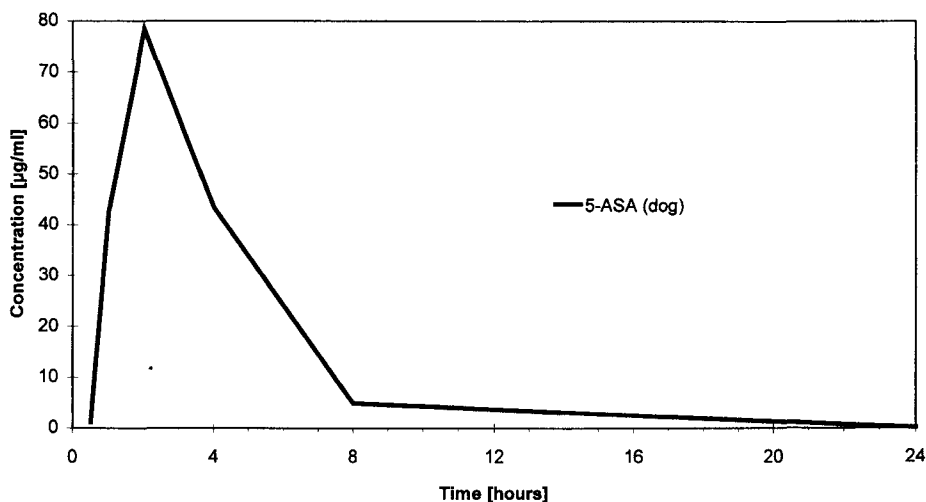
**Figure 2.** Levels of 5-aminosalicylic acid and N-acetyl-5-aminosalicylic acid in human plasma after oral administration of 5-aminosalicylic acid (500 mg).



**Figure 3.** Levels of 5-aminosalicylic acid and N-acetyl-5-aminosalicylic acid in minipig plasma after oral administration of 5-aminosalicylic acid (500 mg).

## Discussion

Man and minipig are known to exhibit nearly similar physiology of the intestinal and kidney tracts (Leucht et al. 1982; Swindle and Smith 1998). Moreover, there



**Figure 4.** Levels of 5-aminosalicylic acid and N-acetyl-5-aminosalicylic acid in dog plasma after oral administration of 5-aminosalicylic acid (500 mg)

**Table 2.** Pharmacokinetic parameters of 5-ASA and N-acetyl-5-ASA in man, minipig and dog (expressed relatively dose to body weight)

	man	minipig	dog
5-ASA			
AUC [ $\mu\text{g h/ml/D}$ ]	$1.4 \pm 0.8$	$1.3 \pm 0.5$	$11.2 \pm 3.1$
$c_{\text{max}}$ [ $\mu\text{g/ml/D}$ ]	$0.4 \pm 0.2$	$0.9 \pm 0.6$	$3.2 \pm 1.8$
$t_{\text{max}}$ [h]	$3.5 \pm 1.1$	$1.4 \pm 0.8$	$1.8 \pm 0.5$
N-acetyl-5-ASA			
AUC [ $\mu\text{g h/ml/D}$ ]	$4.8 \pm 1.1$	$5.4 \pm 1.5$	–
$c_{\text{max}}$ [ $\mu\text{g/ml/D}$ ]	$1.0 \pm 0.4$	$2.4 \pm 0.9$	–
$t_{\text{max}}$ [h]	$4.3 \pm 1.2$	$1.4 \pm 0.8$	–

is a considerable similarity in the activities of the enzymes of biotransformation. It has been shown recently that the enzymes of “Phase I of biotransformation”, mainly the cytochromes P-450, possess similar properties in the minipig and in man (Anzenbacher et al. 1998; Witkamp and Monshouwer 1998). The similarity of substrate specificities of minipig and human drug-metabolizing systems has been confirmed recently (Anzenbacher et al. 1998; Madden et al. 1998). On the other hand, the dog seems to be, at least in some cases, a less suitable or less versatile

experimental animal for studies on drug biotransformation (Chauret et al. 1997; Witkamp and Monshouwer 1998). This is documented in our study by the sole fact that there was no N-acetyl metabolite of 5-ASA found in plasma samples from this experimental animal

The present study represents a contribution to the current discussion on the suitability of various species of experimental animals for modelling of pharmacokinetics in man. For drugs with relatively small "first pass" effect of biotransformation, there is a greater choice of suitable animal species for studies of comparative pharmacokinetics (as e.g. in the case of atenolol, where the pharmacokinetics parameters were similar for man, minipig as well as for dog). On the other hand, when the "first pass" effect is more pronounced, the situation is more complicated as the choice of the suitable species much more depends on mechanisms of biotransformation of the given drug and on the enzymes of biotransformation active or available in the particular animal species.

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