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Effects of non steroidal anti-inflammatory drugs and sulfonamides on hepatic cytochrome P4502C activity *in vitro* in goats and cattle

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Cytochrome P450 monooxygenases (CYP) are involved in the biotransformation of many endogenous and exogenous compounds. In humans, enzymes from the CYP2C subfamily (predominantly CYP2C8/CYP2C9) are known to play a major role in the metabolism of the hypoglycaemic drug tolbutamide (Relling *et al*., 1990; Veronese *et al*., 1990; Doecke *et al*., 1991; Goldstein & Morais, 1994), as well as in the metabolism of a number of other drugs like phenytoin (Doecke *et al*., 1991; Veronese *et al*., 1991), diclofenac (Leemann *et al*., 1993a) and (S)-warfarin (Rettie *et al*., 1992). Furthermore, human CYP2C iso forms are probably involved in the metabolism of several non steroidal anti-inflammatory drugs (NSAIDs) such as tenoxicam, piroxicam (Zhao *et al*., 1992), naproxen (Newlands *et al*., 1992; Rodrigues *et al*., 1996), ibuprofen (Leemann *et al*., 1993b) and flurbiprofen (Tracy *et al*., 1995). In humans, the anti bacterial drug sulfaphenazole is known to be a potent inhibitor of CYP2C-activities *in vivo* and *in vitro* (Baldwin *et al*., 1995). In sheep, sulfadimethoxine was shown to be an inhibitor of the *in vivo* tolbutamide clearance (Thiessen & Rowland, 1977). In goats, a cDNA (CYP2C31) with high homology with human CYP2C9 (Zeilmaker *et al*., 1994) and a liver microsomal tolbutamide 4-hydroxylation activity (Zweers-Zeilmaker *et al*., 1996) were found. In cattle also tolbutamide hydroxylating activity was found and this was inhibited by an antibody against rabbit CYP2C3 (Vendrig, unpublished results). NSAIDs and sulfonamides are frequently used in veterinary medicine, but little is known about their possible interactions with other drugs at the level of biotransformation. In a previous study the inhibition constants of some sulfonamides towards microsomal tolbutamide hydroxylation in dwarf goats were determined (Zweers-Zeilmaker *et al*., 1997). Some sulfonamides, the anti epileptic drug phenytoin and the NSAIDs diclofenac and phenylbutazone were shown to inhibit tolbutamide hydroxylation in a competitive manner. In the present study the *in vitro* inhibitory effects of a number of sulfonamides and NSAIDs on tolbutamide hydroxylation in goat and cattle microsomes are compared.

Microsomal fractions were prepared from representative cross-sections of the caudate lobe of livers obtained from four female dwarf goats (20–30 kg, 3–6 years of age) and four female Meuse-Rhine-Yssel cows (400–500 kg, 3–6 years of age) according to standard procedures (Zweers-Zeilmaker *et al*., 1997). Microsomal tolbutamide (Bufa Chemie, Uitgeest, the Netherlands) 4-hydroxylation rates were determined as described elsewhere (Zweers-Zeilmaker *et al*., 1997). Nine different sulfonamides were tested for their ability to inhibit tolbutamide hydroxylation: sulfamerazine, sulfadiazine, sulfamethoxazole, sulfaphenazole, sulfanilamide and sulfadimethoxine (all obtained from Sigma Chemical Co. St. Louis, MO, USA), sulfadimidine-sodium (Brocacef, Maarssen, the Netherlands), sulfatroxazole (donated by Leo Pharmaceuticals, Weesp, the Netherlands), and sulfadoxine (donated by Hoffmann-La Roche, Basel, Switzerland). Also nine different NSAIDs were tested for their inhibitory activities: diclofenac (as the sodium salt), (±) ibuprofen, (±) flurbiprofen, (±) fenoprofen, (±) ketoprofen, (+) naproxen, tenoxicam and piroxicam (all obtained from Sigma) and phenylbutazone (purchased from BUFA BV, Uitgeest, the Netherlands). Microsomes were incubated with tolbutamide alone (800 μM) or with the different NSAID's and sulfonamides for 60 min at 37°C. Each potential inhibitor was tested at two concentrations, 400 and 80 μM. Statistical calculations were performed using analysis of variance (anova) followed by a Student *t*-test. Values were considered to be significantly different if $P < 0.05$.

The effects of the different inhibitors on the tolbutamide 4-hydroxylation rate are shown in Figs 1 and 2. All tested NSAIDs, except naproxen and ketoprofen (Fig. 1A), as well as sulfaphenazole (Fig. 2A) inhibited the tolbutamide hydroxylation rate by 20–60% in goat microsomal fractions at the lowest inhibitor concentration tested (80 μM). The other sulfonamides did not give a significant inhibition at this concentration. With cattle microsomal fractions only diclofenac and SFZ significantly inhibited tolbutamide hydroxylation at the lower concentration (percentage inhibition of 51.3% and 46.6%, respectively; Fig. 1B & 2B). At the highest concentration tested sulfatroxazole, sulfadoxine, sulfamerazine and sulfadimethoxine also significantly decreased tolbutamide 4-hydroxylation rate in goats, whereas naproxen, sulfadimidine, sulfamerazine, sulfamethoxazole and sulfanilamide still caused no inhibition (Fig. 1A & 2A). In cattle microsomes flurbiprofen, ibuprofen, ketoprofen, phenylbutazone, fenoprofen, piroxicam, tenoxicam, sulfadoxine and sulfatroxazole produced a decrease in the tolbutamide

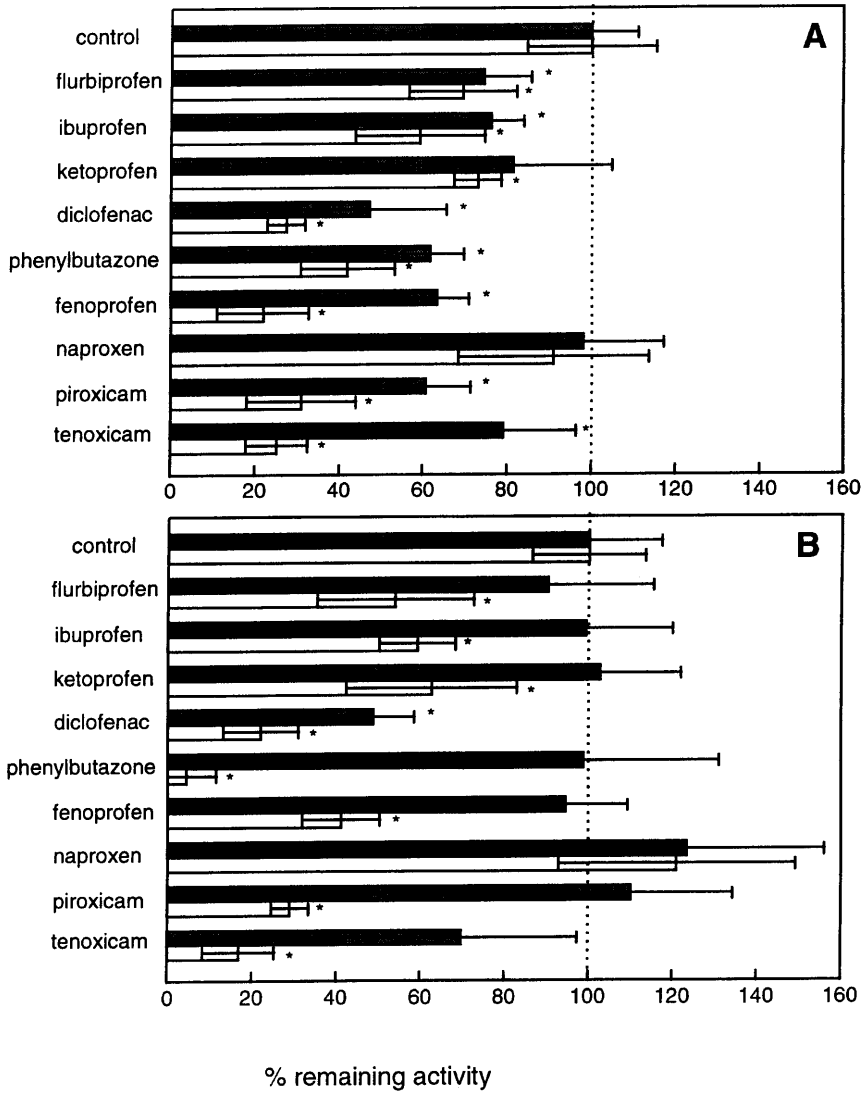

Fig. 1. Effects of NSAIDs on TB hydroxylation in goat and cattle microsomes. Microsomal fractions of goat (A) or cattle (B) were incubated with 800 μM tolbutamide in combination with either 80 μM (solid bars) or 400 μM (open bars) of the NSAIDs investigated. Data are expressed as percentage of control activity (tolbutamide and DMSO alone) and represent the mean of duplicates of four different animals ± SD. *, Significantly different from control ($P < 0.05$).

4-hydroxylation rate only at the higher inhibitor concentration tested (Fig. 1B & 2B). Naproxen, sulfadimidine, sulfamerazine, sulfadiazine, sulfamethoxazole and sulfanilamide had no effect on the 4-hydroxylation of tolbutamide in cattle microsomal fractions even at the highest concentration tested.

For the NSAIDs no clinical interactions related to inhibition of CYP450 have been published so far, but those NSAIDs inhibiting tolbutamide hydroxylation were also found to inhibit human CYP2C activity, or have been reported to be substrates of CYP2C (Zhao *et al.*, 1992; Leemann *et al.*, 1993a Tracy *et al.*, 1995; Kappers *et al.*, 1996; Rodrigues *et al.*, 1996). Fenoprofen appears to behave differently as this compound was found to be a potent inhibitor in cattle and goats, whereas no inhibition was seen in V79 cell-line stably expressing human CYP2C10 (Kappers *et al.*, 1996). The lack of effect of naproxen was in accordance with the finding of Kappers *et al.* (1996) and also of Rodrigues *et al.* (1996). The latter studied the interaction between naproxen and tolbutamide in human microsomes in the reversed way. A 10-fold higher concentration of tolbutamide than naproxen was necessary to inhibit the O-demethylation of naproxen by 36%. The results from the present study suggest that in ruminants differences may exist in the relative inhibiting potency of NSAIDs. By contrast, no differences were found for the group of sulfonamides. In the present study the activity of 4-OH-tolbutamide was taken as a reference. Further studies should elucidate if members of these structurally different classes (NSAIDs and sulfonamides) could give mutual interactions. As far as we know there are no data available in this respect.

In conclusion, our data show that two groups of clinically important drugs are able to inhibit CYP2C activity *in vitro* in goats and cattle in a different and concentration dependent manner. Although the experiments should be seen as screening of the potency of NSAIDs and sulfonamides to inhibit CYP2C activity, interactions occurring in clinical practice might be explained by this mechanism. Whether or not *in vivo* interactions occur is mainly dependent on the concentration of compounds at the active site of CYP2C. This concentration is dependent on factors such as therapeutic dose, protein binding, portal blood flow and the importance of the cytochrome P450 enzyme in the

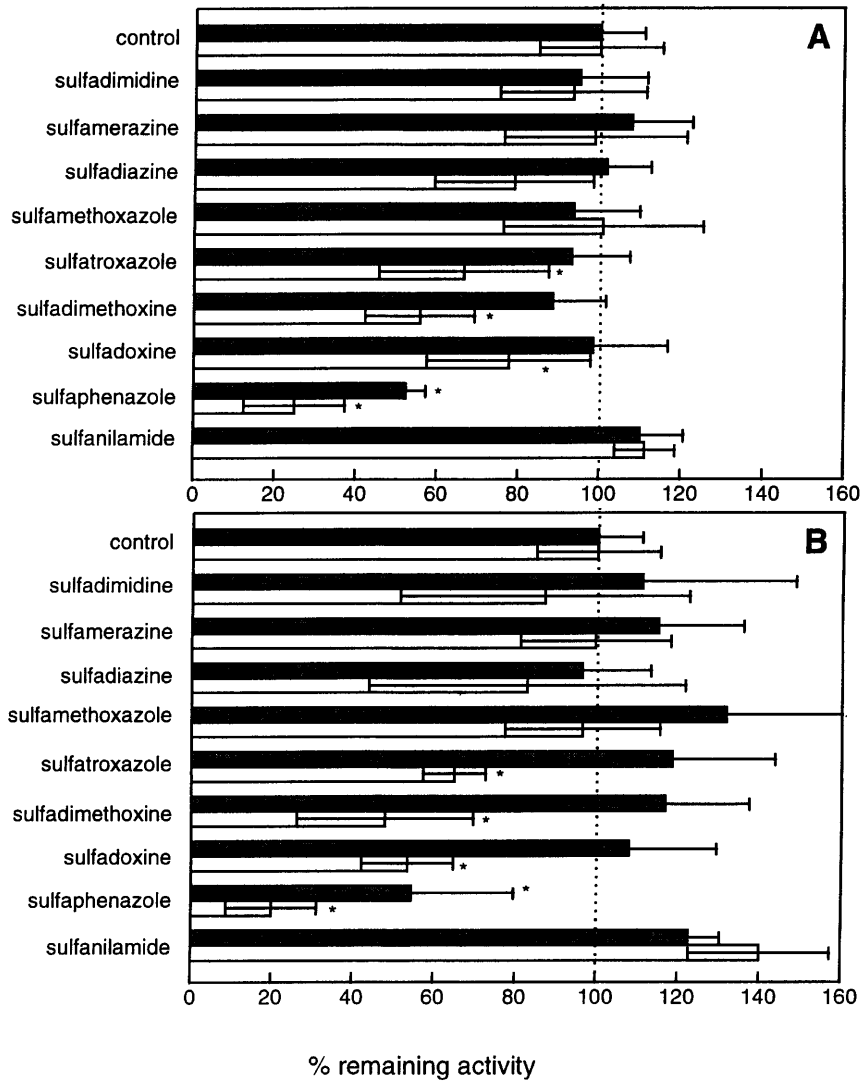

Fig. 2. Effects of sulfonamides on tolbutamide hydroxylation in goat and cattle microsomes. Microsomal fractions of goat (A) or cattle (B) were incubated with 800 μM tolbutamide in combination with either 80 μM (solid bars) or 400 μM (open bars) of the sulfonamides investigated. Data are expressed as percentage of control activity (tolbutamide and DMSO alone) and represent the mean of duplicates of four different animals ± SD. *, Significantly different from control ($P < 0.05$).

overall metabolism of the compounds. In practice plasma concentrations of the sulfonamides will be much higher than those of most of the NSAIDs tested. Stereo–specific interactions with CYP450 may also be relevant for some of the NSAIDs tested here.

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