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METABOLISM OF SULFADIAZINE IN NEONATAL AND YOUNG PIGS

COMPARATIVE *IN VIVO* AND *IN VITRO* STUDIES

POUL NIELSEN, CHRISTIAN FRIIS, NILS GYRD-HANSEN and FOLKE RASMUSSEN

Department of Pharmacology and Toxicology, Royal Veterinary and Agricultural University, 13 Bülowsvej, DK-1870 Frederiksberg C, Denmark

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Abstract—Metabolism of sulfadiazine (SDZ) was studied *in vivo* and *in vitro* during postnatal development of piglets in order to examine whether *in vitro* metabolism approaches the *in vivo* situation. Experiments were performed in 1-day-, 8-day- and 60-day-old piglets.

In vivo: ^{14}C-SDZ was injected intravenously and urine and tissue samples collected after 3 hr. Urinary excretion data as well as data from liver and kidney tissue indicated a relatively high capacity for acetylation at birth, while the capacity for oxidation is low during the first week of life. At 60 days of age the acetylation and oxidation of SDZ is equal each accounting for about 20% of the amount excreted in urine.

In vitro: Incubation of subcellular fractions of liver and kidney showed that acetylation of SDZ in liver reached maximum within 1 week. Oxidative activity was absent at 1 day, present at a low level at day 8, and at a high level at day 60. Neither acetylation nor oxidation of SDZ took place in kidney.

The results show a close correlation between *in vivo* and *in vitro* results with respect to the developmental pattern seen in piglets during the postnatal period of life.

The postnatal development of various enzyme systems associated with biotransformation of xenobiotics has been studied extensively *in vitro* by using different test substances [1]. However, little information is available about the *in vitro* metabolism of drugs during early life and how it approaches the *in vivo* situation.

In order to compare the developmental pattern of the most important metabolic pathways under *in vivo* and *in vitro* conditions the metabolism of trimethoprim, sulfadiazine, and parathion are at present being investigated in newborn and young pigs. The pig was chosen as the species for investigation because maturational changes in the basic drug-metabolizing enzyme systems have been established previously [2, 3].

Previous experiments with trimethoprim describing the development of *O*-demethylation and glucuronidation showed a close correlation between *in vivo* and *in vitro* metabolism [4, 5]. In this study sulfadiazine (SDZ) is examined. Apart from being eliminated by renal excretion SDZ undergoes biotransformation involving acetylation and aromatic hydroxylation [6].

MATERIALS AND METHODS

A total of twenty-four female piglets—twelve for *in vivo* and twelve for *in vitro* experiments—were used for this study. The piglets were divided into three groups according to age: A, 1–2 days; B, 7–9 days; and C, 56–67 days.

In vivo *experiments*. A dose of 60 mg/kg (12.5 μCi/kg) ^{14}C-SDZ (labelled in the benzene ring, purity 98%) was administered by intravenous injection. Urine was collected quantitatively through a Foley catheter. After 3 hr the piglets were sacrificed by decapitation and kidneys and liver were removed and weighed. Tissue, plasma, and urine were stored at −20° prior to analysis.

In vitro *experiments*. Homogenates, 9000 *g* and 105,000 *g* supernatant fractions of liver and kidney were prepared as described elsewhere [5].

The oxidative reactions were measured in a medium containing the following constituents in a total volume of 6 ml 20 mM sodium phosphate buffer with pH 7.4: Nicotinamide adenine dinucleotide phosphate, 4.3 μmol; glucose-6-phosphate, 20 μmol; magnesium chloride, 20 μmol; sodium chloride, 425 μmol; 9000 *g* supernatant fraction equivalent to 500 mg tissue and sulfadiazine (SDZ), 0.4 μmol or *p*-nitroanisole, 4.0 μmol as substrates [7].

The *N*-acetyl-transferase activity was measured in a medium of 2.0 ml 50 mM potassium phosphate buffer with pH 6.8 containing 0.6 μmol acetyl-CoA, 105,000 *g* supernatant equivalent to 200 mg tissue and 0.06 μmol SDZ or 0.5 μmol *p*-aminohippuric acid (PAH) as substrates [8].

The substrates were added after 5 min preincubation. Oxidation reactions were carried out at 37°, while acetylation was measured after incubation at 25°.

Para-nitroanisole and PAH were used as reference substrates in order to check the quality of the tissue preparations. Samples without either tissue, substrate or co-factors were incubated along with test samples in all experiments.

The enzyme reactions were stopped by precipitation of the proteins using $ZnSO_4/Ba(OH)_2$ for samples with *p*-nitroanisole, acetone-ethanol (1 + 1, v/v) for SDZ-oxidation and trichloroacetic acid for acetylation of SDZ and PAH.

Analytical methods. The total concentration of ^{14}C-SDZ and its metabolites in body fluids and tissues was measured by liquid scintillation counting [5].

Proteins in plasma and in tissue samples homogenized in 3 vol. of water were precipitated by adding an excess of ethanol-acetone (1 + 1, v/v). After centrifugation the supernatant was filtered and evaporated to dryness. Following removal of fat by extraction with *n*-hexane the residue was dissolved in water and used for determination of SDZ and its metabolites.

The relative amounts of SDZ and metabolites with free amino-groups were determined spectrophotometrically after separation by thin layer chromatography as described by Nielsen [9] using ethyl acetate–methanol–water–acetic acid (95:5:1:0.5 by vol.) as solvent. However, as N^4-acetyl-SDZ and 2-sulfanilamido-4-hydroxypyrimidine (4′-OH-SDZ) are not completely separated by this method these two metabolites were also quantitated by liquid scintillation counting of the spots visualized under u.v.-light. N^4-acetyl-SDZ was then calculated as the difference between the sum of the two determined by liquid scintillation counting and the amount of 4′-OH-SDZ measured by spectrophotometry. The identity of SDZ, 4′-OH-SDZ, and N^4-acetyl-SDZ was confirmed by TLC of the samples run together with the pure compounds synthesized as described by Atef and Nielsen [10].

SDZ and 4′-OH-SDZ in incubated samples were determined after separation by TLC as described above, while the rate of *O*-demethylation of *p*-nitroanisole to *p*-nitrophenol was determined by measuring the nitrophenol formed [2].

In incubated samples from acetylation experiments SDZ (and PAH) was determined spectrophotometrically according to the method of Bratton and Marshall [11]. Total concentration (SDZ or PAH + their respective N^4-acetyl derivatives) was determined in the same samples after acid hydrolysis and the concentration of the N^4-acetyl derivatives calculated as the difference between the concentration before and after hydrolysis.

For statistical calculations a significance level of $P < 0.05$ was used.

RESULTS

In vivo *experiments*

During the 3 hr collection period 11 ± 3, 20 ± 3, and 56 ± 4% of the dose was excreted in the urine in group A, B and C, respectively. As illustrated in Fig. 1 more than 50% of the ^{14}C-SDZ in urine from newborn piglets consisted of N^4-acetyl-SDZ, while approximately 30% was present as unchanged SDZ and about 1% as the hydroxylated metabolite (4′-OH-SDZ). The pattern was nearly the same in urine from group B, while in group C unchanged SDZ made up more than half with approximately 20% as N^4-acetyl-SDZ and 20% as 4′-OH-SDZ. Urine pH was found to be 6.0 ± 1.2, 5.1 ± 0.3, and 7.0 ± 0.6 in group A, B and C, respectively.

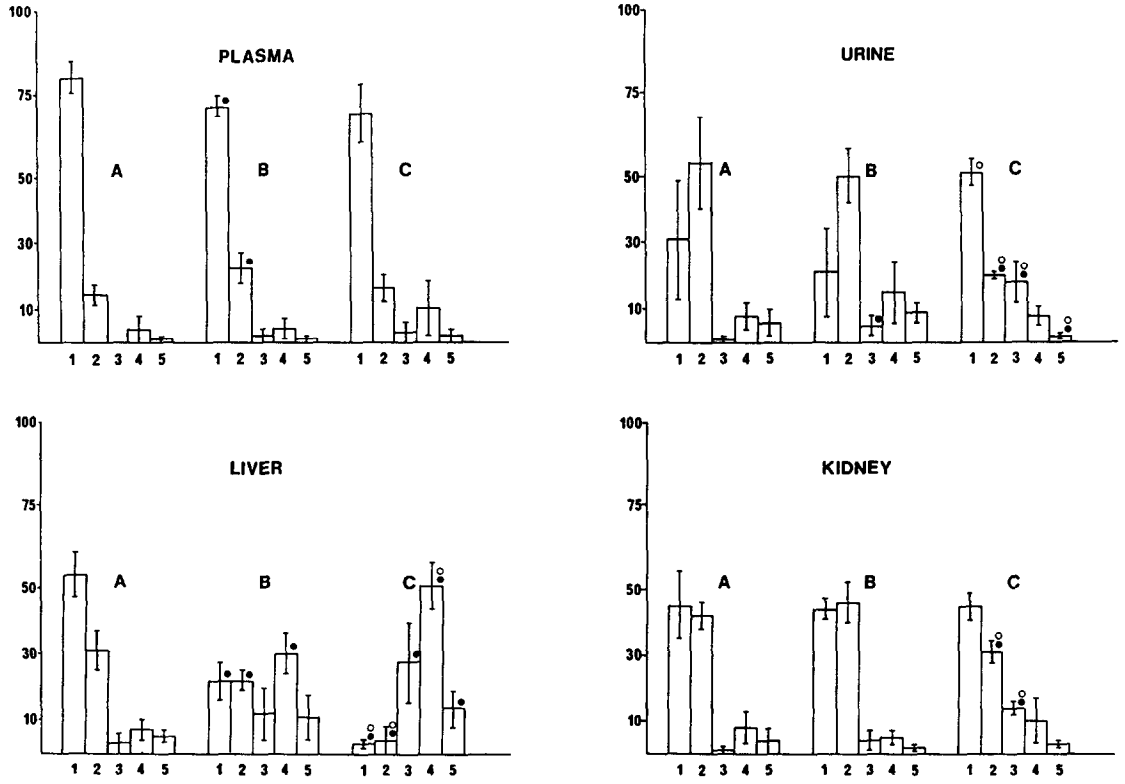

Fig. 1. Distribution of sulfadiazine and its metabolites in plasma, urine, liver, and kidney from 1-day- (A), 8-day- (B), and 60-day-old piglets (C) (mean ± S.D.): (1) sulfadiazine (SDZ); (2) N^4-acetyl-SDZ; (3) 4′-OH-SDZ; (4) other metabolites; (5) conjugates. Ordinate: Percentage of total drug concentration. ●, Significantly different from group A; ○, significantly different from group B.

Table 1. Tissue distribution of ^{14}C-sulfadiazine in piglets (mean ± S.D.)

Group	Concentration (μg/g or ml) A	B	C
Plasma	75 ± 4	74 ± 3	48 ± 3*†
Kidney	108 ± 6	102 ± 4	96 ± 8
Liver	42 ± 2	45 ± 1*	24 ± 4*†

* Significantly different from A.
† Significantly different from B.

At the time of slaughter the concentration of ^{14}C-SDZ in kidney was higher than in plasma, while the opposite was seen for the liver (Table 1). The proportion of N^4-acetyl-SDZ was in both liver and kidney lowest in group C (Fig. 1). The hydroxylated metabolite—4′OH-SDZ—was hardly detectable in the youngest group of piglets, but was a major metabolite in liver and kidney in the oldest group. The "other metabolites", which is a term used for the sum of minor metabolites with free amino groups, were found in large proportions in livers from group B and C. These minor metabolites are most likely oxidation products since they have lower R_f values than both SDZ, N^4-acetyl-SDZ, and 4′-OH-SDZ indicating an increased polarity, although still lower than that of the conjugates.

Conjugates (SDZ or its metabolites conjugated—probably with glucuronic acid or sulfate) were present mainly in the liver, whereas the amounts were small in plasma and kidney.

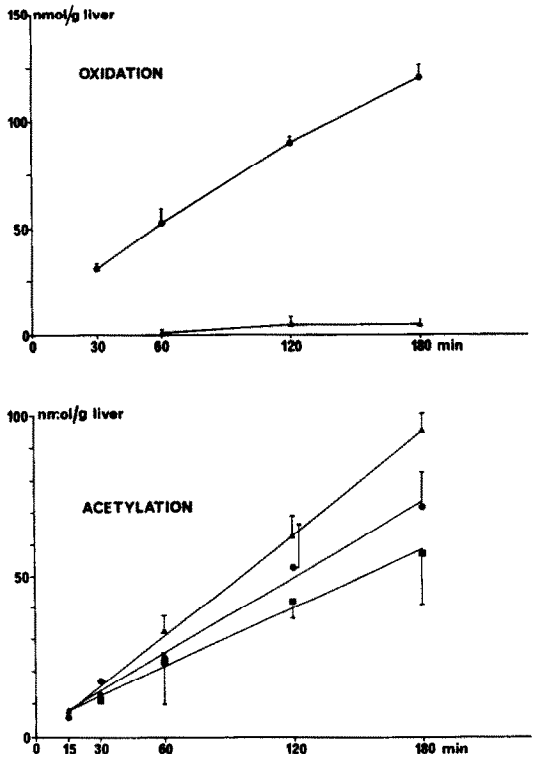

Fig. 2. Oxidation of sulfadiazine (upper graph) incubated with 9000 *g* supernatant of liver homogenates from 8-day (▲) and 60-day-old (●) piglets. Acetylation of sulfadiazine (lower graph) incubated with 105,000 *g* supernatant of liver homogenates from piglets aged 1 day (■), 8 days (▲), and 60 days (●). Each point represents the average of four animals. The vertical bars illustrate one S.D.

In vitro *experiments*

The incubation experiments with 9000 *g* supernatant from liver homogenates showed that SDZ was not oxidized in the 1-day-old piglets (Fig. 2). In group B 5 ± 3 nmol/g liver (wet weight) of 4′-OH-SDZ was found after 3 hr incubation, while in group C the amount of this metabolite had increased to 121 ± 6 nmol/g liver. Oxidation of *p*-nitroanisole took place also among piglets in group A—and was approximately 100 times higher than of SDZ in groups B and C.

Experiments with the 105,000 *g* supernatant from liver homogenates showed that SDZ was acetylated by all three age groups (Fig. 2). The acetylation rate was significantly higher in 1-week-old piglets than in the other two groups. After 3 hr incubation 95 ± 5 nmol/g liver was formed in group B, while the corresponding values for group A and C were 57 ± 16 and 71 ± 11 nmol/g liver, respectively. Under identical conditions PAH was acetylated more than 100 times as fast as SDZ.

In the kidney neither oxidizing nor acetylating activity could be demonstrated in experiments with SDZ as substrate, whereas PAH was acetylated to nearly the same extent as in the liver. Para-nitroanisole was oxidized by the kidney tissue, but at a lower rate than by the liver.

DISCUSSION

Elimination of SDZ takes place both by renal excretion and metabolism, where the latter process is the major one in pigs at all ages [6]. In agreement with this a pronounced increase in the oxidizing activity of liver tissue was seen during the postnatal period with the main rise taking place between weeks 1 and 8. These *in vitro* results correspond well with the *in vivo* experiment—both with increase in the 4′-OH-SDZ content of the liver from group B to C (Fig. 1), and more important, with the marked age-dependent rise in urinary excretion of 4′-OH-SDZ (Fig. 1). A similar development in hepatic oxidizing capacity has been demonstrated by Short and Davis [2] using hexobarbital, zoxazolamine, *p*-nitroanisole, and 1-amphetamine as substrate and by Gyrd-Hansen *et al.* [5] with trimethoprim as substrate.

The SDZ-*N*-acetyltransferase activity is well developed at birth as about 50% of the total renal excretion consisted of N^4-acetyl-SDZ in the two youngest groups (Fig. 1). The *in vivo* observation is in agreement with the *in vitro* experiments, where the hepatic *N*-acetyltransferase was found to reach maximum within the first week (Fig. 2). A similar age course has been observed for *p*-aminobenzoic acid-*N*-acetyltransferase in guinea pigs [12].

In accordance with previous studies by Short and Davis [2] and Short *et al.* [3] the enzymic activity in kidney was found to be very low. Thus oxidation and

acetylation reactions could only be demonstrated for the reference substances, *p*-nitroanisole and PAH.

The higher metabolic rates for the reference substances than for SDZ may be explained either by different enzyme systems being involved, or by differences in substrate affinities for the same enzyme system [1]. The different rate at which SDZ and *p*-nitroanisole are oxidized could be due to the fact that SDZ undergoes aromatic hydroxylation and *p*-nitroanisole *O*-demethylation—processes which involve different enzyme systems. However, trimethoprim, which is metabolized in the same way as p-nitroanisole, has been shown to be oxidized *in vitro* at much slower rate than *p*-nitroanisole [5].

The much higher rate by which PAH is acetylated as compared to SDZ may be related to difference in substrate affinities probably caused by diversity in electronegativity of the group in *p*-position to the amino group. For a series of aniline derivatives the acetylation rate has thus been demonstrated to depend strongly on the electronegativity of the *p*-substituent [13].

In spite of the low metabolic activity with SDZ as substrate in the *in vitro* experiments the present investigation demonstrates a close correlation between *in vivo* and *in vitro* assays with respect to the developmental pattern seen in piglets for both acetylation and oxidation of sulfadiazine.

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