Placental transfer and distribution of pinazepam and its metabolite N-desmethyldiazepam in the maternal and fetal rabbit: effect of the stage of gestation

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SUMMARY

The distribution of pinazepam and its metabolite N-desmethyldiazepam was studied in fetuses of New Zealand rabbits on the 20th and 27th day of pregnancy. The concentrations of both compounds were also measured in the maternal brain, liver and uterus. Pregnant rabbits were sacrificed at 0.5, 2, 4 and 12 h after intravenous administration of pinazepam (5 mg/kg). The concentrations of pinazepam and N-desmethyldiazepam in various biological specimens were measured by a specific gas-chromatographic procedure. Pinazepam and N-desmethyldiazepam rapidly crossed the placenta. In 20 day old fetuses, comparable concentrations of pinazepam were found in the liver, brain, heart, lungs and kidneys. In contrast, the liver of 27 day old fetuses accumulated pinazepam at concentrations higher than the other tissues. The hepatic extraction of pinazepam, already described in adult rabbits (1), develops prenatally. A preferential accumulation of pinazepam rather than N-desmethyldiazepam was also observed in the maternal uterus. In this tissue the concentrations of pinazepam were 5–10 times higher on the 27th rather than the 20th day of pregnancy. The stage of pregnancy influences the distribution pattern of pinazepam in rabbit fetuses and their mothers.

INTRODUCTION

Pinazepam (7-chloro-1-propargyl-5-phenyl-3-2H-1,4-benzodiazepin-2-one) is a benzodiazepine characterized by a high liver accumulation in rabbit (1) and rat (2) liver. A liver first pass extraction and metabolism of pinazepam was suggested in humans (3).

The accumulation of a drug in tissues depends upon several factors including the presence of structures with ‘affinity for’ and/or ‘binding to’ the compound. The rise of such structures may be development-dependent. The aim of this study was to investigate the distribution pattern of pinazepam in the rabbit fetus. The rabbit was chosen as an animal model because a liver first pass extraction of pinazepam has previously been described in this animal (1). In addition, the rabbit fetus is large enough to allow measurement of drug concentrations in single organs.

We measured the concentrations of pinazepam in the liver, brain, heart, kidneys and lungs of fetal rabbits on two different stages of development. We observed that the liver of term fetuses accumulated pinazepam at concentrations several times higher than the other tissues. In contrast, the liver of fetuses at two thirds of gestation accumulated pinazepam at concentrations similar to the other organs.

N-Desmethyldiazepam, the major metabolite of pinazepam, is rapidly formed after administration to
rabbit (1). The distribution pattern of pinazepam differs from that of its metabolite since N-desmethyldiazepam undergoes a lower hepatic accumulation (4). We then studied the distribution of N-desmethyldiazepam in fetal rabbits for comparative purposes. We also describe the distribution of pinazepam and its metabolite in maternal brain, liver and uterus.

**MATERIALS AND METHODS**

**Standards**

Pinazepam and N-desmethyldiazepam were kindly supplied by Zambeletti, Milan, Italy.

**Chemical analysis**

The concentrations of pinazepam and N-desmethyldiazepam were measured in the plasma, amniotic fluid and tissue homogenates by means of a specific and sensitive gas chromatographic procedure, with an electron capture detector, as previously described (5) and modified in order to improve the chromatographic separation of pinazepam from N-desmethyldiazepam (2). Drug analysis in the brain required a purification step. The levels of pinazepam and N-desmethyldiazepam in brain homogenates were measured by the method described by Trebbi et al. (6). Samples were assayed in parallel with calibration curves. The calibration graphs for the drugs were obtained by plotting the ratio of the peak area to that of the internal standard against known amounts of the drug added to the biological specimens.

**Animals and treatment**

Twenty four timed pregnant New Zealand rabbits were used. Twelve animals were on the 20th and the other 12 on the 27th days of gestation. The average ± SD of the body weight of the first and second group was 3.4 ± 0.3 and 3.7 ± 0.3 kg, respectively.

Pinazepam was dissolved (5 mg/ml) in a mixture containing propylene glycol: glycofurol: benzyl alcohol: saline (35:30:2:48). The pH of the mixture was adjusted to 7.4 with Tris-base. The rabbits were given pinazepam (5 mg/kg body weight) intravenously. The treatment of each group of animals was made on the same occasion. Animals (three per time point) were killed by cervical dislocation at 0.5, 2, 4 and 12 h after administration. The blood was obtained by external jugular excision and collected in tubes containing 2 drops of heparin (5000 units/ml). The plasma was obtained by centrifugation at 1800 x g for 20 min in a refrigerated centrifuge and stored at -20°C until analyzed. Tissues were quickly removed and immediately frozen with dry-ice and kept at -20°C until analyzed. The fetuses obtained on 20 and 27 day of gestation had a body weight (mean ± SD) of 2.1 ± 0.1 and 20.6 ± 0.9 g, respectively. The brain, heart, lungs, kidneys, liver, placenta and amniotic fluid were removed from each fetus and immediately frozen at -20°C until analyzed. The tissues of the fetuses from the same mother were pooled and assayed as single samples. Blood samples were collected by decapitation from the oldest fetuses only. The maternal plasma, brain, liver and uterus were collected and stored as described earlier.

Tissue specimens were homogenized (1/5, w/v) in 1 M dipotassium hydrogen orthophosphate buffer pH 7.4, by means of an Ultra-Turrax homogenizer (Janke and Kunkel, Staufen, West Germany). The homogenates were grouped on the basis of the tissue. Each group of specimens was assayed on a single occasion. The liver was pre-analyzed to select the appropriate volume of homogenate to assay.

**Calculation**

The area under the concentration-time curve (AUC) was measured by trapezoidal rule between 0.5 and 12h.

**RESULTS**

Pinazepam and N-desmethyldiazepam concentration-time curves in the liver, brain, heart, kidneys and lungs of 20 and 27 day old fetuses are depicted in Figure 1. Pinazepam reached comparable concentrations in the various tissues of 20 day old fetuses. The AUC for the parent compound and metabolite are summarized in Table I. The liver of 27 day old fetuses accumulated pinazepam at concentrations 2-10 times higher than in the other tissues (Fig. 1). The AUC for pinazepam and N-desmethyldiazepam are reported in Table I as well.

Figure 2 shows the concentrations of both compounds in the placenta and plasma, uterus, liver and brain of mothers on the 20th day of pregnancy. The uterus accumulated pinazepam at concentrations notably higher than the plasma. A reverse picture was