

## Plasma protein binding of an N-pyrrolyl derivative penicillin in several mammalian species

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The extents of protein binding of an N-pyrrolyl derivative penicillin in plasma of different species were determined *in vitro* by the equilibrium dialysis technique and spectrophotometry determination. The percentage of binding was determined in cows, sheep, pigs and dogs. The percentage of drug that was protein bound was independent of drug concentration for this penicillin within the range studied (5 to 40  $\mu\text{g ml}^{-1}$ ). The extent of binding was determined at 20  $\mu\text{g ml}^{-1}$ . There was a significant difference in the extent of penicillin binding between species (*t* test  $P < 0.005$ ) except for between cows and dogs (*t* test  $P < 0.05$ ) and sheep and pigs (where it was not significantly different). In the species studied the extent of penicillin binding ranged from 41 to 55 per cent.

THE interaction of drugs with plasma proteins has been extensively studied. There is considerable scientific evidence, especially for antibiotics but also for other drugs, that there may be a better correlation of pharmacological response with the concentration of unbound drug in plasma than with the total plasma concentration.

The binding of drugs to plasma proteins affects their intensity of pharmacological effect, distribution, biotransformation and glomerular filtration. The unbound fraction of drug in plasma is pharmacologically active, whereas the bound fraction is inactive but serves as a depot. Thus, the determination of the free or bound portions of various drugs in the plasma has important implications concerning the drug's therapeutic activity.

The penicillin studied was an N-pyrrolyl derivative (4-thia-1-*l*-azabicyclo [3.2.0] heptane-3, 3-dimethyl-6-amino-7-oxo-N/2 [1H-pyrrolyl] acetyl/2-carboxylic acid [6R, trans]) synthesised during an investigation into N-pyrrolyl derivatives. This new penicillin presents an interesting spectrum of activity specially against Gram-positive bacteria (Arin et al 1983).

The blood donor animals were clinically healthy adults of the following species: nine sheep, eight pigs, eight dogs and 11 cows.

Blood was collected in tubes containing dipotassium EDTA and plasma was separated by centrifugation. The plasma samples obtained were immediately frozen at  $-20^{\circ}\text{C}$  until further use. Total plasma protein concentrations were determined by using a protein assay kit (Sigma).

The penicillin was added to isotonic phosphate buffer (pH 7.4) at concentrations of 10, 15, 20, 30 and 40  $\mu\text{g ml}^{-1}$ . The extent of binding of penicillin was determined in each species at a concentration of 20  $\mu\text{g ml}^{-1}$  by equilibrium dialysis through Visking membranes at  $5^{\circ}\text{C}$  for 48 hours

(Baggot and Davis 1973). The penicillin concentration in buffer samples was measured according to Bundgaard and Ilver's technique modified by Zapico et al (1987).

The extent of plasma protein-binding of penicillin was independent of the drug concentration within the range of concentrations studied (5 to 40  $\mu\text{g ml}^{-1}$ ).

The fraction of penicillin bound by plasma proteins, at a drug concentration of 20  $\mu\text{g ml}^{-1}$  in the several species of animals is given in Table 1. This table also shows the plasma protein concentration in each species. The statistical calculations were done according to standard methods and the results are given as average  $\pm$  SEM. A significant difference in the proportion of penicillin that was protein bound was found between species other than pigs and sheep.

The authors showed that the percentage of penicillin bound is independent of the penicillin concentration in the range of 5 to 40  $\mu\text{g ml}^{-1}$ , confirming results by others for several penicillins (Keen 1965, Ziv and Sulman 1972).

The extent of binding of the penicillins varies considerably between species and between compounds. Thus the rate of binding for penicillin G in cow plasma is 46.3 per cent and in sheep and pigs is 42.2 per cent and 36.6 per cent, respectively (Keen 1965, Ziv and Sulman 1972). On the other hand, these same authors found the percentage of binding for cloxacillin and ampicillin to be 71.3 per cent and 18 per cent, respectively, in cow plasma. Other authors indicate the extent of binding for cloxacillin in dog plasma to be 64.5 per cent (Acrod 1970).

The N-pyrrolyl derivative penicillin studied by the authors has a different molecular structure than the rest of the reported penicillins. The present results are not comparable

TABLE 1: Extent of plasma protein binding of an N-pyrrolyl derivative penicillin in several species of animals

Species	Number of animals	Percentage bound penicillin (20 $\mu\text{g ml}^{-1}$ ) Mean $\pm$ SEM	Plasma protein concentration (g (100 ml) $^{-1}$ ) Mean $\pm$ SEM
Cow <sup>a</sup>	11	51.07 $\pm$ 2.67	6.7 $\pm$ 0.35
Sheep <sup>b</sup>	9	41.68 $\pm$ 3.34	6.3 $\pm$ 0.23
Swine <sup>c</sup>	8	41.83 $\pm$ 2.58	7.3 $\pm$ 0.40
Dog <sup>d</sup>	8	55.2 $\pm$ 1.06	6.8 $\pm$ 0.37

There was a significant difference in the extent of penicillin binding among the following species (used Student's *t* test to compare mean values of the percentage of bound of penicillin):

$P < 0.005$  for <sup>a</sup> and <sup>b</sup>, <sup>a</sup> and <sup>c</sup>, <sup>b</sup> and <sup>d</sup>, <sup>c</sup> and <sup>d</sup>

$P < 0.05$  for <sup>a</sup> and <sup>d</sup>

with those from other authors as a slight structural modification of the molecule significantly alters the extent of the drug-protein interaction.

The observed differences in the extent of binding of the N-pyrrolyl derivative penicillin among species may reflect different affinities of the various plasma albumins for the drug molecule. Further investigations are needed to confirm this.

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