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Plasma Protein Binding of Levamisole in Several Species

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SUMMARY

The binding of levamisole to total plasma proteins of 6 animal species was determined in vitro *by equilibrium dialysis. The percentage of bound drug protein was independent of levamisole concentration within the range studied, 5-50 µg/ml (ANOVA). Levamisole was bound to a low extent to plasma proteins of each animal species (19.40-25.91%). There were significant differences in the extent of levamisole binding among species (ANOVA). Owing to the low degree of protein binding and the high volume of distribution of levamisole, the variations in protein binding due to different factors would not be of major clinical importance in its therapeutic application.*

Key words: Levamisole - Protein binding - Equilibrium dialysis

INTRODUCTION

Plasma protein binding of a drug limits its concentration both in tissues and in its sites of action since only unbound drug is in equilibrium across membranes. Binding also affects the distribution, biotransformation and glomerular filtration of drugs. Therefore, plasma protein binding is an important parameter for understanding the pharmacokinetics of a drug.

Levamisole is a broad-spectrum anthelmintic widely used in veterinary medicine mainly against gastrointestinal and pulmonary nematodes (1-3). It also has an immunomodulating effect (4, 5).

Currently, there is not a large amount of data available concerning plasma protein binding of levamisole in different animal species. Hence, the purpose of this study was to determine plasma protein binding of levamisole in 6 different animal species.

MATERIALS AND METHODS

Plasma sampling

Six healthy animals of each of the following species were used as blood donors for these experiments: chicken, rabbit, sheep, cow, horse and pig.

Blood was collected in heparinized tubes. Plasma was separated by centrifugation, pooled for each species, immediately frozen and stored at –20 °C until assay. Total plasma protein concentrations were spectrophotometrically determined at 750 nm (6) using a protein assay kit (Sigma).

Equilibrium dialysis

Plasma protein binding of levamisole was studied by equilibrium dialysis using Visking tubing (molecular weight cut-off not greater than 12,000-14,000 daltons). Membranes were prepared according to Chignell (7) and stored at 5 °C before being used within the next 24 h.

TABLE 1. Plasma protein concentration in several animal species[a].

Animal[b]	Plasma protein concentration (g/100 ml)
Chicken[c]	3.71 ± 0.31
Rabbit[c]	5.99 ± 0.05
Sheep[d,e]	6.91 ± 0.81
Cow[d,f]	7.08 ± 0.36
Horse[d,g]	7.79 ± 0.49
Pig[d,h]	8.11 ± 0.33

[a]Each value is the mean ± SD of 5 determinations; [b]significant differences among species (ANOVA); significant differences with: [c]the rest of animal species, [d]chicken and rabbit, [e]cow, [f]sheep, [g]pig, [h]horse (Duncan test).

Levamisole was added to isotonic phosphate buffer (pH = 7.40) obtaining final concentrations of 5, 10, 20, 30, 40 and 50 μg/ml, and the extent of protein binding was determined for each concentration. Three ml of phosphate buffer (pH = 7.40) containing levamisole at each of the different concentrations was placed into glass tubes, and a Visking tubing containing 1 ml of plasma was also introduced.

All the experiments were carried out at 37 °C. Two hours were required to obtain the equilibrium of free drug concentrations between plasma and buffer. The dialysis procedure was repeated 5 times for each concentration investigated. Previous controls demonstrated no adsorption to dialysis membranes or tube walls.

At the end of dialysis, 2.5 ml of buffer containing the free drug was removed from the tubes. Levamisole was extracted and purified according to (8). Levamisole identification and quantification was carried out by spectrophotometry at 212 nm.

Statistical analysis

The data obtained were processed by analysis of variance (ANOVA). The Duncan test was applied when significant differences were found in order to identify where these differences occurred. A $p < 0.05$ was taken as the level of significance for all analyses.

RESULTS

Plasma protein concentrations in each species (chicken, rabbit, sheep, cow, horse and pig) are shown in Table 1. Concentrations range from 3.71 g/100 ml in chicken to 8.11 g/100 ml in pig.

A significant difference in plasma protein content among species was found (ANOVA). The Duncan test indicated differences between species, except between sheep, horse and pig, and between cow, horse and pig.

The values of plasma protein binding of levamisole at different concentrations ranging from 5-50 μg/ml are shown in Table 2.

Two-way ANOVA showed that plasma protein binding of levamisole is not dependent on plasma drug concentration within the range of 5-50 μg/ml, but that it does depend on the animal species studied. The extent of plasma protein binding of levamisole was low in all the animal species studied (from 19.40% in chicken to 25.91% in cow).

The Duncan test determined that the significant differences in these animal species were between chicken and the rest of species studied, except for rabbit, and also between cow and rabbit.

DISCUSSION

The values of total protein concentration obtained in this study were similar to those reported by other authors (9-14) who found the lowest values in chicken (3.0-5.0 g/100 ml) and the highest ones in pig (6.0-10.3 g/100 ml). Similarly, the data shown in this paper indicate that the lowest value for bound levamisole was found in chicken. We have not found data on plasma protein binding of levamisole in the animal species studied in the literature.

The protein binding of levamisole, similar to those of other basic drugs such as amphetamine, desmethylimipramine, morphine or naltrexone, is independent of its concentration (15).

According to several authors, the binding of a drug to plasma proteins affects its distribution, biotransformation and glomerular filtration when more than 80% of the drug is bound. This influence, however, is smaller if the binding of the drug is less than 50% (16). Owing to the low degree of protein binding and the high volume of distribution of levamisole (17-21), the variations in protein binding due to different factors (diseases, displacement by other drugs, *etc.*) will probably not have any major clinical importance in the case of this drug.

TABLE 2. Exent of plasma protein binding of levamisole (%) in several animal species[a].

Animal[b]	Levamisole concentration (μg/ml)[c]						
	5	10	20	30	40	50	Mean ± SD
Chicken	18.29 ± 6.31	24.50 ± 5.12	18.63 ± 4.57	19.06 ± 7.78	15.80 ± 1.74	20.27 ± 5.99	19.40 ± 5.64
Rabbit[d]	21.64 ± 1.75	24.23 ± 9.18	22.87 ± 6.92	23.59 ± 8.86	21.74 ± 4.14	20.11 ± 3.54	22.36 ± 5.96
Sheep[e]	32.91 ± 7.32	25.16 ± 5.28	21.76 ± 5.31	19.24 ± 5.02	21.56 ± 4.76	24.77 ± 4.30	23.93 ± 6.40
Cow[e]	23.57 ± 7.78	23.23 ± 9.08	27.76 ± 5.49	25.27 ± 5.92	28.55 ± 2.35	26.59 ± 2.31	25.91 ± 5.76
Horse[e]	22.75 ± 3.91	19.10 ± 7.26	25.50 ± 2.90	21.92 ± 5.34	27.73 ± 4.69	21.74 ± 7.29	23.12 ± 5.73
Pig[e]	21.93 ± 5.17	24.03 ± 4.90	24.11 ± 8.73	24.16 ± 9.15	27.24 ± 8.21	27.09 ± 3.39	24.70 ± 6.42

[a]Each value is the mean ± SD of 5 determinations; [b]significant differences among species (ANOVA); [c]no significant differences among concentrations (ANOVA). Significant differences with [d]cow and [e]chicken (Duncan test).

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