

IN VITRO INTERACTION OF SULFAMERAZINE WITH THE PLASMA PROTEIN OF BUFFALOES, COWS, SHEEP AND GOATS

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ABSTRACT

In vitro binding of sulfamerazine with the plasma protein of buffaloes, cows, sheep and goats was studied. The binding in buffaloes ranged between 51.9 to 64.5 percent, cows 66.7 to 70.4, sheep 61.9 to 67.7 and in goats 63.7 to 75.2 percent at concentrations of drugs ranging between 50 to 250 µg/ml. Plasma pH affected the binding, being lower at higher pH values and vice versa. When total amount of protein in plasma was decreased the amount of bound drug decreased and vice versa. The studies indicate that binding of sulfamerazine to plasma protein was affected by the drug concentration in plasma, plasma pH, and total proteins.

Keywords: Sulfamerazine, plasma protein, buffaloes, cows, sheep, goats

INTRODUCTION

In assessing the clinical significance of plasma concentration of drugs, it is essential to consider the binding of drugs to plasma proteins (Meyer and Gultmen, 1968). Protein binding of drugs involves, electrostatic interactions, hydrogen bonds and vander wall's forces. Plasma protein binding is often mentioned as a factor playing a role in Pharmacokinetics Pharmacodynamics and drugs interactions (Katzung, 1998).

Some drugs show high tendency of binding with the proteins, some intermediate and some show low tendency of protein binding. The ratio of concentration of free and bound drug can be dependent upon total drug concentration (Brander *et al.*, 1991). Since plasma proteins do not normally cross the capillary endothelium, the drugs which are bound to proteins do not result in a peak intensity of pharmacological action. The protein bound drug is inactive (Akhtar, 1999). Protein binding can influence the distribution of drug, its pharmacological action, and elimination (Nawaz *et al.*, 1988).

Species difference in plasma protein binding could be the major factor responsible for the differences in pharmacological and toxicological response as well as pharmacokinetics of drugs between species. Clinical conditions that can alter blood pH, blood protein concentration, can effect the protein binding and response to the drug. In diseased condition the pH may rise to lethal levels (Lehninger, 1993).

Anton (1960) studied that the sulfonamide bound to plasma albumin was devoid of antibacterial activity but the degree of binding varied greatly in different

species. Therefore, the present study deals with the binding of sulfamerazine to the plasma proteins from buffaloes, cows, goats, and sheep and the affect of changing drug concentration, plasma pH and proteins on the binding.

MATERIALS AND METHODS

The *in vitro* plasma protein binding of sulfamerazine was investigated in four species of domestic ruminants. Fresh blood samples from buffaloes, cows, goats and sheep were collected in heparinized flasks from Municipal Slaughter House, Faisalabad. The samples were immediately brought to the laboratory where these were centrifuged and plasma was separated and used for the study of protein binding.

The protein binding of sulfamerazine was studied by changing the drug concentration, pH and plasma protein concentration. Stock solution of sulfamerazine was used to prepare the drug concentration in plasma ranging from 50, 100, 150, 200 and 250 µg/ml.

Ultrafiltrates of the plasma samples were prepared by ultrafiltration through a cellophane membrane with pore-size of 20-80⁰A (Poulsen, 1956) which permit molecules with a molecular weight upto 5000 to pass through the membrane. The concentration of sulfamerazine in the ultrafiltrates of plasma was determined by method of Bratton and Marshal (1939).

The pH of plasma samples from different species was recorded with the help of Corning model 3D pH meter. The pH of plasma was increased with addition of K₂HPO₄ and decreased with powder mixture of 0.1 M phosphate buffer containing KH₂PO₄/K₂HPO₄. The

extent of protein binding at normal pH of plasma and at increased and decreased pH values were determined at drug concentration of 50 µg/ml in the plasma of buffaloes, cows, sheep and goats.

Influence of plasma protein concentration on sulfamerazine protein binding was determined. The total plasma proteins in buffaloes, cows, sheep and goats were determined by method described by Varley *et al.* (1980). Plasma protein concentration in the samples was varied by 10 and 20 percent, decreased by adding distilled water and increased by evaporating the sample under vacuum at 37 °C. The extent of protein binding in normal and changed plasma protein concentration values was studied at a drug concentration of 50µg/ml.

RESULTS AND DISCUSSION

For the study of plasma protein binding of sulfamerazine the classical method of ultrafiltration (Poulsen, 1956) was employed which still remains the method of choice when reliable data on protein binding of drugs is required (Lindup, 1975; Aubry *et al.*, 1994). The results showing *in vitro* protein binding of sulfamerazine at various drug concentrations (50, 100, 150, 200 and 250 µg/ml) with plasma proteins in buffaloes, cows, sheep and goats have been presented in Table 1. The protein binding of sulfamerazine in buffaloes ranged between 51.9 to 64.5 percent, in cows 66.7 to 70.4, in sheep 61.9 to 67.7 and in goats 63.7 to 75.2 percent at various concentrations of drug (50 to 250 µg/ml). At low levels of drug concentration binding was highest. With the increase in plasma drug concentration the amount of bound drug increased while percentage binding decreased. Fish and Chow

(1997) found that serum protein binding is not concentration dependent (For levofloxacin) and Nelofer (1997) found positive correlation between drug concentration and bound drug. The plasma binding of sulfamerazine varied amongst species. Binding capacity was highest in the plasma of goats and in decreasing order in plasma from cows, sheep and buffaloes.

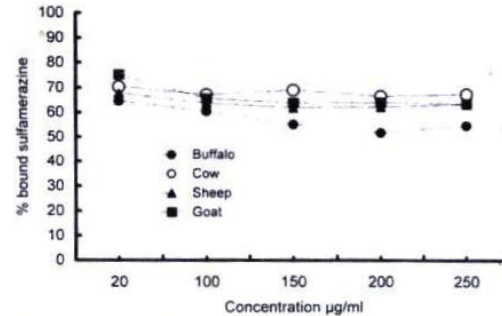


Fig. 1: Effect of Sulfamerazine concentration on *in vitro* plasma protein binding in domestic ruminants. (Each data point is average of 5 determinations)

The influence of plasma pH on the protein binding of sulfamerazine was recorded at a drug concentration of 50µg/ml and the mean results are presented in Table 2. In buffaloes and goats for a pH range of 7.4 to 7.8 percent-bound drug varied from 75.6 to 79.1 and 73.1 to 82.6 percent respectively. While in cows for a pH range 7.25 to 7.65 and in sheep for a pH range of 7.5 to 7.9, percent bound drug varied from 57.1 to 67.5 and 64.4 to 68.2 respectively. The plasma pH affected the binding, being lower at higher pH values and vice versa.

Table 1: Effect of sulfamerazine concentration on *in vitro* binding with the plasma proteins from four species of ruminants. (Each data point is average of 5 determinations)

Species	Total drug µg/ml	Drug conc. µg/ml		Percent drug	
		Free	Bound	Free	Bound
Buffalo	50	17.2	32.8	35.5	64.4
	100	39.5	60.5	39.5	60.5
	150	67.2	82.8	44.8	55.2
	200	96.2	103.8	48.1	51.9
	250	113	137	45.2	54.8
Cow	50	14.8	35.2	29.6	70.4
	100	32.8	67.2	32.8	67.2
	150	46.3	103.7	30.9	69.1
	200	66.5	133.5	33.3	66.7
	250	81.3	168.8	32.5	67.5
Sheep	50	16.6	33.3	33.3	67.7
	100	36.2	63.8	36.2	63.8
	150	57.2	92.8	38.1	61.9
	200	75.5	124.5	37.7	62.3
	250	91.3	158.7	36.5	63.5
Goat	50	12.4	37.6	24.8	75.2
	100	34.4	65.6	34.4	65.6
	150	54.1	95.9	36.0	64.0
	200	71.6	128.4	35.8	64.2
	250	92.3	157.7	36.9	63.7

Table 2: Influence of plasma pH on *in vitro* binding of sulfamerazine 50 µg/ml to plasma proteins (Each data point is average of 5 determinations)

Species	pH	Conc. µg/ml		Percent drug	
		Free	Bound	Free	Bound
Buffalo	7.4	11.0	39.0	22.0	78.0
	7.5	10.4	39.6	22.8	79.1
	7.6	12.0	38.0	23.9	76.1
	7.7	11.9	38.1	23.7	76.3
	7.8	12.2	37.8	24.4	75.6
Cow	7.25	16.2	33.8	32.5	67.5
	7.35	20.4	29.6	40.9	59.1
	7.45	20.3	29.7	40.6	59.4
	7.55	21.4	28.6	42.9	57.1
	7.65	20.9	29.1	41.8	58.2
Sheep	7.5	16.8	33.2	33.6	66.4
	7.6	15.9	33.1	31.8	68.2
	7.7	15.9	33.3	32.9	66.9
	7.8	16.6	33.4	33.2	66.8
	7.9	17.8	32.2	35.6	64.4
Goat	7.4	12.8	37.2	25.6	74.4
	7.5	9.20	40.8	18.4	81.4
	7.6	8.70	41.3	17.4	82.6
	7.7	13.4	36.6	26.9	73.1
	7.8	12.3	37.7	24.6	75.4

Table 3: Influence of plasma protein concentration on *in vitro* binding of sulfamerazine 50 µg/ml to plasma proteins (Each data point is average of 5 determinations)

Species	Protein concentration	Conc. µg/ml		Percent drug	
		Free	Bound	Free	Bound
Buffalo	20% dil	15.2	34.8	30.5	69.5
	10% dil	15.1	34.9	30.2	69.8
	Normal	13.5	36.5	27.0	73.0
	10% conc.	12.4	37.6	24.9	75.1
	20% conc.	10.9	39.1	21.8	78.2
Cow	20% dil	22.3	27.7	44.6	55.4
	10% dil	21.7	28.3	43.5	56.5
	Normal	20.7	29.4	41.3	58.7
	10% conc.	21.8	28.2	43.6	56.4
	20% conc.	23.3	26.7	46.7	53.3
Sheep	20% dil	20.0	30.0	40.0	60.0
	10% dil	18.0	32.0	36.0	64.0
	Normal	15.9	34.1	31.7	68.3
	10% conc.	15.4	34.6	30.8	69.2
	20% conc.	14.5	35.5	29.0	71.0
Goat	20% dil	11.0	39.0	22.0	78.0
	10% dil	12.8	37.2	23.9	76.3
	Normal	12.9	37.1	25.8	74.2
	10% conc.	12.8	37.2	25.7	74.3
	20% conc.	10.8	39.2	21.7	78.3

The influence of plasma protein concentration on *in vitro* binding of sulfamerazine 50 µg/ml to proteins has been presented in Table 3. For normal plasma protein concentration, percent bound drug was 73.0, 58.7, 68.3 and 74.2 for buffaloes, cows, sheep and goats respectively while for 10 and 20% diluted samples, percent bound drug was 69.8 and 69.5 (buffaloes) 65.5 and 55.4 (cows), 64 and 60 (sheep) and 76.3 and 78 percent respectively and for 10 and 20% concentrated plasma, percent bound drug was 75.1 and 78.2 (buffaloes), 56.4 and 53.3 (cows), 69.2 and 71.0 (sheep) and 74.3 and 78.3%, respectively. When total amount of protein in plasma was decreased the amount of bound drug also decreased and vice versa.

The studies indicate that binding of sulfamerazine to plasma protein was affected by the drug concentration in plasma, plasma pH, and total proteins. Percentage of bound drug was more than 5% in all species at studied drug concentrations.

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