Indian J. Pharmac (1987) I9 : 225-229 **INDIAN JOURNAL OF PHARMACOLOGY**

IN VITRO STUDIES ON PROTEIN BINDING OF RIFAMPICIN

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1. **Protein binding of rifampicin to varying albumin concentrations was carried out in vitro. Effect on other antitubercular drugs on protein binding of rifampicin was also studied.**

2. **The results indicated that rifampicin binding was influenced by albumin concentration, suggesting that it was chiefly bound to albumin.**

3. **Presence of other antitubercular drugs along with rifampicin decreased the percentage of bound rifampicin.**

Key Phrases : ***Protein binding of drugs, rifampicin***

Protein binding of drugs is clinically important because it is only the free drug which exerts the pharmacological action (Levy, *1980;* Wise 1983). Individual characteristics of human serum are known to influence protein binding of the drugs (Bridges and Wilson, 1976). One of the host characters which profoundly alters the *in vivo* protein binding is the nutritional status of the individual.

Our earlier studies on rifampicin indicated that protein binding of rifampicin is reduced in undernourished (unpublished data). It was observed that protein binding was further reduced in undernourished tuberculosis patients receiving rifampicin along with other anti-tubercular drugs. The differences in protein binding of rifampicin within undernourished population with similar serum albumin levels were not clear, Hence, in the present studies, extent of protein binding of rifampicin was determined *in vitro,* varying the concentration of albumin as well rifampicin. The effect

of other antitubercular drugs on protein binding of rifampicin was also investigated.

Plasma protein binding was determined on a equilibrium dialysis cell containing two separate chambers separated by cellophone membrane. Plasma (0.5 ml) diluted to give varying concentrations of albumin (both synthetic and fresh human plasma was used) was equilibrated with phosphate buffer pH 7.4 (Boman, 1977). After incubation for 18 h at **37°C,** the bound drug and free drug concentrations were estimated by microbiological assay using ***Bacillus Subtilis*** (ATCC 6633) as the test organism (Kiss et al., 1976). Fresh human plasma obtained from healthy volunteers, human albumin from Calbiochem, La Jolla, California and rifampicin obtained from Sigma, St. Louis, Missouri were used in the experiments. The extent of binding was determined by mixing rifampicin at varying concentrations with plasma and incubating it for one hour at 37°C followed by equilibrium dialysis as described above. The binding of rifampicin was determined at various concentrations of rifampicin. The *in vitro* binding of rifampicin in the presence of other antitubercular drugs namely INH (10 μg/ml) ethambutol (5 μg/ml), pyrazinamide (5 μg/ml) and ethionamide (20 μg/ml) were also studied.

The results of ***in vitro*** binding of rifampicin is given in table. Maximum binding was observed at 4 g/dl of albumin and is significantly more than the binding with 3 g/d1 and 2 g/dl: Binding of rifampicin to reconstituted human albumin was slightly lower at all concentrations of albumin as compared to fresh human plasma. Fresh human plasma was diluted with phosphate buffer to give 3 g/d1 and 2 g/d1 and ***in*** vitro binding to rifampicin was carried out to check if any non specific factors influenced rifampicin binding. The results given in table suggests that rifampicin binds to only albumin. The effect of other antitubercular drugs on rifampicin binding indicate that significant reduction in protein binding of rifampicin occurs when INH (10 μg/ml) and ethionamide (20 μg/ml) were added along with 10 μg/ml of rifampicin when these drugs were added together there was a significant decrease in the binding.

A number of drugs have the property of binding to plasma proteins. This is of importance in determining its volume of distribution, pharmacological activity and elimination (Keen, 1971). Our results on binding of rifampicin in normal plasma ageed well with reported values (Acocclla, 1978) The decreased binding of reconstituted albumin to rifampicin as compared to fresh human plasma implies that it is not as good as fresh plasma in its binding characteristics. It has been reported that with frozen plasma, the protein binding tended to be lower (Aoyagi, 1973).

Rifampicin binding was reduced in the presence of other drugs like LNH, ethambutol, pyrazinamide and ethionamide added individually as well as when added together suggesting that administration of other drugs may displace rifampicin from binding

Table-1. Binding rate of rifampicin to plasma proteins at varying concentrations

Rifampicin (ug/ml)	Binding rates %							
	A Fresh human albumin plasma			B Reconstituted human albumin			C Fresh human plasma (diluted with phosphate buffer pH 7.4)	
	(4.02)	(3.33)	(2.99)	(4.0)	(3.0)	(2.0)	(3.0)	(2.0)
2	84.1 ± 1.12	80.2 ± 0.62***	78.3 ± 1.21 ***	79.6 ± 0.41	75.5 ± 0.64***	52.7 ± 1.06***	73.8 ± 1.06	61.0 ± 0.58***
4	79.3 ± 1.61	76.4 ± 1.41*	75.0 ± 0.96**	77.4 ± 0.36	62.4 ± 0 81***	52.7 ± 1.06***	65.8 ± 0.56	53.0 ± 0.71***
6	77.2 ± 1.01	69 ± 0.82***	67.0 ± 1.41***	74.1 ± 0.62	58.9 ± 0.39***	48.9 ± 0.98***	57.6 ± 0.61	61.0 ± 0.58***
8	75.5 ± 1.21	64 ± 0.58***	63 ± 1.01***	72.7 ± 0.19	52.4 ± 0.76****	43.7 ± 0.84***		

Figures in parenthesis indicate concentration of albumin (g/dl)
The above values are mean of quadruplicate observations with their standard deviation
Values given under second and third columns were compared to the first column for 'A' & 'B' at each concentration of rifampicin values under second column is compared to the first for 'C' at each concentration of rifampicin
Significance was tested by students 't' test *P∠0.05; **P∠0.01; ***P∠0.001.

sites. It is well known that drugs such as ethambutol and INH are also protein bound (Kucers and Bennet, 1979). It has also been reported that in binding experiments using rifampicin, PAS decreased the binding of rifampicin (Aoyagi, 1973). There are no other reports on the effect of other drugs on the binding of rifampicin ***in vitro.*** The observation that presence of concomittantly administered drugs ***in vitro*** decreases the binding of rifampicin supports our ***in vivo*** binding reaults (Polasa and Krishnaswamy, 1984) where decreased rifampicin binding was observed in plasma of individuals receiving combination therapy for tuberculosis. Our earlier data (Polasa and Krishaswamy, 1984) indicated that protein binding of rifampicin was positively correlated to $Auc^{\circ}\text{-}\alpha$ (total) ($r=O.5$, $p<0.05$) indicating that bioavailability (AUC) of drugs is influenced by the extent of protein binding. These results on ***in vitro*** as well as ***in*** vivo indicate that in therapeutic practice, free levels of rifampicin are likely to be increased by concomittant administration of other antitubercular drugs. This in turn could lead to prolonged drug action or toxic drug reactions.

Acknowledgement :

The authors are grateful to Dr. B. S. Narasinga Rao, Director, National Institute of Nutrition for his encouragement. The technical assistance of Miss G. Gajalakshmi and typing assistance of Mrs. D. Lakshmi Rani is gratefully acknowledged.

References :

ACOCELLA, G. (1978). Clinical Pharmacokinetics of Rifampicin. ***Clin. Pharmacokinet., 3*** : ***108-127.***

AOYAGI, T. (1973). Protein binding of rifampicin to different individual sera. Scand. ***J. Res. Dis., 84*** : 44-49.

BOMAN, G. (1973). Protein binding of rifampicin : a Review. ***Scand. J. Res. Dis., 84*** : ***40-49.***

BRIDGES, J. W. and WILSON. **A. G.** E. (1976). Drug **serum** protein interactions and their biological significance In: ***Progress in drug metabolism.*** Vol. 1., eds. J. W. Bridges and L. F. Chasseud, p. 193-247, London: John Wiley.

CRAIG, W. **A.** and WELLING, P. G. (1977) Protein binding of antimicrobials : Clinical pharmacokinetic and therapeutic implications. ***Clin. Pharmacokinet. 2*** : ***252-268.***

KEEN, P. (1971). Effect of binding to plasma proteins on distribution, activity and elimination of drugs In: ***Concepts in biochemical pharmacology, Part I,*** ed. B. Gillette p. 2 13, Berlin : Springer-Verlag.

KISS, J. I., FRAGO, E., JUHAS, Z. I., BASCA, S. and FABIAN, E. (1976). Investigation on the serum and lung tissue of rifampicin in man. Int. ***J. Clin. Pharmac. 13*** : ***42-47.***

KUCERS, A. and BENNET, M. N. (1974) Edr Rifamycin. In: The use ***of antibiotics.*** p. ***552-585*** : London: William Heinemann Medical Books Ltd.

LEVY, G. (1980). Clinical implications of interindividual differences in plasma protein binding of drugs. ***Acta Pharmaceut Sci, 17*** : ***92-94.***

POLASA, K. and KRISHNASWAMY, K. (1984). Rifampicin kinetics in undernutrition. ***Br. J. clin. Pharmac. 17*** : ***481-484.***

WISE, R. (1983). Protein binding of beta-lactams: The effects on activity and pharmacology, particularly tissue penetration. ***J. Antimicrob.*** Chem. 12 : 1-18.

Received after revision, November 20th, 1988

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