The Sciences 1 (2): 87-90
March-April, 2001

Study of Protein Binding of Levofloxacin in Human Beings

Sheikh, M.A., S. Khanum, A. Ahmad, T. Iqbal,
Z. Hydair and Shakeela Naz

Levofloxacin is a quinolone antimicrobial which acts through the inhibition of bacterial topoisomerases that has been developed mostly for clinical use in human medicine. Its protein binding was investigated in human beings under indigenous conditions. Drug concentration of 1, 2, 3, 4 and 5μg/ml were added to the plasma and binding of levofloxacin was determined by ultrafiltration and its concentration in ultrafiltrates was determined by microbiological assay. The binding of levofloxacin showed positive correlation between drug concentration and percentage bound drug and maximum binding was observed at 5 μg/ml and 59% in human beings. The pH of plasma also affected the binding, being highest at 7.4 in human beings, and it was 53%. *In vitro* binding of levofloxacin was maximum at normal level of blood protein.

Key words: Protein binding, levofloxacin

A. Ahmad
Department of Chemistry, University of Agriculture, Faisalabad, Pakistan

Department of Chemistry, University of Agriculture, Faisalabad, Pakistan

Introduction

In systemic circulation and tissues, some concentrations of the drug may bind with plasma and tissue proteins. Plasma protein and It may significantly affects the distribution, biodisposition clearance and action of the drug (Lindup, 1975). Forces involved in the binding of drugs include ion-ion interactions, hydrogen bonding, dipole-dipole interactions and vander waal forces (Ladu, 1972). Many drugs are bound to plasma protein, mostly acidic drugs to plasma albumin and basic drugs to α-acid glycoproteins, binding to other plasma proteins generally occur to much smaller extent (Gillman, 1985). There is a dynamic equilibrium between the bound and free drug. When free drug leave the circulation, bound drug is released to restore the balance and bound drug can be regarded as storage depot (Brander *et al.*, 1991).

The difference in geonetics make the difference in protein s concentration and type between indigenous and exogenous populations. These genetic factors affects the pharmacokinetics and pharmacodynamics of the drugs.

Levofloxacin is a flouroquinolone antibiotic, having broad spectrum of *in vitro* activity against gram-positive and gram-negative bacteria. Levofloxacin like other flouroquinolones, exerts its antibacterial effects through inhibition of deoxyribonucleic acid (DNA) gyrase, a type of II topoisomerase. Levofloxacin is widely distributed in body with a mean volume of distribution 1.1L/kg (Fish and Chow, 1997). So, protein binding of levofloxacin in human beings was investigated to see the effect of drug concentration, plasma protein concentration, and plasma pH on the protein binding, under indigenous conditions.

Materials and Methods

Plasma protein binding of levofloxacin was determined by using blood samples of human beings. Human blood was obtained from the blood bank of Allied Hospital, Faisalabad and Chiniot Dialysis Center, Faisalabad. The blood samples were collected in test tubes containing heparin at the rate of one drop per 10 ml of the blood sample. Blood samples were centrifuged at 3000 rpm for 10-20 min. and plasma was separated for protein binding studies. Pure levofloxacin was obtained from Hilton Pharma Pvt. Ltd., Karachi. The stock solution of 100mg/100ml was prepared in deionized water. The protein binding of levofloxacin was determined by ultrafiltration through a cellophane membrane of pore size 20-80°A. The pores would permit the molecules upto 5000 molecular weight to pass through the membrane (Poulson, 1956). The influence of drug concentration, plasma pH and protein concentrations, biuret reaction (Gornall *et al.*, 1949) was determined. The concentration of levofloxacin in standard solutions and ultrafiltrates was estimated by microbiologicl assay according to disc agar diffusion method (Arret *et al.*, 1971) using *Staphylococcus aureus* as test organism.

Results and Discussion

Influence of drug concentration on binding: In human beings, binding of levofloxacin has increased with increasing drug concentration. At 1μg/ml, binding was 38% or 38μg/ml to plasma proteins, as drug concentration increased from 1 to 2 μg/ml, binding was increased and become 48% of the total drug. At 3, 4, and 5 μg/ml binding was 52, 54 and 59% respectively. Binding was highest at 5μg/ml (Table 1). With increase in drug concentration (moles) the % bound of the drug was also increased (Fig. 1). Under normal condition with increase in drug concentration, binding also increased but after saturation, no further increase is found. Low drug concentration, used in this study are much lower than the saturation points. At 5 μg/ml the binding of levofloxacin studied (Fish and Chow, 1997) was 24-38%. The difference may be due to genetic factors.

Effect of plasma pH on binding: The binding of levofloxacin in human being maximum at pH 7.4 (53%) which is the normal pH of human plasma. At pH 7.2, 7.3, 7.5 and 7.6 binding of Levofloxacin was 37, 48, 41 and 34% as represented by Fig.2 and Table 3. The pH below 7.3 leads to acidosis and above 7.5 leads to alkalosis (Chatterjea and Shinde, 1993). However, in animals a pH range of 7.00 to 7.80 have been stated as compatible with life range (Pitts, 1966) and animals tolerate variations (Nawaz and Shah, 1985). The indigenous cows, goats and sheep showed even longer variation and higher values of blood pH (Nawaz *et al.*, 1988). The pH change in plasma can change the ionization characteristics of both drug and protein. In any particular interactions, either ionized or non-ionized groups of both drug and protein involved in the reactions and very complex pH effects can result (Curry, 1977). Theoretically all changed sites of albumin acts as binding sites and change in pH, change these binding sites and hence binding of the drugs. The % values of protein bindings is 35, 32% at 10 and 20% dilution and 41, 45% at 10 and 20% concentration respectively as shown in Table 3 and Fig.3.

Effect of plasma protein concentration on binding: A graphic representation of protein binding of levofloxacin at various protein concentrations is shown in Fig. 4. Curry (1977) was investigated that most organic compounds used as drugs interact with albumin and not bound to a significant amount

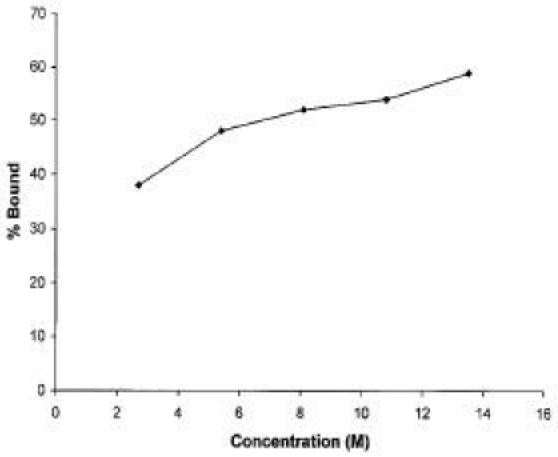

Fig. 1: Curve showing the effecto fo drug concentration on *in vitro* plasma protein binding of levofloxacin in human begins

to other plasma protein and any change in plasma protein effect the binding of drugs to the proteins. Amount and type of plasma protein vary in different species and even in individuals (Kaneko, 1980). The effect on protein binding becomes important when drug binding capacity of albumin molecules or concentration is abnormally low (McLaren, 1982). The low level of albumin in human has exhibited poor protein binding of tetracyclines in blood, so rapid elimination and clearance of drug from the body (Raghuan and Krishnaswamy, 1981). *In vitro* protein binding of levofloxacin was increased by increasing protein concentration because more binding sites available and binding decreased when protein concentration low because of decreased binding sites. These variations may be attributed to the change in the micro environment of binding sites resulting differences in

Table 1: Showing molar concentration of levofloxacin and its binding with plasma protein in human

Total drug (μg/ml)	Total M	Free M(D)	Bound M	% bound	G/alb M	r	1/r	1/d	r/D
1	2.7×10^{-6}	1.7×10^{-6}	1.0×10^{-6}	38	5.94×10^{-4}	1.68×10^{-3}	5.95×10^{2}	5.88×10^{5}	9.88×10^{2}
2	5.4×10^{-6}	2.8×10^{-6}	2.6×10^{-6}	48	5.94×10^{-4}	4.37×10^{-3}	2.28×10^{2}	3.57×10^{5}	15.60×10^{2}
3	8.1×10^{-6}	3.9×10^{-6}	4.2×10^{-6}	52	5.94×10^{-4}	7.07×10^{-3}	1.41×10^{2}	2.56×10^{5}	18.12×10^{2}
4	10.8×10^{-6}	5.0×10^{-6}	5.8×10^{-6}	54	5.94×10^{-4}	9.76×10^{-3}	1.02×10^{2}	2.00×10^{5}	19.52×10^{2}
5	13.5×10^{-6}	5.6×10^{-6}	7.9×10^{-6}	59	5.94×10^{-4}	13.29×10^{-3}	0.75×10^{2}	1.78×10^{5}	23.73×10^{2}

M = Moles of drug; r = Moles of drug bound per moles of albumin; D = free moles of drug

Table 2: Influence of pH on binding of levofloxacin

pH	Total drug μg/ml	Free drug	Bound drug	Free %	Bound %
7.2	3	1.878	1.122	63	37
7.3	3	1.550	1.450	52	48
7.4	3	1.410	1.590	47	53
7.5	3	1.770	1.230	59	41
7.6	3	1.990	1.010	66	34

Table 3: Influence of protein concentration on binding levofloxacin

Protein g/dl	Free drug	Bound	drug	Free %	Bound %
20% dil.	5.756	2.04	0.96	68	32
10% dil.	6.446	1.97	1.03	65	35
Normal	7.246	1.41	1.59	47	53
10% conc.	8.110	1.78	1.22	59	41
20% conc.	8.86	1.66	1.34	55	45

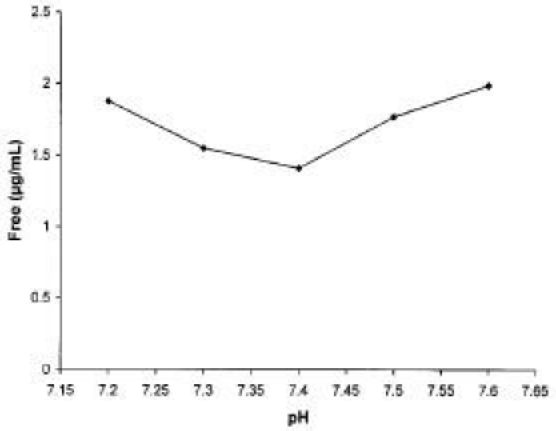

Fig. 2: Curve showing the effect of pH on *in vitro* plasma protein binding of levofloxacin in human beings

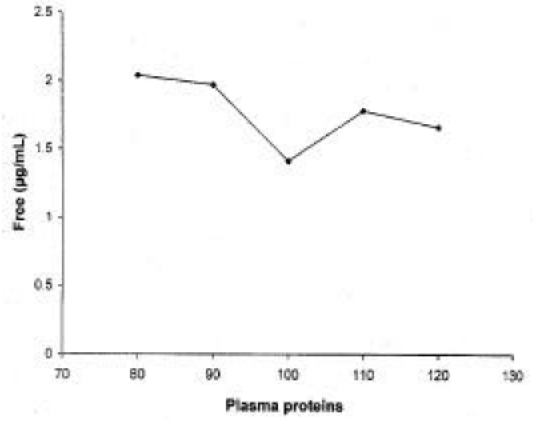

Fig. 3: Curve showing the effect of plasma protein concentration on *in vitro* plasma protein binding of levofloxacin in human beings

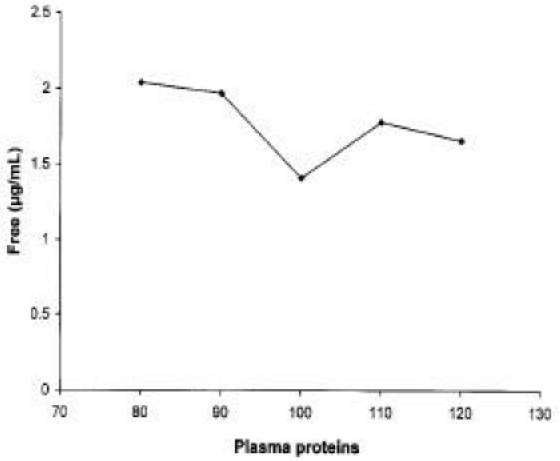

Fig. 4: Curve showing the effect of plasma protein concentration *in vitro* plasma protein binding of levofloxacin in human beings

binding. It should be needed to determine the protein binding of drugs under indigenous conditions to evaluate the drugs.

References

Arret, B., D.D. Johnson and K. Amiel, 1971. Outlines of detail for microbiological assay of antibiotics. J. Pharmaceut. Sci., 60:373-378.

Brander, G.C., D.M. Pugh, R.J. Bywater and W. L. Jenkins, 1991. Veterinary applied pharmacology and therapeutics. 5th Ed. Bailliere Tindall, London.

Curry, S.H., 1977. Drug disposition and pharmacokinetics with a consideration of pharmacological and clinical relationships. 2nd Ed. Blackwell Scientific Pub., U.K.

Chatterjea, M.N. and R. Shinde, 1993. Textbook of Medical Biochemistry. J. P. Brothers Medical Pub. New Dehli. 108-116.

Fish, D.N. and A.T. Chow, 1997. The clinical pharmacokinetics of levofloxacin. Department of Pharmacy Practice, University of Colorado Health Sciences Center, Denver, U.S.A. Clin. Pharmacokinet., 32: 101-119.

Gillman, A., L. Goodman, S. Goodman, W. Theodore, Rall, F. Murad, 1985. The Pharmacological Basis of Therapeutics. 7th Ed. Macmillan Pub. Co., New York.

Gornall, A.G., C.S. Bradawill and M.M. David, 1949. Determination of serum proteins by means of biuret reaction. J. Biol. Chem., 177: 751-766.

Kaneko, J.J., 1980. Clinical Biochemistry of Domestic Animals. 3rd Ed. Academic Press, New York.

Ladu, B.N., H.G. Mandel and E.L. Way, 1972. Fundamentals of drug metabolism and drug disposition. The Williums and Wilkins Co., Baltimore.

Lindup, W.E., 1975. Drug protein binding. Biochemical. Soc. Trans., 3: 635-640.

Mclaren, D., 1982. Nutrition and its disorders. Livingstone, Edinburgh.

Nawaz, M. and B.H. Shah, 1985. Geonetica considerations in the quality assurance of pharmaceuticals. Proc. Int. Seminar on policies, management and quality assurance of pharmaceuticals. 21-25th April WHO and Ministry of Health, Govt. of Pakistan.

Nawaz, M., T. Iqbal and R. Nawaz, 1988. Geonetical considerations in disposition kinetics evaluation of chemotherapeutic agents. Vet. Pharmacol., Toxicol and Therapy in food producing animal Vol.2, P.260, Proc. 4th Cong. Europ. Assoc. Vet. Pharmacol. Therap., Budapest.

Pitts, R.F., 1966. Physiology of the kidney and body fluids. An Introductory Text Year Book Medical Publications Inc., Chicago, USA.

Poulson, E., 1956. Renal clearance in cows. C. Fr. Mortensen, Copenhagen, Denmark.

Raghuan, T.C. and K.K. Krishnaswmy, 1981. Tetracyclines kinetics in undernourished subjects. J. Clin. Pharmacol. STher. Toxicol., 19: 409-413.