RESEARCH PAPER

Accepted 6 March 1998
Received 2 September 1997
Indian J. Pharm. Sci., 1998, 60(3) pp. 153-157

In vitro Studies on Effects of Cefotaxime Sodium and Metoprolol Tartrate on Goat Whole Blood Phospholipids

K. ROY*, S. RUDRA, A. U. DE AND CHANDANA SENGUPTA*
Division of Medicinal Chemistry
Department of Pharmaceutical Technology
Jadavpur University, Calcutta 700 032

Considering the importance of drug-lipid interaction in mechanism of action of drugs, *in vitro* effects of metoprolol tartrate and cefotaxime sodium on goat blood phospholipids have been studied in an attempt to correlate phospholipid binding capacity of the drugs to their biological activity. Statistical analysis of the findings indicates linear relationship of cumulative phospholipid loss with incubation period for both drugs with significant correlation.

DRUG response phenomenon is a complicated multistep process having a great degree of prob ability factors which overshadow the mechanistic aspects[1]. Prior to exhibiting biological response, a drug has to cross lipid barriers (membranes) and bind with the target site having complimentary surface property through highly specialized hydrophobic, polar, electronic and/or steric interactions depending on lipophilicity, electron density distribution and polarizability pattern at the surface of the drug and receptor[2], and undergo a favourable chain of physico-chemical events[3,4]. In nonspecific biological activities, partitioning and distribution of the drug in a certain membrane compartment is responsible for the activity[5,6]. Lipophilicity (partition co-efficient) and ionization parameters are the most important regarding transport, distribution and binding of drugs in biological systems[7,8]. Changes occurring in bio-membranes seem to be highly important in drug action mechanisms[9] and effects of drugs on phospholipids are likely to throw some light on pharmacodynamics of drugs since phosphopidis are important constituents of bio-membranes.

*For correspondence
*Present Address: Seemanta Inst. of Pharm. Sci., Mayurbhanj, Orissa - 757 086

In our previous communications, interactions of different categories of drugs with blood lipid constituents were reported[10-15]. In the present study, *in vitro* effects of cefotaxime sodium (CS), a chemotherapeutic drug, and metoprolol tartrate (MT), a cardiovascular drug, on goat blood phospholipids have been quantitatively measured.

Since blood is the transporting tissue by which drugs are carried to the target site, whole blood was chosen as the *in vitro* experimental model which may be considered to be simulative of more complex biological system. Goat whole blood was chosen as the lipid source because of its easy availability and close similarity to human blood[16].

MATERIALS AND METHODS

Whole blood collected from goat *(Capra capra)* and reagents of analytical grade purchased locally were used for the present study. The samples of cefotaxime sodium and metoprolol tartrate were supplied by Roussel India Ltd., Bombay and Concept Pharmaceuticals Ltd., Bombay respectively.

Collection of goat blood and its incubation with/without drug: Goat blood was collected in a sterile vessel containing anticoagulant solution (sodium citrate in saline) and filtered through sterile cloth to remove foreign

materials like hair and dust and was kept in a refrigerator under nitrogen atmosphere for further work.

A portion of the collected blood was treated with a calculated dose (CS 40 mg%; MT 0.2 mg%) of drug solution in saline, continuously stirred for one hour below 15° and kept in refrigerator under nitrogen atmosphere for 10 days. Control sample (drug untreated) was also incubated under the similar condition.

Extraction of total lipid from incubated blood: Ten ml of blood (control) was extracted twice according to the method of Bligh and Dyer[17] by shaking for 1 hour each time in a BOD incubator shaker with a mixture (45 ml) of methanol and chloroform (2:1 v/v) below 20° followed by centrifugation (after each extraction) at 3000 rpm for 15 minutes and transfer of the supernatant fluid (extract) to a separate container. The combined total lipid extract was made upto 75 ml with the same solvent mixture. This constituted the control (zero day incubation).

Using similar procedure total lipid was extracted from both control and drug treated blood on 1st, 2nd, 4th, 6th, 8th and 10th days of incubation.

Estimation of phosphorus in total lipid: The inorganic phosphate content of total lipid was estimated by colour development from reaction between phosphomolybdate and amidol (2,4-diaminophenol) according to the method of Allen[18]. Different aliqouts (0.5, 1.5, 3, 4, 5 ml) of total lipid (75 ml) were used to estimate the phosphate content on 1st, 2nd, 4th, 6th, 8th and 10th days of incubation for both control and drug-treated blood samples. Control sample phosphate content was determined also on day 0 (the day on which drug was added to the test sample). The colour developed was analysed by EC digital spectrocolorimeter GS5700A at 680 nm.

RESULTS AND DISCUSSION

The estimated inorganic phosphate contents of different samples were compared to that of control sample (0 day incubation). The control sample did not show any significant change in phosphate content in different days. Drug-treated blood samples showed significant difference in phosphate content with the control.

The findings are presented in the tables 1 and 2 which show incubation of blood with both drugs leads to reduction in inorganic phosphate content which may be accounted due to their binding with phospholipids.

Phospholipids binding of cefotaxime is in good correlation with its capability of permeation through lipid barriers as exemplified by the facts that cefotaxime can penetrate into cerebrospeinal fluid in sufficient concentration to be useful for the treatment of meningitis and it can also cross placenta and penetrate into aqueous humor[19]. The present findings is further corroborated by relatively more activity of cefotaxime against gram-negative bacteria than gram-positive strains[19]. It is known that gram-negative bacteria are inherently more resistant to antimicrobial agents because they have additional complex lipid envelop, composed of lipoproteins, lipopolysaccharides and phospholipids, beyond the peptidoglycan layer[20]. The outer lipid layer restricts uptake, penetration and attainment of sufficient concentration of antimicrobial agents in the cells.[21].

Phospholipid binding of metoprolol, a β1 adrenoceptor antagonist having high lipid solubility (log P = 2.15)[22], may be correlated with its hydrophobic interaction with the lipophilic receptor site and it may be presumably a prerequisite for its cardiovascular activity.

Regression analysis of data to fit cumulative percent reduction (PR) of phosphorus content as a function of incubation period (D) into a linear model has been done using method of least squares and in case of both drugs good correlation is found with statistical significance ($P < 0.001$) (tables 1A and 2A).

Comparison between slopes of the two regression lines shows $t = 0.0428$ (D.F. = 32) which indicates that there is no significant difference in rates of phospholipid binding with respect to incubation period (d(PR)/dD) between the two drugs. Analysis of variance (tables 1B and 2B) indicates influence of both biological source and period of incubation in the variations of the data.

In summary, both drugs (CS and MT) have phospholipid binding affinity which might be an important factor for the mediation of their therapeutic activity.

Table 1: Cumulative percent reduction (PR)* in blood phosphorus content as a function of incubation period (D) after treatment with Cefotaxime sodium

Days of incubation (D)	#1	#2	#3	Average ± S.E.
1	17.95	14.24	12.28	14.82 ± 1.66
2	26.02	22.67	17.48	22.06 ± 2.48
4	27.69	28.54	23.16	26.46 ± 1.67
6	31.79	33.38	27.76	30.98 ± 1.67
8	34.03	37.47	31.96	34.15 ± 1.30
10	36.50	39.64	34.40	36.85 ± 1.52

* Averages of five observations. #1, #2 and #3 indicate three separate experiments using blood from a different animals each time.

Table 1A - Regression analysis of data of table 1

Regression equation[a]	PR = 2.2716 D + 15.8167
Correlation Coefficient (r)[b]	[b]0.9184
Significance of correlation (r versus 0)	t = 9.28 (D.F. = 16) P < 0.001
Estimate of variance of PR	10.9241 (D.F. = 16)
Standard error of estimate (of PR on D)	3.1162
95% Confidence interval of slope	2.2716 ± 0.5187
95% Confidence interval of intercept	15.8167 ± 3.1479

a. Method of least squares b. Pearson's correlation coefficient
Note - The last column of Table I is excluded in calculations for obvious reason.

Table 1B - Analysis of variance of data of table I

	Between animals	Between days
F ratio	15.140	72.811
Degrees of freedom	(2,10)	(5,10)
Significance level	p < 0.01	P < 0.01

Table 2 - Cumulative percent reduction (PR)* in blood phosphorus content as a function of incubation period (D) after treatment with metoprolol tartrate

Days of incubation (D)	#1	#2	#3	Average ± S.E.
1	12.09	6.94	12.55	10.53 ± 1.80
2	16.65	13.29	14.38	14.77 ± 0.99
4	20.36	17.67	19.89	19.03 ± 0.83
6	26.06	20.94	23.41	23.47 ± 1.48
8	28.74	27.57	26.64	27.65 ± 0.61
10	34.40	30.75	30.56	31.90 ± 1.25

* Averages of five observations, #1, #2 and #3 indicate three separate experiments using blood from a different animal each time.

Table 2 A - Regression analysis of data of table 2

Regression equation[a]	PR = 2.2838 D + 9.4720
Correlation coefficient (r)[b]	0.9687
Significance of correlation (r versus 0)	t = 15.61 (D.F. = 16) P < 0.001
Estimate of variance of PR	3.9067 (D.F. = 16)
Standard error of estimate (of PR on D)	1.8634
95% Confidence interval of slope	2.2838 ± 0.3102
95% Confidence interval of intercept	9.4720 ± 1.8825

a. Method of least squares b. Pearson's correlation coefficient

Note - The last column of Table 2 is excluded in calculations for obvious reason.

Table 2B - Analysis of variance of data of table 2

	Between animals	Between days
F ratio	11.053	113.973
Degrees of freedom	(2,10)	(5,10)
Significance level	P < 0.01	P < 0.01

ACKNOWLEDGEMENTS

The authors thanks the U.G.C., New Delhi., for financial assistance to K.R. and S.R.

REFERENCES

1. Hansch, C. and Fujita, T., **J. Am. Chem. Soc.**, 1964, 86, 1616.
2. Kubinyi, H., In: Wolff, M.E., Ed., **Burger's Medicinal Chemistry and Drug Discovery**, 5th Ed, Vol. I, John Wiley & Sons, New York, 1995, 552.
3. Williams, D.H., **Aldrichimica Acta.**, 1991, 24, 71.
4. Sharp, K., Nicholis, A., Friedman R. and Honig, B., **Biochemistry.**, 1991, 30, 9686.
5. Herbette, L.G., Chester, D.W. and Rhodes, D.G., **Biophys. J.**, 1986, 49, 91.
6. Mason, R.P., Rhodes, D.G. and Herbette, L.G., **J. Med. Chem.**, 1991, 34, 869.
7. Waterbeemd, H. van de and Testa, B., **Drug Res.**, 1987, 16, 85.
8. Taylor, P.J., In: Hansch, C., Sammes, P.G. and Taylor, J.B., Eds., **Comprehensive Medicinal Chemistry. The Rational Design, Mechanistic Study & Therapeutic Applications of Chemical Compounds**, Vol. 4, Pergamon Press, Oxford, 1990.
9. Seydel, J.K., Velasco, M.A., Coats, E.A., Cordes, H.P., Kunz, B. and Wiese, M., **Quant. Struct - Act. Relat.**, 1992, 11, 205.
10. Dutta, H., Mehta, N.K.D., Sengupta, M., Pal, D.K., Sengupta, C. and De A.U., **Ind. J. Pharm. Sci.**, 1988, 50, 328.
11. Dutta, H., De, A.U., Pal. D.K., Sengupta M. and Sengupta, C., **Ind. J. Biochem. Biophys.**, 1991, 28, 210.
12. Dutta, H., Pal, D.K., Sengupta, M. and Sengupta, C., **Ind. J. Biochem. Biophys.**, 1993, 30, 128.
13. Sengupta, M., De, A.U., Dutta, H., Pal, D.K. and Sengupta, C., **Ind. J. Exp. Biol.**, 1993, 31, 21.
14. Sengupta, M., De, A.U. and Sengupta C., **Ind. J. Biochem. Biophys.**, 1995, 32, 302.
15. Dutta, H., De. A.U. and Sengupta, C., **Ind. J. Biochem. Biophys.**, 1996, 33, 76.
16. Hilditch T.P. and Williams P.N., **The Chemical Constitution of Natural Fats**, Chapman & Hall, London, 1964, 13, 100, 128.
17. Bligh, E.G. and Dyer, W.J., **Can. J. Biochem. Biophys.**, 1959, 37, 911.
18. Allen, R.J.L, **Biochem. J.**, 1940, 34, 858.
19. Mandell, G.L. and Petri, W.A. Jr., In: Hardman, J.G. and Limbard, L.E., Eds., **Goodman & Gilman's The Pharmacological Basis of Therapeutics.**, 9th Ed. McGraw Hill, New York, 1996, 1094.
20. Hugo, W.B., In: Hugo, W.B. and Russell, A.D. Eds., **Pharmaceutical Microbiology**, 4th Ed, Blackwell Scientific Publications, Oxford, 1987, 5.
21. Franklin, T.J., In: Hugo, W.B. and Russell, A.D., Eds., **Pharmaceutical Microbiology**, 4th Ed Blackwell Scientific Publications, Oxford, 1987, 203.
22. Hoffman, B.B. and Lefkowitz, R.J. In: Hardman, J.G. and Limbard, L.E. Eds., **Goodman & Gilman's The Pharmacological Basis of Therapeutics**, 9th Ed. McGraw Hill, New York, 1996, 236.