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Effects of Ceftizoxime, Ceftriaxone and Acyclovir on Goat Whole Blood Phospholipids in Relation to Their Therapeutic Activities

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***In vitro* studies on effects of two cephalosporin antibacterials, ceftizoxime sodium (CZS) and ceftriaxone sodium (CTS), and one antiviral, acyclovir (AC), on goat whole blood phospholipids were carried out. Significant reduction in inorganic phosphorus content was found after 24 h of incubation, in cases of CZS and CTS with respect to the corresponding control samples. This finding suggests that CZS and CTS might have affinity for phospholipids and this may be correlated with their ability to penetrate through complex outer lipid envelop of gram-negative bacteria. The same may also be correlated well with their good penetration capacity into cerebrospinal fluid. On the other hand, AC treated samples did not show significant difference with the control. Thus, AC may be devoid of phospholipid binding capacity.**

Biological membranes are composed of amphipathic phospholipids and sterols forming a bilayer with intrinsic and extrinsic proteins in varying combinations[1]. Lipids being important constituents of all types of biomembranes that act as barriers during absorption and partitioning of drugs into various compartments prior to reaching and subsequent binding with the receptive tissues, effects of drugs on lipid constituents may explain some facets of their mechanistic aspects[2-10]. However, drug action on membrane constituents[11] has hitherto remained the most neglected field of drug research[12].

In our ongoing effort to explore drug lipid interactions[2-10] effect of drugs on phospholipids has been considered as one of the parameters of drug-induced changes in lipid constituents that may be related to the biological action of drugs. In a previous communication possible binding of antiamoebic diloxanide furoate with phospholipids was suggested for enhanced derangement of the lipid cyst matrix and consequent decystification of *E. hystolytica*[5]. Phospholipid binding of some other drugs was also related to their biological actions, e.g., cardiovascular action of propranolol[4], metoprolol[8], digitoxin[7] and digoxin[7] and local anesthetic and antiarrythmic action of lignocaine[3] etc.

The present communication is an attempt to document the effects of two cephalosporin antibacterials, ceftizoxime sodium (CZS) and ceftriaxone sodium (CTS) and one antiviral, acyclovir (AC) on goat whole blood phospholipids in relation to their therapeutic activities.

MATERIALS AND METHODS

Blood being the transporting tissue by which drug are carried to the critical reaction site where a favourable chain of physicochemical events[13] occurs to trigger ultimate therapeutic response, whole blood was chosen as the *in vitro* experimental model that may be considered simulative of a more complex biosystem. Goat whole blood was chosen as the lipid source because of its easy

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availability and close similarity to human blood in composition[14].

Collection and preservation of goat whole blood : Goat whole blood was collected in sterile vessel containing anticoagulant solution (normal saline, 5%; sodium citrate, 0.35%) and then filtered through cotton to remove dust and hair and stored below 15° under nitrogen atmosphere for further work.

Incubation of blood samples with or without drug : Whole blood samples were incubated with (test) or without (control) drug samples for one day and phosphorus content of drug treated samples (24 h) was compared to those of control (0 h) and control (24 h). The drugs were used in doses that are within the ranges of normal human therapeutic doses. CZX and CTS were treated with blood as solutions in saline at effective concentrations of 40 mg% and 80 mg% respectively. A homogenous suspension of AC in saline was treated with blood at an effective concentration of 4 mg%.

Determination of phosphorus content of whole blood: Phospholipid binding capacity of drugs was estimated in terms of loss in inorganic phosphorus content in whole blood as detailed below.

Extraction of total lipid from whole blood :

Extraction of total lipid from whole blood (drug treated or untreated) was done in a stoppered vessel following the method of Bligh and Dyer[15] with a mixture of methanol and chloroform (45 ml, 2:1 v/v) for 1 h in a B.O.D. incubator shaker below 15°. The mixture was centrifuged (15 min., 3000 rpm) and the supernatant was filtered through Whatman No. 1 filter paper on COORS no. 3 Buchner funnel under mild suction and collected. The residue was again extracted with the same solvent mixture (45 ml) for 1 h followed by centrifugation of the mixture and separation of the supernatant layer by filtration. The filtrate was added to the previously collected filtrate. The total volume of the extracted lipids was made upto 75 ml with the same solvent mixture. All the extraction and centrifugation operations were done below 15°.

Estimation of phosphorus in total lipids :

The phosphorus content was determined by the procedure of Allen[16]. In this colorimetric estimation method, a solution of amidol (2,4-diaminophenol hydrochloride) in sodium sulphite was used as the reducing agent and ammonium molybdate (8.4%) was used as the colour developing reagent. During the estimation of phosphorus in total lipids (75 ml, extracted from 10 ml of whole blood), five aliquots each of 3 ml were taken in digestion tubes and were digested with perchloric acid (70%) till the contents became colourless. After cooling the tubes, the digested materials were diluted to 12.5 ml with glass distilled water and mixed well with the help of a vortex mixture. Amidol solution (2 ml) and ammonium molybdate solution (1 ml) were added to each tube and mixed well. It was then kept for 20 minutes to develop a blue colour. After the colour development, the solutions were quantitatively transferred to 25 ml volumetric flasks and the volume was made up with glass distilled water. Absorbance of each solution was noted at 680 nm using EC GS5700B spectrocolorimeter against a reagent blank set at 100% transmittance. The amount of phosphorus in blood samples was determined from the regression equation that was obtained from standard curve data using standard sodium dihydrogen phosphate.

Inorganic phosphorus contents of drug treated blood samples were estimated after 24 h of incubation and compared to those of corresponding control values at 0 h (at the beginning of experiment) and 24 h of incubation. For each sample (control or drug treated) five measurements were taken and experiment with each drug was repeated in five animal sets.

RESULTS AND DISCUSSION

The results supported with statistical analyses by 't' test are listed in Tables 1, 2 and 3. From the tables it is evident that control values did not show significant change in phosphorus content after 24 h of incubation. In the case of CZS, significant reduction ($p \leq 0.05$) of inorganic phosphorus content was found in drug treated blood samples after 24 h of incubation with respect to the control values and this may be attributed to phospholipid binding of the drug that may occur possibly by interactions with polar groups of phospholipids. Percent change in inorganic phosphorus content with respect to control (0 h) was not found statistically significant (at $p \leq 0.05$) in case of CTS when the calculation was done on average data of five animal sets (Table 2). However, when the percent changes were calculated in individual animal sets of CTS, significant loss (at $p \leq 0.05$) in inorganic phosphorus was found in all cases. To ascertain the statistical significance of the observed changes, multiple comparison analysis according to the least significant difference procedure[17] was performed and it was found

TABLE 1 : EFFECTS OF CEFTIZOXIME (CZX) AND ACYCLOVIR (AC) ON GOAT WHOLE BLOOD PHOSPHOLIPIDS AFTER 24 H OF INCUBATION

Animal sets	Average# phosphorus content (µg/ml)±S.E. (n=5)			
	Control (0 h)	Control (24 h)	CZX (24 h)	AC (24 h)
A1	21.43±0.21	21.59±0.25	20.45±0.42	21.44±0.11
A2	22.68±0.52	22.54±0.38	19.21±0.23	22.36±0.39
A3	20.03±0.37	20.27±0.25	18.59±0.44	19.52±0.17
A4	21.48±0.16	21.74±0.42	20.36±0.17	21.40±0.12
A5	21.58±0.13	21.51±0.16	19.11±0.23	21.53±0.28
Average*±SE	21.44±0.42	21.53±0.36	19.54±0.37	21.25±0.47
Per cent change$ with respect to control (0 h)	—	+ 0.42%[b]	-8.86%[a]	-0.89%[b]

+ Same source of blood was used for the experiments with CZX and AC # Average of five observations
* Average of five animal sets SE = Standard error (degree of freedom = 4)
$ Calculated according to the average data of five animal sets (degree of freedom = 8)
a. Significant at $p < 0.05$; b. Not significant at $p \leq 0.05$.

TABLE 2 : EFFECTS OF CEFTRIAXONE (CTS) ON GOAT WHOLE BLOOD PHOSPHOLIPIDS AFTER 24 H OF INCUBATION

Animal sets	Average# phosphorus content (µg/ml) ±S.E (n=5)		
	Control (0 h)	Control (24 h)	CTS (24h)
A1	24.28±0.56	23.09±0.94	20.45±0.52
A2	17.33±0.32	17.55±0.20	14.12±0.28
A3	16.41±0.33	16.64±0.35	12.80±0.39
A4	12.48±0.30	12.84±0.22	10.82±0.42
A5	13.84±0.54	14.44±0.52	12.14±0.41
Average*±SE	16.87±2.05	16.91±1.75	14.07±1.68
Per cent change$- with respect to control (0 h)	------	+ 0.24%[b]	- 16.60%[b]

# Average of five observations * Average of five animal sets
SE = Standard error (degree of freedom = 4) $ Calculated according to average data of five animal sets (degree of freedom = 8)
b. Not significant at $p \leq 0.05$.

TABLE 3 : RELATIVE PERCENT CHANGES IN INORGANIC PHOSPHORUS CONTENT IN GOAT WHOLE BLOOD AFTER 24 HOURS OF INCUBATION WITH CEFTIZOXIME (CZX), CEFTRIAXONE (CTS) AND ACYCLOVIR (AC)

Animal sets	Per cent changes* in inorganic phosphorus content					
	with respect to control (0 h)			with respect to control (24 h)		
	CZS	CTS	AC	CZS	CTS	AC
A1	-4.57[b]	-15.75[a]	0.04[b]	-5.25[a]	-11.43[a]	-0.67[b]
A2	-15.29[a]	-18.50[a]	-1.40[b]	-14.79[a]	-19.51[a]	-0.81[b]
A3	-7.20[a]	-22.00[a]	-2.53[b]	-8.28[a]	-23.05[a]	-3.66[a]
A4	-5.19[a]	-13.27[a]	-0.38[b]	-6.33[a]	-15.72[a]	-1.58[b]
A5	-11.44[a]	-12.28[a]	-0.25[b]	-11.15[a]	-15.97[a]	0.08[b]
Average#	-8.74	-16.36	-0.90	-9.16	-17.14	-1.33
±S.E.	±2.03	±1.78	±0.47	±1.73	±1.96	±0.64

*Degree of freedom of 't' values = 8 [test for equality of two means] a. Significant at $p < 0.05$; b. Not significant at $p \leq 0.05$

Average = Mean of five animal sets calculated form relative percent change data of individual animal sets

S.E. = standard error of mean (degree of freedom = 4).

(Table 4) that the CTS treated samples were statistically significantly different (at $p \leq 0.05$) from the control samples. In analogy to the case of CZX, the observed change may be due to phospholipid binding potential of the drug. In the case of AC no significant loss (at $p \leq 0.05$) in phosphorus content was found after 24 h and the values matched with those of the control samples. This suggested that AC did not bind with the phospholipids to any significant extent.

The possible affinity of CZX and CTS for phospholipids as suggested above is in good agreement with their proven effectiveness and record of clinical successes for treatment of meningitis that becomes possible due to their good penetration capacity into cerebrospinal fluid (CSF)[18]. The blood - CSF barrier located in choroid plexus is composed of lipoidal choroidal epithelium having tight junctions that limits entry of polar drugs into CSF[19]. Despite having a number of polar functional groups and high water solubility[20], these drugs may enter into CSF in sufficient quantities to be useful for treatment of meningitis[18], possibly through polar interactions and subsequent binding with amphipathic phospholipids. Loss in inorganic phosphorus was reported also in case

TABLE 4 : EFFECTS OF CEFTIZOXIME, CEFTRIAXONE AND ACYCLOVIR ON GOAT WHOLE BLOOD PHOSPHOLIPIDS AFTER 24 HOURS OF INCUBATION : MULTIPLE COMPARISON ANALYSIS OF DATA

Reference table	Pooled variance (s^2)$	Critical difference (p = 0.05)* (least significance difference)	Ranked means+
Table I	0.2590 [df = 12]	0.7013	(C24, C0, AC) (CZX)
Table II	0.3643 [df = 8]	0.8803	(C24, C0), (CTS)

$ Error mean square of two way ANOVA df = Degree of freedom * Ref. 17

+ Two means not included within same parentheses are statistically significantly different at $p \leq 0.05$.

Keys : CO = Control (0 h), C24 = Control (24 h), CZX = CZX (24 h), CTS = CTS (24 h), AC = AC (24 h)

of another third generation cephalosporin, cefotaxime, in an earlier study[8]. Cephalosporins are also reported to cross various other lipoidal barriers, e.g., they cross placenta and are found in high concentrations in synovial and pericardial fluid[18]. Their penetration into aqueous humor of eye is relatively good.

Phospholipid binding potential of CZS and CTS is in good relation with that these are active against gram-negative bacteria[18] that have extra outer lipid envelop composed of lipopolysaccharides, lipoproteins and phospholipids beyond the peptidoglycan layer making the cell well more impermeable to antimicrobial agents[21]. Phospholipid binding of these drugs may play critical role for penetration through lipid barrier of relatively resistant gram-negative bacteria and subsequent attainment of sufficient intracellular concentration[22]. In this context the finding of lack of binding capacity of AC with phospholipids seems to be interesting, since virus particles have an outer protein coat (instead of lipid layer) surrounding their genetic materials.

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