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Centchroman: Effects on Blood-Lipid

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Considering the high lipophilicity (log P=7.41) of centchroman playing a critical role in the mediation of its biological activities (both therapeutic and toxic), effects of the drug on blood lipid constituents were studied using goat's whole blood as the lipid source. The results reveal that the drug has significant affinity in binding with phospholipid which is further corroborated by changes in fatty acid composition. Lipid binding potential of the drug may have role in its therapeutic effect. Lipid peroxidation induction potential of centchroman has been quantitatively measured in the context of its toxicity. However, the drug does not cause significant extent of lipid peroxidation and this is in good aggreement with its safety record.

Partition coefficient of a drug plays a vital role in absorption, transport and subsequent concentration in the appropriate site of action[1]. While passing through the cell membrane, the partition coefficient factor of a drug may be equally responsible for changes in the lipid constituents that may be reflected in changes in composition of fatty acids and extent of lipid peroxidation[2-4].

The present investigation has been designed to explore any possible relationship existing amongst the biological responses (both therapeutic and toxic) of a non-steroidal, non-hormonal contraceptive agent, centchroman, its partition coefficient and the changes in the lipid constituents of blood such as lipid-loss, change in fatty acid composition and induction of peroxidation. Lipid-loss and fatty acid-changes are linked with therapeutic effect[5,6] while the lipid peroxidation breakdown products with toxic effects of the drug[7].

MATERIALS AND METHODS

Goat blood collected from five different sources, silica gel (E. Merck, West Germany) and authentic samples of fatty acid methyl ester (FAME) (V.P. Chest Institute, Center for Biochemical Technology, New Delhi) were used for the present work. Estimation of fatty acid composition was carried out at Regional Sophisticated Instrument Center, Bose Institute, Calcutta.

Total lipids were extracted according to the method of Bligh and Dyer[8] with methanol-chloroform mixture (2:1 v/v) and estimated in terms of phosphorus contents according to the procedure of Allen[9,10]. The colour developed was measured using colorimeter (E C model GS 5700A) at 680 nm.

For estimation of fatty acid composition, saponification of whole lipid was done with methanolic sodium hydroxide and the free fatty acids were converted to their corresponding methyl esters using methanolic hydrochloric acid (2.5%) according to the method of Kates[11,12]. The FAMEs were first purified by TLC[13] on silica Gel G-coated plates using n-hexane:diethyl ether:glacial acetic acid (80:20:0.25% v/v) as solvent system. Then the purified FAMEs were finally analyzed by GLC on a Pye Unicam (model 104), equipped with FID and glass column packed with 10% DEGS supported on 100-120 mesh chromosorb WAW, with a nitrogen flow rate of 60ml/min, in an oven maintained at temperature 190°. Detector and injection port temperatures were 269° and 240° respectively.

* For correspondence

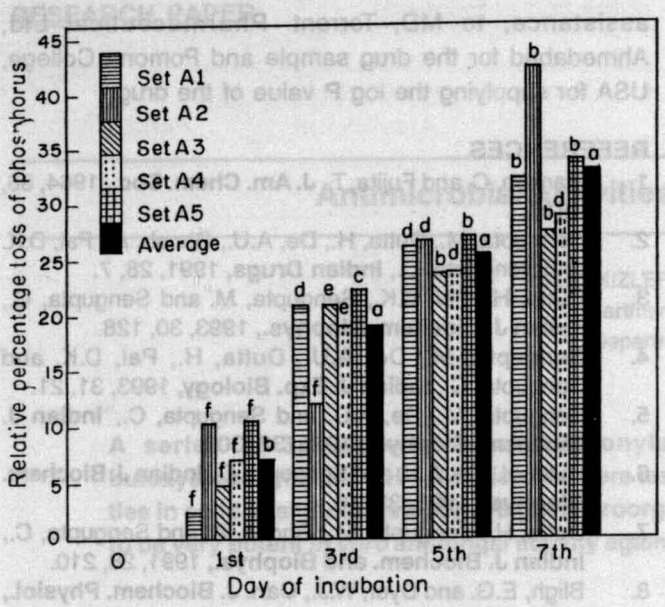

Fig. 1 : Relative per cent loss of phosphorus in blood-lipid due to centchroman ($2.6x10^{-4}$ µmol/ml of blood)

Probability levels (P) of per cent loss of phosphorus : a<0.1%; b<1.0%; c<2.0%; d<5.0%; e<10.0% and f>10%.

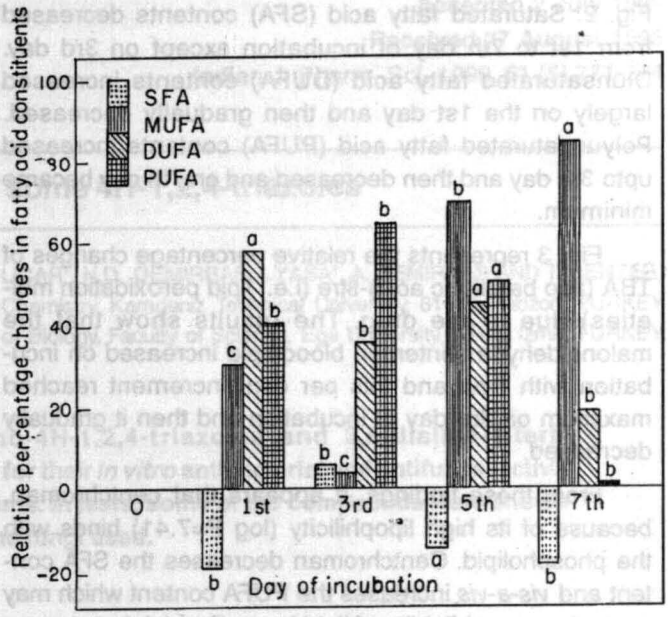

Fig. 2 : Relative per cent changes* in fatty acid constituents of blood-lipid treated with centchroman

Probability level (P) of per cent changes: a<10%; b<20% and c<30%.
*Mean of 3 sets.
SFA, Saturated fatty acids ($C_{14:0}$; $C_{16:0}$; $C_{18:0}$; $C_{20:0}$; $C_{22:0}$) ;
MUFA, Monounsaturated fatty acids ($C_{18:1}$; $C_{20:1}$)
DUFA, Diunsaturated fatty acid ($C_{16:2}$)
PUFA, Polyunsaturated fatty acids ($C_{18:3}$; $C_{18:4}$; $C_{20:5}$; $C_{22:3}$; $C_{22:4}$)

FAMEs were identified by using standard methyl palmitate and methyl stearate as primary standards and comparing the relative retention time of the sample peaks with those of standard FAMEs separated on the same column under identical operational conditions. To estimate lipid peroxidation the method of Tarladgis *et al.*[14-16] has been utilized. The amount of lipid peroxide end product (malonaldehyde) was calculated from the standard curve.

In all the experiments stated above, centchroman was used at $2.6x10^{-4}$ µmol/ml of blood concentration which is maximum in blood-serum[23]. Both the control and drug treated blood were incubated upto 7 days, i.e.; the biological half-life of centchroman[23] and experiments were performed on 1st, 3rd, 5th and 7th day.

RESULTS AND DISCUSSION

Fig. 1 shows relative per cent reduction in phosphorus content of total lipid extract due to treatment of whole blood with drug. The results show that the average loss of phosphorus in whole lipid due to drug effect is significant from 1st day to 7th day of incubation. This may be attributed to the phospholipid binding capacity of the drug.

Lipid binding of the drug is further corroborated by changes in fatty acid composition as indicated from

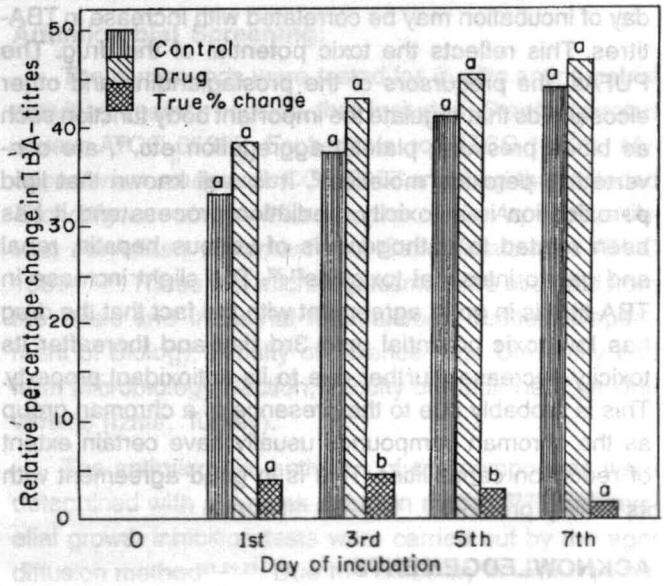

Fig. 3 : Relative per cent changes* in TBA-titres due to centchroman

Probability level (P) of percent change : a<0.1% and b<1.0%
*Mean of 5-sets of 4-samples each

Fig. 2. Saturated fatty acid (SFA) contents decreased from 1st to 7th day of incubation except on 3rd day. Diunsaturated fatty acid (DUFA) contents increased largely on the 1st day and then gradually decreased. Polyunsaturated fatty acid (PUFA) contents increased upto 3rd day and then decreased and on 7th day became minimum.

Fig. 3 represents the relative percentage changes of TBA (thio barbituric acid)-titre (i.e., lipid peroxidation moieties) due to the drug. The results show that the malonaldehyde content in blood-lipid increased on incubation with time and this per cent increment reached maximum on 3rd day of incubation and then it gradually decreased.

From these findings, it appears that centchroman, because of its high lipophilicity (log P=7.41) binds with the phospholipid. Centchroman decreases the SFA content and *vis-a-vis* increases the PUFA content which may cause increased fluidity of lipid layers[17] which in turn may increase binding of centchroman with phospholipid. This may be explained by its capability of permeation through lipid barriers as evidenced by the fact that centchroman can penetrate into estrogen receptors in sufficient concentration to act as an anti-estrogenic agent[18]. This causes the loss of lipid phosphorus and SFA content leading to therapeutic activity[7].

The high PUFA content due to drug effect upto 3rd day of incubation may be correlated with increase in TBA-titres. This reflects the toxic potential of the drug. The PUFAs, the precursors of the prostaglandins and other eicosanoids that regulate the important body function such as blood pressure, platelet aggregation etc.[19], are converted to peroxide moieties[20]. It is well known that lipid peroxidation is a toxicity mediation process and it has been related to pathogenesis of various hepatic, renal and gastro intestinal toxicities[21,22]. The slight increase in TBA-titre is in good agreement with the fact that the drug has low toxic potential upto 3rd day and thereafter its toxicity decreases further due to its antioxidant property. This is probably due to the presence of a chroman group as the chroman compounds usually have certain extent of reduction capabilities. This is in good agreement with its safety profile.

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