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Effect of Contraceptive β-Estradiol on Blood-Lipid

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Considering the importance of lipophilicity of contraceptive β-estradiol (ES) a significant contributor to its mechanism of action, interactions of the drug with total lipids of goat whole blood have been investigated using phospholipid binding, fatty acid composition and peroxidation phenomena as the parameters under investigation. The objective was to derive an insight into the pharmacodynamic behavior of the drug. Significant loss in phospholipid along with changes in fatty acid composition was observed after incubation of whole blood with ES at 150 pg/ml (effective contraceptive concentration in blood) in varying periods of time. This may be ascribed to binding affinity of ES with lipid constituents in blood. Lipid binding potential of the drug may have a role in its therapeutic effect. The effect of ES on lipid peroxidation phenomenon has been quantitatively measured and the results reveal that ES caused significant inhibition of lipid peroxidation which is in good agreement with its cardioprotectant action.

Drug-lipid interaction is a complicated multistage phenomenon. Lipophilicity and ionization parameters are the most important regarding transport, distribution and binding of drugs in biological systems[1,2] and may be equally responsible for changes in the lipid contents and lipid peroxidation phenomena. Lipid content changes are linked with therapeutic effect and lipid peroxidation breakdown products with toxic effects of the drug[3-5].

The present investigation has been designed to explore any possible relationship existing amongst the biological responses (both therapeutic and toxic) of a steroidal estrogenic compound, β-estradiol with its partition coefficient and the blood-lipid pattern changes as both the lipid loss and lipid peroxidation are related to biological activity of the drug[3-5]. ES is used in replacement therapy in postmenopausal women and contraception, but dose used in these two settings are substantially different[6]. ES has also considerable effect on various lipid constituents in human body[7,8]. Considering high lipophilicity of contraceptive ES, its effects on lipid constituents may seem to be significant contributor to its pharmacodynamics. An attempt has been made to study the effects of ES on phospholipid content, fatty acid composition and lipid peroxidation phenomenon, using goat whole blood as the lipid source[9].

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MATERIALS AND METHODS

Appropriate quantity of blood, as per the requirement for determination of a specific parameter, was collected from the jugular vein of female goat (8-10 m of age and weighting 6-8 kg). Silica gel was procured from E. Merck, Germany and authentic samples of fatty acid methyl ester (FAME) were obtained from Centre for Biochemical Technology, CSIR, New Delhi. Fatty acid estimation was carried out at RSIC, Bose Institute, Calcutta. β-Estradiol was provided by Novartis Pharma AG, Switzerland.

Whole blood was used for the determination of different parameters that included phospholipid binding, fatty acid composition and lipid peroxidation end products. The experimental steps consisted of the following: i) extraction of total lipid from blood using the method of Bligh and Dyer[10], ii) estimation of phosphorus in total lipid according to the procedure of Allen[11], iii) saponification of whole blood lipid and esterification of fatty acid to methyl ester (FAME) according to the method of Kates[12,13], iv) quantitative determination of lipid peroxidation end product, i.e., TBA-titres, using the method of Tarladgis *et al.*[14-16]. The detailed procedures were described in a previous communication[5].

In all these experiments, ES was used at 150 pg/ml of blood (maximum plasma concentration for contraception[17]). Both the control and drug-treated blood samples were incubated for 3 h (biological half-life of ES is biphasic: 20 and 70 min[18]) and determination of various parameters mentioned above was performed at 20th, 45th, 70th, 120th and 180th min. Replicate determination of all the parameters were done in five animal sets, except fatty acid estimation which was done in three animal sets.

RESULTS

The results supported with statistical analysis by 't' test are shown in figs. 1, 2 and 3. Fig. 1 shows the relative percentage reduction of inorganic phosphorus content in goat blood-lipid, treated with ES in comparison to control (0 h incubation). The control sample did not show any significant change in phosphorus content in different time periods. The results show that the average loss of phosphorus in whole lipid due to drug is significant throughout the experiment. This may be accounted due to phospholipid-binding capacity of the drug.

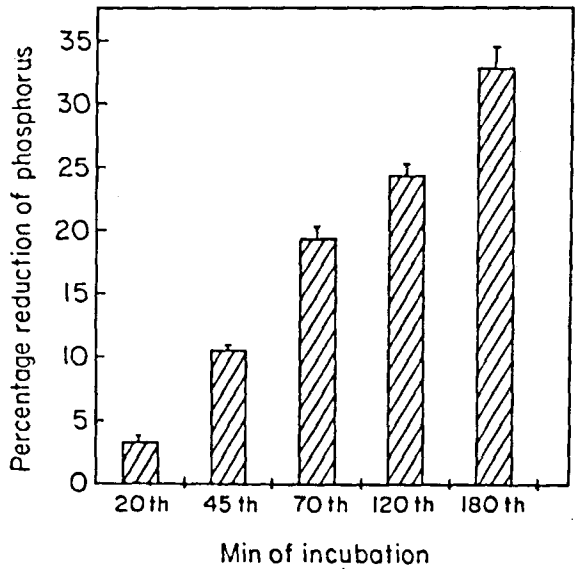

Fig. 1: Average percentage reduction of phosphorus in blood-lipid due to β-estradiol.
Each point is a mean of 5 animal sets. Statistical comparison was made between test and control values by 't' test. The values are significant at $P< 0.05$.

The relative percent changes with respect to control in FAMEs of total lipids due to drug are represented in fig. 2. There is increase of saturated (SFA) and monounsaturated (MUFA) fatty acid contents. Di (DUFA) and poly (PUFA) unsaturated fatty acid contents decrease after drug administration.

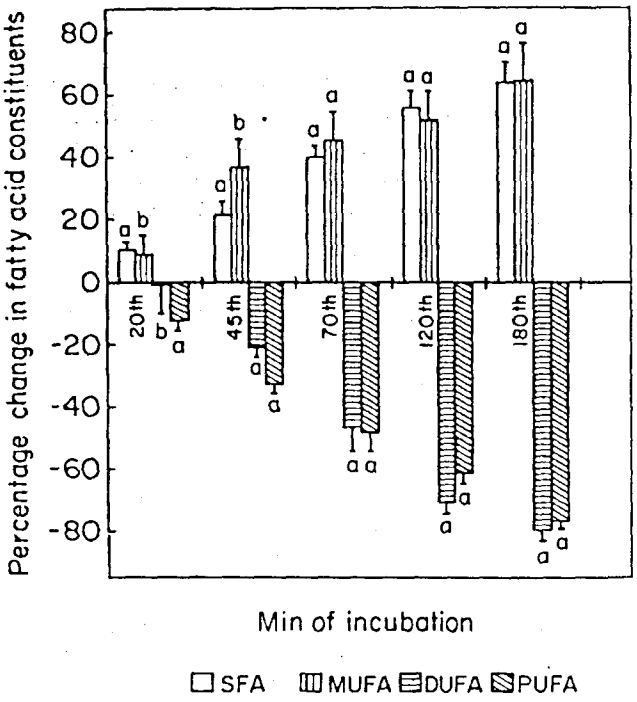

Fig. 2: Average changes in fatty acid constituents of blood-lipid with β-estradiol.
SFA: saturated fatty acid ($C_{12:0}$; $C_{14:0}$; $C_{16:0}$; $C_{18:0}$; $C_{20:0}$; $C_{22:0}$)
MUFA: monounsaturated fatty acid ($C_{16:1}$; $C_{18:1}$; $C_{20:1}$)
DUFA: diunsaturated fatty acid ($C_{16:2}$; $C_{18:2}$; $C_{20:2}$; $C_{22:2}$)
PUFA: polyunsaturated fatty acid ($C_{18:3}$; $C_{18:4}$; $C_{20:3}$; $C_{20:4}$; $C_{20:5}$; $C_{22:4}$; $C_{22:5}$)
Each bar is a mean of n=3. Probability level (P) of changes are significant at a< 0.05 and b< 0.05. The variance ratios (between fatty acids and between animal sets) obtained from ANOVA are shown above the corresponding bar diagrams. Significance level of F values: between fatty acids (d. f. 3, 6), $P< 0.05$ and between animal sets (d.f. 2, 6), $P> 0.05$.

Fig. 3 represents the relative percentage changes of TBA (thiobarbituric acid)-titres (peroxide end products) due to drug. The results show that the TBA-titres in ES-induced blood decrease significantly in comparison to control in a time-dependent manner.

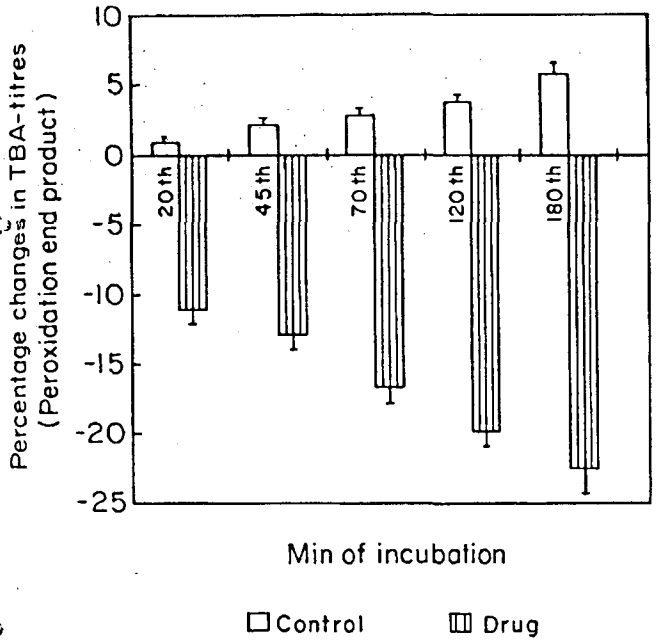

Fig. 3: Average[1] percentage changes in TBA-titres induced by β-estradiol with respect to control.
[1]Mean of 5 animal sets ☐ Control ▥ Drug. The values of percent change are significant at $P<0.05$

DISCUSSION

The results reveal that ES appreciably lowers the phosphorus content of the blood-lipid which may be due to its binding with phospholipids of the blood. Because of its high lipophilicity (log P= 4.01), it may diffuse passively through cellular membrane[6] and bind to a receptor present in the nucleus[19]. As the phosphorus content decreases, the availability of drug at the receptor site increases[3,5]. The receptor interacts with specific nucleotide sequences termed estrogen response elements (EREs)[6] present in target organs (here it may be pituitary gland) and when this interaction increases, it suppresses FSH release[20]. This effect could contribute to the lack of follicular development observed in oral contraceptive users. On the other hand, when EREs are present in uterus, such interactions suppress uterine proliferation for implantation and interfere with nidation, exert contraceptive effect. ES increases SFA content and vis-à-vis decreases the DUFA and PUFA content which is in good relation with binding of drug with phospholipid. ES has an important role as antioxidant[21] and cardioprotectant[22]. The antioxidant activity of ES is revealed from the reduction of TBA-titre in the present experiment. This is in good agreement with the literature report of antiperoxidative effect of ES[7].

The decrease in PUFA level may be responsible for antioxidant effect of the drug (150 pg/ml blood concentration) which may be correlated with decrease in lipid peroxidation end products as PUFAs are converted to peroxide end products[23]. In lipid peroxidation, a hydrogen atom is liberated from the fatty acid by a reactive free radical resulting in the formation of lipid radical[24] which on attack by molecular oxygen produces a lipid peroxy radical forming either a lipid hydroperoxide or endoperoxide. The formation of lipid endoperoxide in PUFAs leads to the formation of peroxide endproduct[25]. As lipid peroxidation is a molecular mechanism of cell injury with potential injurious consequences[23], decrease in peroxide end product due to ES in contraception purpose may be related with its cardioprotectant activity.

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