concentration. Variability in synovial fluid concentration is also determined by distribution into and out of the synovial cavity, which is thought to be diffusion limited and altered in synovitus (Wallis & Simkin 1983) and by variability in the corresponding plasma concentration. It is yet to be determined to what extent variability in binding contributes to the overall variability seen in synovial fluid concentration.

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REFERENCES

Aarons, L., Grennan, D. M., Siddiqi, M. (1983) Eur. J. Clin. Pharmac. 25: 815–818

Bowmer, C. J., Lindup, W. E. (1978) J. Pharm. Sci. 67: 1193–1195

Cardoe, N., de-Silva, M., Glass, R. C., Risdall, P. C. (1977) Curr. Med. Res. Opin. 5: 21–25

Kober, A., Sjöholm, I. (1980) Molec. Pharmacol. 18: 421–426

Wallis, W. J., Simkin, P. A. (1983) Clin. Pharmacokin. 8: 496–522

Wanwimolruk, S., Brooks, P. M., Birkett, D. J. (1983) Br. J. Clin. Pharmac. 15: 91–94

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The effect of xanthine derivatives on red blood cells: microelectrophoretic studies

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The effect of xanthine derivatives on the variation of surface charges of red blood cells has been investigated. Results of mobility curves showed that the derivatives increase the surface charges on the cells, whereas there is little effect on the surface charges of liposomes.

Pentoxifylline (xanthine derivative) can improve red cell (RBC) deformability; decrease blood viscosity, platelet aggregation and serum fibrinogen level; increase fibrinolytic activity and cerebral microcirculation and reduce peripheral vascular resistance (Grigoleit et al 1976; Volker 1976; Angelkort et al 1979; Marcel 1979; Takamatsu et al 1979). It was found to be useful in occlusive cerebral and peripheral vascular diseases (Gorbatschova et al 1978; Itoh & Satoh 1979; Satewachin et al 1978).

The mechanism of pentoxifylline in reducing the rigidity of red cell membranes to improve deformability due to the elevation of ATP concentrations, has been reported by Nakao (1974) and Stefanovich (1978). However, little work has been published on the effect of pentoxifylline on the surface charges of RBCs, as the magnitude of the surface charges on the cells can influence their aggregation. Therefore, we have used microelectrophoresis to investigate the variation of the surface charges of RBCs in the presence of the xanthine derivatives: pentoxifylline, aminophylline, xanthinol niacinate, caffeine and theophylline.

Materials and methods

Materials. Pentoxifylline (Hoechst, ROC), aminophylline (Sigma, USA), xanthinol niacinate (Italchimici, Italy), caffeine (Sigma, USA), theophylline (Delta Synthetic Co., ROC), cholesterol (Sigma, USA) and dicetyl phosphate (P.L. Chemicals Inc., USA) were used as received. Phosphatidylcholine was purified from fresh egg yolk by a two-step column procedure i.e. an alumina column and a silicic acid column (Singleton et al 1965).

Methods. A chloroform solution of phosphatidylcholine cholesterol and dicetyl phosphate at a molar ratio of 1·60 : 1·00 : 0·15 was prepared in a 500 ml round bottle flask and evaporated under reduced pressure at 37 °C to form a thin film on the flask. Phosphate buffer (0·067 M, pH 7·4) was added to the flask to give a concentration of lipid 1 mg ml^{-1}. Multilamellar liposomes were formed by constant vortexing for 5 min. The liposome dispersion was hydrated at 37 °C for 2 h.

Stock solutions of xanthine derivatives made in the phosphate buffer were added to 0·1 ml of fresh blood obtained from normal adult donors or 1 ml of liposome dispersion. The volume was made up to 20 ml with phosphate buffer to give the required concentration of the xanthine derivatives. The dispersion was incubated for 30 min at 25 °C before the measurement. The total volume (20 ml) of the dispersion was used for each measurement.

Mobility determinations on the RBCs and liposomes were carried out at 25 °C using a Rank MK II Microelectrophoresis Apparatus (Rank UK). The flat cell assembly and platinum electrodes were used. Ten individual particles were timed in both directions of the electric current to minimize the polarization of electrodes. The mobility is expressed by U = V/E where V is

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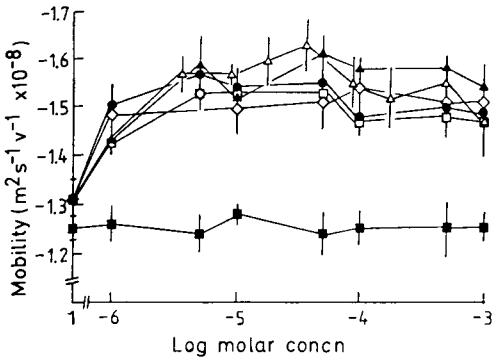

FIG. 1. Mobility curves of red blood cells in the presence of xanthine derivatives. △, pentoxifylline; ▲, aminophylline; ◇, xanthinol niacinate; □, caffeine; ●, theophylline; ■, liposomes in the presence of pentoxifylline.

the velocity of the particle migrating and E is the field strength calculated from the applied voltage divided by the distance between electrodes.

Results and discussion

The mobility curves for RBCs and liposomes in the presence of xanthine derivatives are given in Fig. 1. The mobility of RBCs in phosphate buffer was $-1{\cdot}31 \pm 0{\cdot}05 \times 10^{-8}$ m^2 s^{-1} v^{-1} which is in good agreement with the literature value of $-1{\cdot}31 \times 10^{-8}$ m^2 s^{-1} v^{-1} (Abramson 1942).

After incubation with xanthine derivatives, RBC mobility increased. For example, at concentration of 2×10^{-5} M of pentoxifylline, the mobility increased to $-1{\cdot}62 \times 10^{-8}$ m^2 s^{-1} v^{-1}. The mobility of liposomes in the presence of xanthine derivatives showed no significant change over the concentration range studied (the mobility curve of liposomes in the presence of pentoxifylline is shown in Fig. 1 as representative of the xanthine derivatives).

Pentoxifylline inhibited platelet aggregation by increasing the surface charges of platelets, possibly because of its binding to the platelet membrane by occupying the membrane phosphodiesterase active site. Inhibition of membrane phosphodiesterase leads to an increase in cyclic (c) AMP concentration at the membrane. cAMP activates protein kinase and catalyses the phosphorylation of the membrane protein by ATP. This causes an increase in negative charge of the membrane and results in inhibition of platelet aggregation (Stefanovich 1978).

From Fig. 1, it is clear that xanthine derivatives have the effect of increasing the surface charge on the RBCs which resulted in a rise in the mobility. This suggests that xanthine derivatives have an effect by increasing the surface charge on RBCs as well as on platelets. The mechanism of surface charge increase on RBCs may be the same as that on platelets (to inhibit membrane phosphodiesterase).

Xanthine derivatives have no effect on the surface charges of liposomes. This supports the biochemical mechanism of surface charge increase generated by xanthine derivatives.

Pentoxifylline, aminophylline, xanthinol niacinate, caffeine and theophylline studied here show a similar biochemical effect on the increase of surface charge of RBCs. However, among these drugs the magnitude of the increase in surface charge on the cells is not significant.

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REFERENCES

Abramson, H. A., Mayer, L. S., Gorin, M. H. (1942) Electrophoresis of Proteins and Chemistry of Cell Surface, Reinhold, New York

Angelkort, B., Maurin, N., Boateng, K. (1979) Curr. Med. Res. Opin. 6: 255–258

Gorbatschova, F. E., Turaschwili, G. A., Kvassov, V. T., Milovanova, M. M., Tschistjakova, M. A., Lifwinova, V. P., Sokolovski, G. A. (1978) Pharmatherapeutica 2: 100–104

Grigoleit, H. G., Porsche, E., Stefanovich, V., Jacobi, G. (1976) Ibid. 1: 241–247

Itoh, T., Satoh, T. (1979) Ibid. 2: 159–164

Marcel, G. A. (1979) J. Med. 10: 409–416

Nakao, M. (1974) in: Yoishwa, N., Rappaport, M. (eds) Cellular and Molecular Biology of Erythrocytes, Urban and Schwarzeberg, Munchen, p. 35

Satewachin, I. I., Iljin, V. N., Schestakov, V. A., Alexadrova, N. P., Rodionov, S. V. (1978) Pharmatherapeutica 2: 109–117

Singleton, W. S., Gray, M. S., Brown, M. L., White, J. L. (1965) J. Am. Oil Chem. Soc. 42: 53–56

Stefanovich, V. (1978) Pharmatherapeutica 2: 5

Takamatsu, S., Sato, K., Takamatsu, M., Sakuta, S., Mizuno, S. (1979) Ibid. 2: 165–172

Volker, D. (1976) Ibid. 1: 154–159