Lipid adsorption/absorption on polycarbonate surfaces an understanding

CHANDRA P SHARMA and THOMAS CHANDY

Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Poojapura, Trivandrum 695 012, India

MS received 6 January 1982; revised 7 September 1982

Abstract. Thrombus formation on a foreign surface is a complicated process, involving many factors. The adhesion and aggregation of platelets play an important role in the initial events of thrombus formation on such surfaces. In this work, adsorption/absorption studies using saturated fatty acids were carried out on polycarbonate surface to evaluate how such surfaces may affect blood-polymer interaction. The surface change was investigated by measuring the interfacial energies as derived from advancing contact angle technique. The plasma recalcification time and platelet adhesion studies were also carried out to further develop an understanding.

Keywords. Fatty acid adsorption/absorption; advancing contact angle; platelet adhesion and aggregation; plasma recalcification time; Interfacial tension; Blood compatibility; surface energy; thrombosis.

1. Introduction

Normal vascular endothelium is the ideal nonthrombogenic surface, which is generally considered to be resistant to platelet adhesion and aggregation. On the other hand, a foreign surface in contact with blood is thrombogenic due to platelet adhesion and aggregation. Thrombosis and coagulation induced by blood-polymer interaction are of key concern in the area of medical implants. Although several investigators have considered the nature of various polymer surfaces in the hope of developing a nonthrombogenic surface, the mechanism of thrombus formation is still not known due to the complicated nature of the process.

It has been found that concentrations of fatty acids, within the range of plasma—free fatty acid concentrations in man, can produce hypercoagulability (Warner *et al* 1967). Other investigators (Hoak *et al* 1967; Connor *et al* 1963; Poole 1955) indicated that fatty acids, mainly longchain saturated fatty acids, promote thrombosis *via* platelet aggregation mediated by ADP and accelerated the clotting by activating Hageman factor. Kim *et al* (1977) proposed that platelet aggregation at a polymer interface is initiated and/or accelerated by the presence of fatty acid suspension formed through the release of adsorbed fatty acid. In the paper we have attempted to correlate the surface energy parameters towards saturated fatty acid adsorption/absorption and release at the interface and the relationship with thrombosis. Platelet adhesion and plasma recalcification time studies were also carried out for a better understanding of the subject.

2. Experimental

2.1 Adsorption/absorption of fattyacids on polycarbonate

Polycarbonate solution was prepared in dichloromethane (10 mg% w/v), films (~ 0.1 mm thickness) cast on clean glass plates (4.5×4.5 cm) were cut out, cleaned with 0.1% soap solution (Teepol), distilled water and finally rinsed with ethanol. The clean films were then dried in a vacuum oven at 60°C for 4 hrs.

The films were then exposed to saturated fatty acids of varying chainlength and a control surface in buffer. Bare surface (I set) – 0.1 M phosphate buffer (pH 7.4). Fatty acids adsorbed/absorbed surface (II to V sets) – 0.1 M fatty acid solution combined with 0.4 g of NaOH was dissolved in 0.1 M phosphate buffer (pH 7.4). After dissolving, the solution was standardized to a pH of 7.4, and made up the volume to 100 ml with the same buffer.

Fatty acids used were butyric acid: $\text{CH}_3\text{-CH}_2\text{-CH}_2\text{-COOH}$ (LR, BDH Chemicals Ltd, Poole, England, 99% pure), caproic acid: $\text{CH}_3\text{-(CH}_2)_4\text{-COOH}$ (LR, BDH Chemicals Ltd, Poole, England); Heptanoic acid: $\text{CH}_3\text{-(CH}_2)_5\text{-COOH}$ (AR; Wilson Laboratories, Bombay) and stearic acid: $\text{CH}_3\text{-(CH}_2)_{16}\text{-COOH}$ (AR, Wilson Laboratories, Bombay). Dissolving stearic acid completely was difficult, however since the adsorption/absorption is in μg levels, the experimental data were used to study relative variations.

The films were kept in the respective fatty acid solutions for varying time intervals, taken out; rinsed with phosphate buffer under controlled flow rate to remove excess fatty acids on the surface. The films were dried overnight in vacuum oven at room temperature and the contact angle studies were carried out using a goniometer (Kernco Instruments, Texas) and platelet adhesion.

Surface energy parameters of various fatty acid adsorbed/absorbed surfaces were obtained from advancing contact angle technique. The contact angles for each set of film were measured using a goniometer and spectroscopically pure solvents. From the contact angle, the values like γ_{SV} and γ_{SL} were obtained from the conversion tables of Neumann *et al* (1980). Contact angle experiments were repeated at different lengths of time (short time exposures in minutes of 10, 20, 30, 40, 60, 80 and 120 and long terms of 5, 20, 35, 50 and 70 days): however no significant changes were observed with time of exposure and molecular length of the fattyacid. Therefore the data for 10 min exposure to butyric acid alone has been presented in table 1.

2.2 Platelet adhesion on fattyacid adsorbed/absorbed surfaces

Washed human platelets were prepared as described (Sharma and Chandy 1982) and suspended in tyrode solution (Lee and Kim 1979) for the adhesion studies.

The vacuum-dried films were exposed to platelet suspension for 1 hr in a dust-free area, rinsed with buffer of pH 7.4, under controlled flow rate for 3 min, and the platelets fixed with 2.5% glutaraldehyde [(50% w/v) Koch-Light Laboratories, England] – solution in 0.1 M phosphate buffer pH 7.4, further stained with Coomassie Blue G. The platelet density was detected using an optical microscope. Different vision fields were read randomly and averaged in similar fashion for all samples.

Table 1. Surface energy components on butyric acid exposed polycarbonate films (10 min exposure).

Liquids	γ_{LV}^b	Bare Surface			Butyric acid adsorbed surface		
		θ^a	γ_{SV}^b	γ_{SL}^b	θ^a	γ_{SV}^b	γ_{SL}^b
Water	72.5	70 ± 1	41.2	16.4	72.5 ± 1	39.7	17.9
Glycerol	63.0	62 ± 1	38.3	8.7	64 ± 1	37.2	9.5
Formamide	58.0	54 ± 1	39.0	4.9	55 ± 1	38.5	5.2
Ethylene-glycol	47.7	44 ± 1.5	36.3	1.8	46 ± 1	35.4	2.1

$$\text{Mean } \gamma_{SV}^b = 38.7 \pm 2.5$$

$$\text{Mean } \gamma_{SV}^b = 37.69 \pm 2$$

^a Contact angle $\pm$ S.D.; ^b ergs/cm².

2.3 Coagulation studies

2.3a Plasma recalcification time: Citrated human plasma was collected and tested for recalcification time under controlled pH and temperature with various saturated fatty acid adsorbed/absorbed polymer surface.

Glass tubes (dimension 10 × 75 mm) were coated with 10% polycarbonate and were dipped in 0.1 M fatty acids (as described earlier) for varying time intervals. After adsorption/absorption with time, the tubes were taken out and rinsed carefully with buffer to remove excess fatty acids. The tubes were then dried overnight in vacuum oven at room temperature. Control PC-coated tubes were also prepared.

The clotting time was determined as described by Austen and Rhymes (1975). The test was repeated until reproducibility was assured.

3. Discussion

It is proposed that the presence of saturated fattyacids (within the range of plasma-free fattyacid concentrations in humans) can produce hypercoagulability (Warner *et al* 1967) and can promote thrombosis (Hoak *et al* 1967; Prost-Dvojakovic and Samma 1973). Our experimental observations basically support that fattyacid adsorption/absorption on a polymer surface has adverse effects towards blood compatibility, since the platelet adhesion is increased and there is a decrease in plasma recalcification time (PRT) relatively compared to bare surface (table 2). Further interfacial tension of lipid adsorbed/absorbed surface is also increased probably due to encouraged hydrophobic behaviour of the surface (table 1). So it seems that the surface properties of the material play an important role for other blood components to adhere to the surface, which may again enhance the chances of thrombus formation.

Although, no significant variations are observed with exposure time and increase in molecular length from butyric acid to stearic acid in our contact angle data, it

Table 2. Platelet adhesion and plasma recalcification time on fattyacids exposed polycarbonate films (10 min. exposure)

Surfaces	No. of platelets adhered to fatty acids adsorbed surfaces $\pm$ S.D.	PRT in seconds $\pm$ S.D.
Bare	6 $\pm$ 1	148.5 $\pm$ 1.5
Butyric acid	9 $\pm$ 2	137 $\pm$ 6
Caproic acid	9 $\pm$ 2	126 $\pm$ 1.5
Heptanoic acid	9 $\pm$ 2	128 $\pm$ 3
Stearic acid	10 $\pm$ 3	128 $\pm$ 3

appears that it encourages the process of platelet adhesion and decreases the plasma recalcification time. This shows that the initial events of lipid adsorption/absorption on a polymer surface is extremely important towards the process of thrombosis.

It has been suggested (Kim *et al* 1977) that the fattyacid suspension may result in a high Ca^{2+} concentration at the platelet-extracellular region and subsequently increased biochemical release from the cells. Also, negatively-charged colloidal particles of fatty acid (micelle) may accelerate the platelet aggregation and clotting. This supports our observations, since a number of platelet aggregates were present on the polymer surface precoated with excess fatty acids. Also, there was a decrease in PRT. So we feel that the increased Ca^{2+} ion concentration and the negative charge of the micellar lipid alter the platelet function towards encouraged adhesion of the cells and triggering of the coagulation cascade system.

The charge of the micelles will influence the possibilities for both Ca^{2+} ions and charged proteins to approach the micelle and attach to its surface by electrostatic forces (Kahn and Kemker 1969). Apart from charge, hydrophobicity of the micelle also helps in determining its coagulation activity. Our surface energy and contact angle data suggest an increase in interfacial tension and a decrease in surface energy on fatty acid adsorbed/absorbed surfaces, which clearly indicates the hydrophobic character of the surface and the possible increase in interactions at the blood-polymer interface.

It is obvious that (Harry P Gregor 1975) electrostatic forces are only one of a number of noncovalent interactions and that it is the overall balance of these forces including hydrogen bonds, electrostatic forces, Van der Waals London dispersion forces and hydrophobic "forces" that determines not only the structure of micelles and polymers but also the interactions that occur between them. In addition to its importance in determining structure, hydrophobic effects are also the primary driving force involved in micelle-polymer interaction. So the possible interaction of lipids with polycarbonate can be demonstrated as follows. Both polycarbonate and micellar lipid are amphipathic in nature, i.e. each contains both hydrophilic and hydrophobic moieties due to polar and nonpolar groups distributed on the surface.

Adsorption/absorption of lipids on to polycarbonate take place satisfying the affinities of relative polar and nonpolar groups of both interacting molecules. During this process of adsorption/absorption net variation of surface energy parameters have not been much significant, however the slight shift observed seems to be towards the increase in the hydrophobic behaviour of the surface. Such a net hydrophobic effect and the variations caused by the chemical entities of the lipid adsorbed/absorbed surface, encourages the platelet adhesion and reduces the PRT, which ultimately causes clotting.

Acknowledgements

We appreciate the help received from the Blood Bank Group of the Institute for providing the blood. Thanks are also due to Miss M K Sheela for assistance.

References

- Austen D E G and Rhymes I L 1975 *A laboratory manual of blood coagulation* (Oxford: Blackwells) p. 35
Connor W E, Hoak J C and Warner E D 1963 *J. Clin. Invest.* **42** 860
Harry P Gregor 1975 *Biomedical applications of polymers* (New York and London: Plenum) p. 19
Hoak J C, Warner E D and Connor W E 1967 *Circ. Res.* **20** 11
Kahn M J P and Hember H C 1969 *Thrombos. Diathes. Haemorrh.* **22** 417
Kim S W, Wisniewski S, Lee E S and Winn M L 1977 *J. Biomed. Mater. Res. Symp.* No. **8** 23
Lee E S and Kin S W 1979 *Trans. Am. Soc. Artif. Int. Organs* **25** 124
Neumann A W, Absolom D R, Francis D W and Vanoss C J 1980 *Separation and purification methods* **9** 69
Poole J C F 1955 *Br.J. Exp. Pathol.* **36** 248
Prost-Drojakovic R J and Samma M 1973 *Haemostatis* **2** 73
Sharma C P and Chandy T 1982 *J. Colloid. Interface Sci.* **89** 479
Warner E D, Hoak J C and Connor W E 1967 *Thromb. Diath. Haemorrh. Suppl.* **26** 249