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Medical application of microcalorimetry in human toxicology. A study of blood compatibility of hemodialysis membranes

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Abstract

In the present study we have measured total metabolism of human granulocytes and mononuclear cells (lymphocytes and monocytes) in contact with artificial surfaces. The quantitative assessment of resting metabolism was calculated from their rates of heat production. Three polymers commonly used as membranes in artificial kidneys were tested: polyacrylonitrile (AN); polyetherpolycarbonate (PC), and regenerated cellulose (Cu). The functional activity of the granulocytes was tested after exposure to artificial materials by stimulation of phagocytosis with zymosan. Biocompatibility was reflected by the level of heat production. Polymers with the lowest degree of biocompatibility (Cu) show the highest stimulation of granulocyte basal metabolism and the lowest granulocyte reactivity to zymosan. The ability to record the metabolic activity in cellular systems by microcalorimetry offers a potential to the evaluation of biomaterials.

INTRODUCTION

In the last 20 years there has been an increasing development of medical devices based on use of artificial materials. Examples in the surgical field include the replacement of parts of joints, heart, lungs, liver, eyes, ears, vessels. Instances where blood comes in contact with artificial materials can be represented by the use of extracorporeal blood circulation, such as blood purification. Adverse reactions might occur by contact of human tissues with biocompatible materials. This can be the case when the blood comes in contact with artificial materials and reactions occur between blood cells or plasma and the artificial surface. The degree of biocompatibility of the materials used is particularly important for hemodialysis membranes in artificial kidneys due to the fact that the area made of artificial material is very large and the duration of exposition to blood is long. In the present study we have assessed quantitatively the metabolism of granulocytes and mononuclear cells (lymphocytes and monocytes) from their rates of heat production when the cells were in contact with different polymers commonly used as membranes in artificial kidneys.

MATERIAL AND METHODS

Venous blood (20 cm^3) was collected from each of the healthy donors into Vacutainer tubes containing 13 U/ml heparin. Granulocytes and mononuclear cells were obtained by centrifugation on a density gradient in a one-step procedure using Mono-Poly Resolving Medium (Flow Laboratories, USA (1,2). Granulocytes and mononuclear leucocytes were separated into two distinct bands on this gradient. Contaminating erythrocytes were removed by hypotonic shock. Washed granulocytes and mononuclear cells were separately resuspended in cell-free autologous plasma buffered with Tris to pH 7.4. Calorimetric analysis was performed at 37 °C using a multichannel static ampoule heat conduction calorimeter (3), TAM 2277, (ThermoMetric, Sweden). 0.1 cm^3 of the suspension containing 10^6 cells was enclosed in sealed 1-cm^3 cylindrical stainless-steel (SS) ampoules. The inside of each ampoule was lined with one of four different polymers. The polymer membranes were obtained from commercially available hemodialyzers: polyacrylonitrile (AN) from Biospal AN 69 (Hospal, France); polyetherpolycarbonate (PC) from Lundia Pro, regenerated cellulose (Cu) from Lundia IC (Gambro, Sweden), and fluorinated ethylene propylene (FEP; Dupont, USA). The lining was achieved by fitting the polymer membranes in the form of a cup with a cylindrical piston into the ampoule in such a way

that the ampoule surface was completely covered. Wetting the ampoule with 0.1 cm^3 of physiological saline was carried out to keep the polymer membrane in position. The polymer membranes were washed and stored in distilled water at 4 °C before use, the purpose of this was to remove surface contaminants and membrane stabilizers and allow for polymer swelling. Before insertion of the membranes into the ampoules, they were blotted on a filter paper and equilibrated in physiological saline at 37 °C. Numerical data are expressed as mean ± standard deviation (SD). Differences between groups have been tested by Wilcoxon's nonparametric tests for paired data.

DESIGN OF THE EXPERIMENTS

Two groups of experiments were conducted. In one, using mononuclear cells as the blood component, SS ampoules were used as reference material. In the other series, using granulocytes, FEP was used as reference material. The basal rate of heat production, P (dQ/dt), of the cell suspensions was recorded, and the P value after 2 hours has been used to characterize the metabolic status of the cells in contact with the artificial surface. The residual metabolic activity was evaluated for the granulocytes by addition of 15 mg of zymosan (Sigma, USA) in 50 µl physiological saline (final concentration of 0.1 mg zymosan per microliter suspension) to the cells after the initial 2-hour incubation with the polymers outside the calorimeter. The thermogenesis of this secondary activation was recorded for another period of 2 hours.

RESULTS AND DISCUSSION

The interaction between the mononuclear cell suspensions and the two polymers Cu and PC, revealed a two component calorimetric profile, a rapidly descending linear component and a steady state component after about two hours. Results of P-values after 2 hours (TABLE 1) differed significantly ($p=0.005$) between the reference material and the investigated polymers Cu and PC, but no significant difference was obtained between Cu and PC.

TABLE 1. Rate of heat production (P/ pW per cell) in 0.1 cm^3 suspension of mononuclear cells 10^6 in contact with two types of membrane polymers after 2 hours at 37 °C. SS ampoules were used as reference material. Mean values ± standard deviations (SD) are given (n = 10).

Material	Mean	SD
SS	2.63	0.53
PC	3.61	1.18
Cu	4.19	1.12

TABLE 2. Rate of heat production (P/ pW per cell) in 0.1 cm^3 suspension of granulocytes 10^6 in contact with 4 polymers after 2 hour at 37 °C. The increase after zymosan stimulation is given in percent of the basal value. Results are presented as mean ± standard deviation (SD).

Material	Basal values		Activation	
	X	SD	X	SD
FEP	1.47	0.31	1237	471
AN	3.15	0.63	586	155
PC	5.48	2.05	304	82
Cu	8.87	6.09	130	188

When performing calorimetric measurements on plasma suspensions of granulocytes in SS ampoules, an initial activation process of the cells was recorded. Activation was prevented by the introduction of FEP-lined ampoules (4). The appearance of the calorimetric curve was similar to the one obtained for mononuclear cells. Granulocyte heat production rate was found to be significantly higher ($p < 0.0001$) in the presence of each of the 3 polymers (Cu, PC, AN) than the reference value obtained in the presence of FEP (TABLE 2). The highest basal value was found for Cu, indicating that this material has the lowest degree of biocompatibility. The functional activity of the granulocytes was tested following exposure to polymers by stimulation of phagocytosis with zymosan and measurement of the increase in the rate of heat production. Microcalorimetry has previously been found to be useful for a quantitative assessment of phagocytosis (ref. 5,6). In the present study, Cu was found to have the lowest degree of response to zymosan, significantly lower than the corresponding value obtained with reference material and lower than found when testing the other two membrane polymers used (PC and AN); (TABLE 2). An inverse correlation was thus found between the degree of activation of basal metabolism due to polymers and the degree of response to zymosan thereafter. We interpret this phenomenon as a state of cell energy deprivation due to the interaction of granulocytes with polymers possessing different degrees of biocompatibility. A decreased capability of phagocytosis represents a lowered defense potential against infections. It should be emphasized that uremic patients on hemodialysis are particularly prone to infections. It is still unclear to what extent the results of our present experiments reflect the situation

in vivo. We do not know, for example, whether the decreased phagocytic capability is a temporary phenomenon or a long lasting one. Based on our present experience, we conclude that microcalorimetry has been found in this initial evaluation of biocompatibility to be a useful instrument that seems to offer rewarding possibilities for future investigations of the degree of compatibility of biomaterials.

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