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Effect of blood cooling on cuprophan-induced anaphylatoxin generation

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Effect of blood cooling on cuprophan-induced anaphylatoxin generation. We investigated whether cooling of the extracorporeal blood during hemodialysis could prevent anaphylatoxin generation and leukopenia caused by blood-cuprophan contact. After preliminary in vitro studies confirming the temperature dependence of C5a generation, we carried out hypothermic dialysis on nine patients by manipulating blood and dialysate temperature in such a way that blood temperature within the dialyzer averaged 25°C. In comparison with the control procedure (blood temperature within the dialyzer 35°C) hypothermic dialysis reduced peak C5a generation from 41.7 ± 17 ng/ml to 9.7 ± 3.4 ng/ml ($P < 0.01$), and white blood cell fall from 72 ± 15 to 25 ± 20% ($P < 0.01$). Arterial PO_2 decreased less in hypothermic dialysis (−19 ± 9% of pre-HD value) than in the control procedure (−30 ± 11%) ($P < 0.05$). We conclude that blood cooling attenuates cuprophan-induced anaphylatoxin generation and leukopenia.

Exposure of blood to certain foreign surfaces as occurs during hemodialysis (HD), cardiopulmonary bypass and nylon fiber leukoapheresis, causes complement (C) activation via the alternative pathway mechanism [1–3]. The intensity of C activation varies with the material employed for extracorporeal circulation, new cuprophan membrane causing a much more marked activation than reused cuprophan, polyacrylonitrile or polymethylmethacrylate membranes [4, 5]. A manifestation of C activation is transient but profound leukopenia occurring early during HD treatment, which is thought to result from pulmonary sequestration of granulocytes aggregated by the complement-derived fragment C5a [6–8].

In the course of studies aimed at assessing the effect of varying dialysate temperatures (T) on cardiovascular stability during HD treatment, we noted that in the 38° to 20°C range the lower the dialysate T was set, the milder the leukopenia [9]. This observation led us to investigate the influence of T on C activation by cuprophan membrane. To this end we evaluated the intensity of C activation from the amount of C5a antigen generated both in vitro and in vivo. The results of the investigation form the object of the present report.

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Methods

In vitro study

Four heparinized plasma samples from each of eight subjects (4 staff members and 4 patients) were incubated with cuprophan membrane (1 ml/plasma per 20 cm^2 membrane) in revolving plastic tubes, immersed in thermostatic water baths set at temperatures of 16°, 20°, 30°, and 37°C, respectively. After 60 minutes EDTA was added to plasma and its C5a concentration determined.

In vivo study

Nine patients (5 males, 4 females), who had been undergoing thrice weekly hemodialysis (HD) treatment for one to seven years (average 2.7), took part in this study. They were free of any related disease, their ages ranged from 25 to 71 years (average 52.5), and they were not taking any medication other than phosphate binders and vitamins. Diagnosis of renal disease was interstitial nephropathy (3), unknown etiology (4), diabetic nephropathy (1), and renal vascular disease (1).

Each patient randomly underwent one isothermic and one hypothermic HD treatment. For the purpose of this study, HD treatments were carried out with a special extracorporeal line containing a serpentine tube 200 cm long in both the arterial (dialyzer afferent) and venous (dialyzer efferent) segments. During hypothermic HD, dialysate fluid flowed unwarmed through the dialyzer while the arterial serpentine tube was kept immersed in a bath of running tap water. Before re-entering the patient, blood was rewarmed by immersing the venous serpentine tube in a thermostatic bath set at 39° to 41°C. During isothermic HD, both the arterial and the venous serpentine tubes were kept in a water bath at 37°C while dialysate fluid flowed through the dialyzer at a T of 37°C. All HD treatments were carried out with new Cuprophan, hollow fiber dialyzers of 1.2 m^2 diameter (GF 120H Gambro, Lund, Sweden), at a blood flow of 250 ml/min and a dialysate flow of 500 ml/min. Dialysate composition was: Na^+ 138, K^+ 1.5, Ca^{++} 3.8, Mg^{++} 1.0, acetate 36.9 mEq/liter.

Temperature was monitored by means of thermocouple needles (Ellab) placed in the blood and dialysate circuits. Blood T within the dialyzer was calculated as the average of pre- and post-dialyzer blood T. Blood gases, leukocyte and differential count were determined in duplicate on blood drawn from the

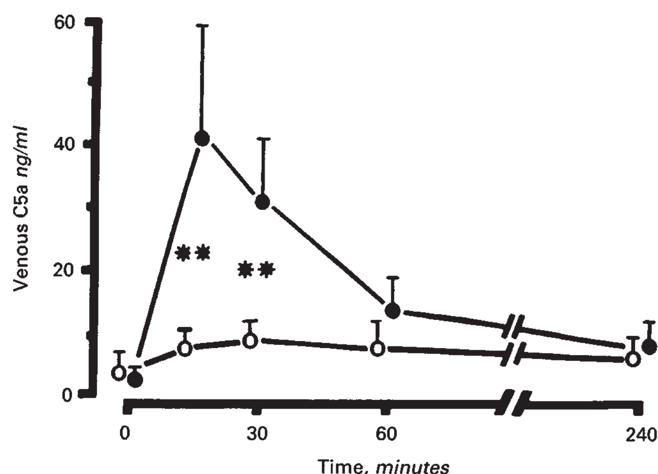

Fig. 1. *C5a in the venous blood of extracorporeal circuit during hypothermic (○) and isothermic (●) HD.* $^{**}P < 0.01$.

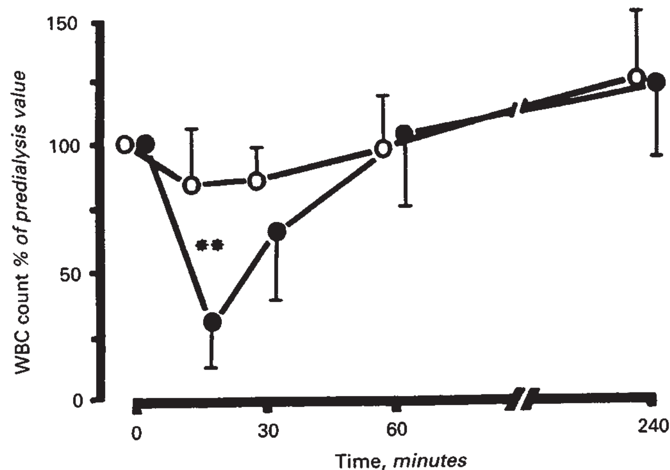

Fig. 2. *Changes in WBC during hypothermic (○) and isothermic (●) HD.* $^{**}P < 0.01$.

arterial line at 0, 15, 30, 60, 120 and 240 minutes of HD. At the same time intervals, blood for measurement of C5a antigen was obtained from both the arterial (afferent) and venous (efferent) line of the dialyzer. Blood was drawn in tubes containing EDTA, immediately centrifuged, separated and stored at −80°C until processing.

C5a antigen was determined by RIA (Upjohn Kalamazoo, Michigan, USA); white blood cell and differential count was determined by a manual method. Dialyzer urea and creatinine clearance were determined by standard methods at the end of the second hour of HD treatment. Blood gases were determined by a Radiometer ABL2 gas analyzer.

Statistical analysis was performed by paired Wilcoxon test. Correlated parameters were done by using linear and exponential regression analysis. Data are presented as mean ± standard deviation.

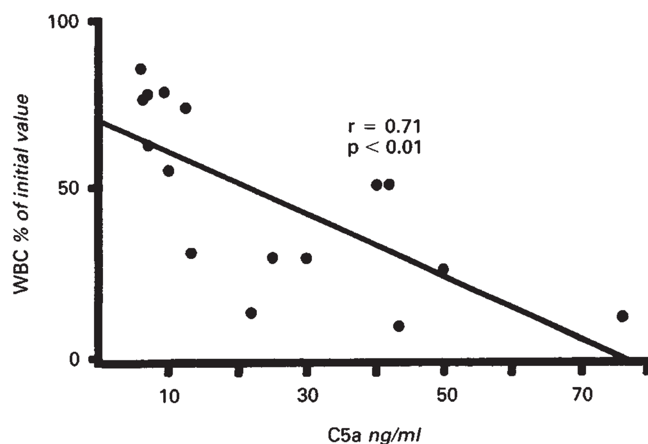

Fig. 3. *Relationship between C5a release and white blood cell nadir changes.*

Results

In vitro study

C5a generation after 60 minutes was, as expected, strictly T dependent. The relationship between the T changes in Celsius degrees (x) and in vitro C5a generation (y) is defined by the equation $y = 6.41 \cdot 2.718^{0.109x}$ (in terms of Kelvin degrees $y = 7.5^{-13} \cdot 2.718^{0.109x}$) ($r = 0.917$; $P < 0.001$). According to this equation, lowering the T from 35° to 25°C decreases C5a generation by 60%. A further lowering to 15°C reduces its generation by 90%.

In vivo study

Calculated blood T within the dialyzer averaged 24.9° ± 1.3°C during hypothermic HD, 35.3° ± 1.0°C during isothermic HD. Rewarming the venous line was not sufficient to bring blood T to the same level as in isothermic dialysis. Probably as a result of this greater heat loss from the extracorporeal circuit, rectal T rose less during hypothermic HD, even though the difference was not statistically significant.

Venous C5a concentration increased from 3.1 ± 1.5 ng/ml to a peak of 41.7 ± 17 ng/ml during isothermic HD, and from 4.6 ± 2.6 ng/ml to 9.7 ± 3.4 ng/ml during hypothermic HD (Fig. 1). The difference in C5a variation between the two procedures was highly significant ($P < 0.01$). The peak concentrations occurred after 15 or 30 minutes in isothermic HD and in six out of nine hypothermic HD patients, while in the remaining three hypothermic treatments the peak was delayed for 60 minutes. In arterial blood the C5a concentration increased slightly during isothermic HD after 15 minutes, whereas it remained almost unchanged through the hypothermic HD.

Concurrently with this effect on C5a generation, hypothermic HD reduced the degree of white blood cell count maximal fall from 72 ± 15% to 25 ± 20% ($P < 0.01$) (Fig. 2). In both procedures the fall in white blood cells was caused mainly by neutrophil depletion. The maximum fall in WBC (y) bore a significant relationship with the concurrent rise in C5a concentration (x) in venous blood ($r = 0.71$; $y = -0.918\ x + 70.349$; $P < 0.01$) (Fig. 3). It always occurred at 15 minutes in isothermic HD, while in hypothermic HD it was observed in three patients at 15 minutes, in four at 30 minutes, and in two at 60 minutes.

PaO_2 dropped significantly less during hypothermic than

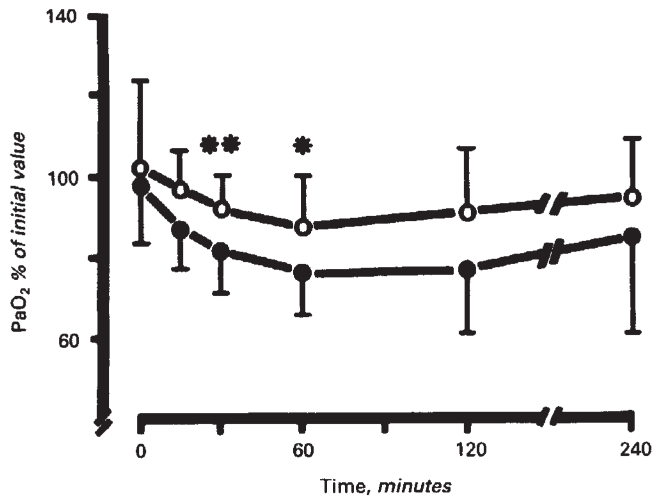

Fig. 4. *PaO_2 changes during isothermic (●) and hypothermic (○) HD,* **$P < 0.01$; *$P < 0.05$.

during isothermic HD, the maximal drop occurring after 60 minutes to 81 ± 9 and 71 ± 11% of the predialysis values, respectively ($P < 0.05$) (Fig. 4). $PaCO_2$ changes, on the other hand, were similar during both iso- and hypothermic HD.

In comparison with isothermic HD, hypothermic HD caused a decrease in both urea and creatinine clearance from 182 ± 21 to 151 ± 16 ml/min, and from 150 ± 14 to 126 ± 15 ml/min, respectively ($P < 0.01$).

Discussion

Like other polysaccharidic substances cuprophan membrane is capable of activating the alternative pathway of complement cascade [1, 2]. As originally shown by Pillemer et al in the early fifties [10], fully confirmed by our in vitro studies employing a completely different assay system, activation of the alternative pathway is temperature dependent. The dose-response curve between T and anaphylatoxin generation conforms to an exponential relationship, such as generation of C5a, is strongly curtailed when the T of reaction is lowered below 30°C.

The results obtained in vivo with hypothermic dialysis were substantially in line with the in vitro findings. With our home-made system, the lowest obtainable T value of blood contacting the dialyzer membrane was 25°C. On comparing the actual peak concentrations of anaphylatoxins during the two dialytic procedures, we found that hypothermic dialysis caused a 77% decrease in C5a peak values. The diminished C activation was mirrored by a lower drop in white blood cell count, thus lending support to the notion that C activation is the cause of granulocytopenia [4, 5, 8].

Together with the milder leukopenia and the blunted C activation, there occurred less PaO_2 decrease during hypothermic than during isothermic dialysis. Arterial hypoxia occurring during acetate dialysis is currently believed to result from C activation [11] and/or reflex hypoventilation evoked by the loss of CO_2 through the dialyzing membrane [12, 13]. Blood cooling might have decreased PaO_2 drop by reducing the degree of both C activation and CO_2 loss through the dialyzer. To the extent that arterial hypoxia may depend on increased O_2 consumption, as suggested by some authors [13], even the lesser increase in core T occurring during hypothermic dialysis might have contributed to the decreased PaO_2 fall. However, since this study was not designed to explore these mechanisms, we cannot draw any conclusions about the cause of decreased hypoxia during hypothermic HD.

Dialysis-borne C activation can be largely avoided if reused Cuprophan or non-cellulosic membranes are employed [4, 5]. Even dialysis hypoxia, which can be deleterious in some patients, can be largely prevented if bicarbonate instead of acetate is used as buffer in the dialysis bath [13]. It appears more practical to resort to these means than to cool the blood within the dialyzer, not least because these means, unlike blood cooling, do not entail any reduction in dialyzing efficiency.

Perhaps, the effect of cooling on C activation can be exploited to greater advantage in other types of extracorporeal circulation such as cardiopulmonary bypass [3]. In this case, as against in that of dialysis, optional materials devoid of the property of activating the C system are not presently available. Activation of C cascade is suspected as a key factor in the pathogenesis of clinical catastrophies occasionally occurring following an uneventful cardiopulmonary bypass. To shed some light on this problem a means of preventing C activation, such as cooling of extracorporeal blood to an appropriate degree, appears worthy of being exploited.

Acknowledgments

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