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EFFECT OF IRON OVERLOAD ON SUPEROXIDE ANION PRODUCTION BY GRANULOCYTES IN HAEMODIALYSIS PATIENTS

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Summary

To investigate the possible influence of iron overload on the phagocytic function of 40 patients on chronic maintenance haemodialysis, we measured the generation of superoxide anion (O_2^-) by leucocytes in whole blood after stimulation by opsonized zymosan or phorbol myristil acetate (PMA). Iron overload (21 patients), as defined by a serum ferritin >1000ng/ml in polytransfused patients, was associated with decreased metabolic responses of leucocytes to both stimulating agents. These data suggest that the increased susceptibility of some haemodialysis patients to bacterial infections may be partly related to a deleterious affect of iron overload on leucocyte function.

Introduction

Bacterial infections represent a major threat to patients undergoing maintenance haemodialysis [1]. The possible involvement of polymorphonuclear leucocyte changes in this increased susceptibility to pyogenic agents remains controversial [2]. Among the various factors which could affect polymorphonuclear leucocyte function in haemodialysis patients, iron overload has to be considered since iron excess has been shown to impair their function [3–6]. In the present study, polymorphonuclear leucocyte responsiveness of haemodialysis patients with or without iron overload was assessed by measuring superoxide anion (O_2^-) generation after in vitro stimulation by phagocytisable particles (opsonized zymosan) or by a soluble activating agent (phorbol myristil acetate (PMA).

Material and methods

Twenty-one patients (group A) with serum ferritin >1000ng/ml (range 1000 to 14,370ng/ml, median 3770ng/ml) and transferrin saturation above 50 per cent were compared to 19 patients (group B) with serum ferritin <1000ng/ml (range

13 to 950ng/ml, median 73ng/ml) and transferrin saturation lower than 25 per cent. All patients were on chronic maintenance dialysis for at least three months. At the time of the study, none had acute hepatitis or active bacterial infection. There was no significant difference between the two groups regarding age, sex, duration of haemodialysis, serum 25-hydroxy vitamin D_3 or serum parathormone.

The measurement of O_2^- production was performed as described elsewhere [7]. Briefly, 5ml of heparinized blood was drawn from the fistula immediately before the beginning of a dialysis session. One hundred microlitres of whole blood were incubated in the presence of ferricytochrome c without or with a stimulating agent (opsonized zymosan or PMA) for 15 minutes at 37°C. The extracellular release of O_2^- was assayed by measuring O_2^- dependent ferricytochrome c reduction. After appropriate dilution, the absorbance of reduced cytochrome c in the cell-free supernatant was read at 550nm in a split-beam spectrophotometer. The absorbance units were converted into nmoles of reduced cytochrome c by using an absorbance coefficient at $21.1 mM^{-1}\ cm^{-1}$.

Specificity of ferricytochrome c reduction by O_2^- was evaluated by adding superoxide dismutase in control tubes. Results were expressed as nmoles of $O_2^-/10^6$ PMN x 15 minutes on the basis of the total and differential counts of white blood cells.

Results

As shown in Table I, baseline O_2^- production without stimulus was not significantly different in the two groups. In contrast, the metabolic responses to opsonized zymosan as well as to PMA were significantly decreased in iron overloaded patients (group A) as compared to the others (group B).

TABLE I. Superoxide anion production by leucocytes in whole blood

	No stimulus	Opsonized zymosan	PMA
Group A (n=21)	6.3±4.6*	86.5±6.3	91.2±6.3
Group B (n=19)	11.5±8.3	120.4±8.2	141.9±12.3
p**	NS	<0.002	<0.005

* nmoles $O_2^-/10^6$ cells x 15 min (mean ± SEM)

** as determined by Student's 't' test
Group A: serum ferritin >1000ng/ml;
Group B: serum ferritin <1000ng/ml

Discussion

The process of phagocytosis is accompanied by a dramatic increase of the oxidative metabolism of phagocytic cells which results in the production of O_2^- and hydrogen peroxide. These products are indeed required for the generation of

microbicidal oxydants [8]. Recently, the measurement of O_2^- production by stimulated polymorphonuclear leucocytes in whole blood has been proposed as a relevant test for the quantitative evaluation of phagocytic function [7]. Using this assay we found that iron overload is associated in haemodialysis patients with decreased polymorphonuclear leucocyte response to opsonized zymosan as well as to PMA. Deleterious effects of iron overload on polymorphonuclear leucocyte function has been previously reported. In vitro incubation of polymorphonuclear leucocytes from normal donors with iron excess results in reduced uptake of bacteria [3,6]. Moreover, there is some evidence that the functional capacity of polymorphonuclear leucocytes from patients with iron overload is impaired [3,6]. The putative mechanisms of the noxious influence of iron excess on polymorphonuclear leucocytes involve an enhanced production of toxic oxygen radicals [5]. Multiple transfusions and resulting iron overload have been found to affect several parameters of the immune responses in haemodialysis patients [9,10]. Our data indicate that these polytransfused patients also display impairment in phagocytic functions which could results in an increased susceptibility to bacterial infections.

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