Superoxide Anion Production and Dietary Treatment in Chronic Renal Failure

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Dear Sir,

Chronic renal failure (CRF) is associated with several functional defects of leukocytes (chemotaxis, phagocytosis and chemoluminescence), which may partly account for the increased risk of infections in uremic patients [1–3]. Recent studies have shown that the oxidative metabolism of polymorphonuclear leukocytes from uremic patients on conservative treatment, as well as in patients on dialysis treatment, is impaired [3]. Aparicio et al. [4] demonstrated that in patients treated with a low-protein, low-phosphorus diet supplemented with essential amino acids and keto-analogues, the chemoluminescence response of leukocytes improved.

The aim of this study was to evaluate the effect of a very-low-protein (0.3 g/kg/day) diet, supplemented with a mixture of essential amino acids and keto-analogues (SD), on superoxide anion (SA) production by granulocytes from patients with severe CRF in the predialytic phase. We performed a comparative study of 26 patients with severe CRF; they were divided into two groups: group 1, 13 patients on SD; group 2, 13 patients on standard low-protein diet (LPD) x (0.6 g/kg/day); 18 normal subjects were used as controls.

Diabetics, heavy smokers and patients with infectious diseases, neutrophilia, monocytosis or eosinophilia were excluded. Serum levels of immunoglobulins, C3 and C4 were within the normal range in all.

SA production by granulocytes was measured using a colorimetric assay (Fago-Test, Far Divisione Diagnostici, Verona, Italy), which has been derived from the method described by Bellavite et al. [5]. According to the instructions of the manufacturer, heparinized (10 U/ml) blood samples were used in order to study SA production by zymosan-stimulated granulocytes in the presence of cytochrome C (0.25 nmol/l). The incubation was carried out in plastic tubes for 15 min at 37°C in a water bath under continuous shaking. Appropriate controls were carried out in order to evaluate the SA-independent reduction of cytochrome C by granulocytes incubated in the absence of zymosan. The incubation was stopped by adding N-ethylmaleimide in cold Krebs-Ringer phosphate buffer and the tubes were centrifuged at 1,500 g for 10 min. The absorbance of the cell-free supernatants was then measured at 550–468 nm (absorbance at 550 nm minus that at 468). The absorbance resulting from the absorbance values of test tubes minus the absorbance values of control tubes was multiplied by a correction factor

of 121, which was calculated according to the manufacturer's instructions by means of the Fagotest control kit. SA production was expressed in nanomoles of SA/106 granulocytes. The results were expressed as mean $\pm$ SEM and statistical analysis was performed by Student's t test for unpaired data.

Creatinine clearance and serum creatinine were not significantly different between group 1 and group 2, indicating an equally severe CRF. However serum and urinary urea were much higher in group 2 (table 1).

Table 1. Renal function, serum and urinary levels of urea of the studied patients

Parameters	Group 1	Group 2	p
Creatinine clearance, ml/min	7.0 $\pm$ 0.5	8.3 $\pm$ 0.5	NS
Serum creatinine, mg/dl	7.3 $\pm$ 0.6	6.7 $\pm$ 0.5	NS
Serum urea, mg/dl	34.5 $\pm$ 4.9	102.0 $\pm$ 11.6	< 0.001
Urinary urea, g/day	2.4 $\pm$ 0.3	7.5 $\pm$ 0.8	< 0.001

Group 1: patients on SD (n = 13); group 2: patients on LPD (n = 13); results are expressed as means $\pm$ SEM.

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SD LPD Controls

Fig. 1. Superoxide anion production by activated granulocytes from patients affected by CRF, treated with SD or LPD, and from normal controls. Means $\pm$ SEM.

The production of SA in group 1 was higher than in group 2 (57.0 $\pm$ 8.1 vs. 38.2 $\pm$ 4.1, $p < 0.05$), and not different from normal controls; SA production in group 2 was lower than that of controls (38.2 $\pm$ 4.1 vs. 56.6 $\pm$ 4.8, $p < 0.01$) (fig. 1). No correlation was found between SA values and serum and urinary urea or creatinine clearance.

Our data confirm the existence of a defect in the oxidative metabolism of granulocytes of patients with severe CRF [3], which is not corrected by conventional LPD and can instead be improved by SD [4].

The better detoxication obtained by SD, as indicated by the lower serum urea levels, may explain why it ameliorates the reactive oxygen production by activated granulocytes.

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