EFFECT OF ZINC TREATMENT ON CELL MEDIATED IMMUNITY OF CHRONIC RENAL FAILURE PATIENTS

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Summary

This study was performed on 13 normal subjects and 34 patients with chronic renal failure (CRF). Oral elemental zinc at a dose of 60mg/day for four weeks was administered. Delayed type hypersensitivity (DTH) skin tests were performed using the multitest method. Monocyte accessory cell function (MAF) was tested by their ability to support T-cell colony growth in semi-solid cultures stimulated by staph-protein A (SpA). After zinc treatment a significant increase of DTH and MAF was found in all patients with advanced CRF in comparison with controls. These results provide evidence that zinc treatment may restore the impaired cellular mediated immunity of uraemia.

Introduction

Most patients with chronic renal failure (CRF) or on maintenance haemodialysis (HD) have plasma zinc values below those observed in normal subjects [1]. The significance of hypozincæmia in uraemia is however controversial since low plasma zinc levels do not always reflect tissue zinc deficiency [2].

Studies of uraemic patients and of animals with experimentally induced uraemia have shown that the immune response is severely altered. The most pronounced changes are found in cell-mediated immunity [3]. Zinc deficiency affects the development, maintenance, and function of immunocompetent lymphocytes in both non uraemic animals and man [4]. Human zinc deficiency, seen in acrodermatitis enteropathica or in parenterally aliments patients not supplemented with zinc, causes T-cell lymphopenia, cutaneous anergy and depressed PHA-stimulated T-cell mitogenesis [5,6].

The aim of the present study was to examine the effect of hypozincæmia and zinc repletion on the in vivo and in vitro status of cell-mediated immunity (CMI) in chronic renal failure patients.
Patients and methods

Twenty-six patients with CRF and eight patients on maintenance haemodialysis were included in the study. The mean age of these patients was 43.7 ± 8.8 years and 36.2 ± 10.9 respectively. CRF patients were divided in two groups: CRF$_1$ of 12 patients with moderate uraemia (Ccr ≥25ml/min) and group CRF$_2$ of 14 patients with advanced uraemia (Ccr <25ml/min). Thirteen normal subjects aged 42 ± 13 years were used as sex and age matched healthy controls. CRF patients were on a protein restricted diet and received daily supplements of multivitamins, folic acid and aluminium hydroxide antacids. Haemodialysis patients were on a non-restricted diet and were dialysed for four hours thrice weekly with hollow fibre dialysers. All patients were in good clinical condition at the time of the study.

Oral elemental zinc was administered as zinc acetate (E Merck, Darmstadt) at a dose of 60mg/day (200mg of zinc acetate) for four weeks in both controls and patients. Before and after zinc treatment an assessment of CMI was made by in vivo and in vitro studies. For the in vivo study, delayed type hypersensitivity (DTH) skin tests were performed with the multitest method (IMC, Institut Merieux). The antigens employed were: streptococcus (SK), tuberculin (PPD), candida albicans (CA) and trichophyton (TR). For in vitro study monocyte accessory cell function (MAF) was tested by the ability of these cells to promote T-cell colony formation (TcCF) in semi-solid agarose cultures stimulated by staph-protein A (SpA, Pharmacia). All microcultures were performed in flat-bottomed microwell plates (Falcon Plastics, Oxnard, CA) by using the single layer technique. 5X10$^4$ T-cell enriched preparations and 2X10$^4$ macrophages (MΦ) per microwell were used. All T-cell cultures were stimulated with 10µg/ml SpA. Colony responses were enumerated after incubation for seven days at 37°C in a humidified atmosphere of 95 per cent air and five per cent CO$_2$. Distinct aggregates consisting of 15 or more cells were scored as colonies with a Zeiss 135 inverted phase contrast microscope. The number of colonies were expressed as the mean ±SD of triplicate samples.

For zinc analysis blood samples were drawn from patients and controls after overnight fasting and were collected in plastic tubes to avoid zinc contamination. Plasma zinc concentrations were measured by atomic absorption spectrophotometry (Perkin Elmer 305 A) after dilution 1:1 with deionised water.

Results

At the dose of 200mg daily (60mg of elemental zinc), zinc acetate was well tolerated and no side effects were recorded. Plasma zinc concentration was also restored within normal limits (70–130µg/100ml) in both groups of patients after four weeks with this regimen.

Table I illustrates the results of zinc treatment on skin testing (mean of all antigens) in controls and patient groups. The score was measured as the mean of two diameters of the skin induration [4]. Score equal to or greater than 2mm was considered as positive. The response to skin tests after zinc treatment indicated slight increase of DTH in both groups of controls and CRF$_1$, but was significantly
increased in CRF\textsubscript{2} and haemodialysis patients (p<0.001 and p<0.05 respectively) in comparison with the pre-treatment skin response. Twenty-five to thirty per cent of uremic patients who were anergic to skin testing before zinc treatment showed positive skin reactions in one or more skin antigens after zinc treatment. The effect of zinc treatment on TcCF is shown in Table II. TcCF of patients before treatment showed a significant difference (p<0.01) when compared to controls. After zinc treatment a significant increase (p<0.01) of TcCF was found in both groups of patients in comparison to their pre-treatment mean values of TcCF. The addition of indomethacin in the cultures pre- and post-zinc treatment did not change the above results (data not shown).

**TABLE I.** Effect of zinc treatment on delayed type hypersensitivity skin testing. The score measured by the mean (±SD) of two diameters of the skin induration. Score >2mm was considered as positive

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Before</th>
<th>After</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>13</td>
<td>3.3 ± 3.5</td>
<td>4.7 ± 3.9</td>
<td>NS</td>
</tr>
<tr>
<td>CRF\textsubscript{1}</td>
<td>12</td>
<td>2.6 ± 2.4</td>
<td>3.1 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>CRF\textsubscript{2}</td>
<td>14</td>
<td>0.6 ± 1.3</td>
<td>2.3 ± 2.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HD</td>
<td>8</td>
<td>0.9 ± 1.7</td>
<td>2.3 ± 2.8</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

CRF\textsubscript{1}: patients with moderate uraemia
CRF\textsubscript{2}: patients with advanced uraemia
HD: haemodialysis patients

**TABLE II.** Effect of zinc treatment on T-cell colony formation. The number of colonies were expressed as the mean ±SD of triplicate samples

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Before</th>
<th>After</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>4</td>
<td>140.2 ± 17.5</td>
<td>141.2 ± 17.2</td>
<td>NS</td>
</tr>
<tr>
<td>CRF\textsubscript{1}</td>
<td>6</td>
<td>90.5 ± 18.7*</td>
<td>125.7 ± 16.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CRF\textsubscript{2}</td>
<td>7</td>
<td>88.6 ± 19.8*</td>
<td>127.6 ± 19.5</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*p<0.01 (compared to controls)
CRF\textsubscript{1}: patients with moderate uraemia
CRF\textsubscript{2}: patients with advanced uraemia

**Discussion**

The importance of zinc in maintaining normal CMI is emphasised by the severe immunodeficiency found in animals with experimental zinc deficiency and in patients with either congenital or acquired zinc deficiency [3,4,6–8]. These
studies indicate that zinc is required for normal immune function and may have a key role in regulating some lymphocyte functions.

Our data showed depression of both in vivo and in vitro T-lymphocyte response during hypozincæmia in most uraemic patients. Reversion to normal or nearly to normal limits occurred four weeks after the administration of zinc as a sole supplement of nutritional therapy. Skin test reactivity also reappeared in 25–30 per cent of uraemic patients who were anergic before zinc supplementation.

The present results tend to confirm the findings of previous investigations [4,6–8]. First, the restoration of plasma zinc levels was accompanied by similar increase of DTH reactivity. Second, the increased plasma zinc levels was also associated with an improvement of T-cell colony responses. Since the clonal growth of T-lymphocytes is crucially dependent on the presence of MΦ [9] it can be said that the increase responses of T-lymphocytes reflected a better accessory function of MΦ induced by zinc treatment.

One possible interpretation for the improved responses of T-lymphocytes might be an increase in the interleukin-1 production of MΦ after zinc treatment. However, our preliminary results showed that there were not significant changes in the secretion of this monokine before and after zinc treatment. At the moment the likeliest explanation for the immuno-enhancement seen after zinc treatment may be caused by an improvement of cell membrane functions. The latter is probably related to the requirement of zinc for DNA synthesis [10]. Thus, the ability of zinc to promote T-cell responses may represent a beneficial effect of zinc treatment on the defective antigen processing or presentation function of monocytes in hypozincæmic uraemic patients.

Therefore, although the above mechanisms are not yet clear, the present data justify a prospective study of the long-term effects of dietary zinc supplementation in uraemic patients. This treatment is inexpensive, non-toxic and could significantly improve immune function in CRF.

References

2. Kerr DNS. Proc 8th Int Congr Nephrol 1981; 1014
Open Discussion

RAMZY (Cairo) How can you explain the correction of skin anergy by zinc supplementation only when it is known that other serum factors, such as middle molecules, are present and your patients are still uraemic?

GREKAS It is well known that the plasma of uraemic patients can inhibit leucocyte activation in cultures. From our data we can’t say that other factors don’t contribute in uraemic patients to depressed lymphocyte activity and after zinc in activation of leucocyte activity. I can say that we can’t exclude other uraemic factors.

RAMZY But have your patients become reactive to skin tests by the administration of zinc? In your study you have excluded all other possible factors and concentrated only on zinc.

GREKAS During the study all our patients were in the same clinical state. None were given multivitamins or other preparations or other medicinal support and in this way we can say that the zinc was the only additional treatment which influenced the activities.

BIANCHI (Chairman) Is the restoration of delayed hypersensitivity complete or partial?

GREKAS We used more than one skin antigen and with the test we assess all seven of them. We showed that uraemic patients after zinc supplementation had a positive skin reaction to at least one skin antigen or more. It means that delayed type hypersensitivity is restored in uraemic patients after zinc supplementation.

UNKNOWN Do you have any evidence that the humoral immunity is improved after zinc treatment?

GREKAS We measured the immunoglobulins pre and post zinc treatment but there was no difference.