Lymphocyte Enzymes of DNA-Synthesis in Chronic Renal Failure

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Summary

Incubation of healthy lymphocytes in uraemic serum was found to induce depression of the intracellular activity of the enzyme thymidine kinase (EC 2.7.1.75), whereas DNA polymerase (EC 2.7.7.7) remained unaltered.

Higher molecular weight urine metabolites from healthy persons, which caused accelerated cell destruction in further investigations, had no influence on either enzyme.

Therefore depressed enzymatic DNA synthesis is proposed as the main reason for disturbed lymphocyte transformation in chronic uraemia, whereas a separate cytotoxic effect may be due to retained higher molecular weight toxins.

Introduction

The transformation of the T-lymphocytes into lymphoblasts with mitotic activity after antigen stimulation is known to be depressed in chronic renal insufficiency. The resulting loss of cellular immunity is demonstrable by measurement of $^3$H-thymidine uptake in lymphocyte cultures after phytohaemagglutinin (PHA) stimulation. Addition of uraemic serum to the cultures decreases metabolic DNA synthesis as well as impairs cell viability (Korz et al, 1975). The latter effect may be caused by retained higher molecular weight metabolites traceable in urine from healthy persons, which induce dose dependant depression of transformation by accelerated lymphocyte destruction (Korz et al, 1975).

As the activity of various enzymes is known to be reduced in chronic uraemia (Dobbelstein, 1971), enzymatic disturbance of DNA synthesis seems to be possible. Therefore two of the main enzymes, thymidine kinase and DNA polymerase,
were estimated in lymphocytes from healthy subjects after incubation in uraemic serum and in dissolved higher molecular urine metabolites, and also in lymphocytes from patients with chronic renal failure. The first enzyme regulates the synthesis of thymidine nucleotide by phosphorylation of thymidine, while DNA polymerase catalyses the polymerisation of the four nucleotides to deoxyribonucleic acid (DNA).

METHODS

After separation of healthy lymphocytes by Leuco-Pac-Filter (Fenwal Lab. USA) passage and dextran-sedimentation of the erythrocytes the resulting cell suspension was dispensed in four portions and then after resuspension in one ml each of Medium TC 199, control serum and sera from two patients receiving regular dialysis treatment were incubated for one hour at 37°C. Subsequently the cells were washed, resuspended in Medium TC 199 and the activity of both enzymes was estimated.

In a second series of investigations, the enzymes were analysed in the lymphocytes of patients on regular dialysis treatment.

In a third series healthy lymphocytes were incubated for one hour in three different quantities of higher molecular weight urine fraction (0.1; 0.5 and 1 ml of a solution containing 2.22 mg/ml TC 199; final volume: 1 ml). Then the intracellular activity of both enzymes was measured.

The higher molecular weight urine fraction was isolated by dialysing 24-hour urine from healthy persons against demineralised, and twice distilled water. The lyophilised, non-dialysable residue amounted to 200 to 300 mg/24 hours.

Although substances up to 10,000 molecular weight will pass through the membrane, about 40% of the residue was found to be substances of molecular weights below 5000 after disaggregation by 8-molar urea solution; the reason may be the formation of complexes. Therefore the fraction was defined: 'higher molecular'.

The estimation of thymidine kinase was performed after cytolysis by phosphorylation of ³H-labelled thymidine and subsequent separation of the thymidine monophosphate so formed by column adsorption chromatography (Weissman et al, 1960).

To analyse DNA polymerase the incorporation of ³H-labelled thymidine triphosphate into acid-insoluble polynucleotides was measured (Smith & Gallo, 1972; Stombolova et al, 1973).

The activity of both enzymes was defined as pmoles substrate turnover per hour per mg protein.

RESULTS

After incubation of healthy lymphocytes in uraemic sera DNA polymerase remained unchanged, while the activity of thymidine kinase showed significant
TABLE I. Enzyme-activity in Healthy Lymphocytes After Incubation in Uraemic Serum

<table>
<thead>
<tr>
<th></th>
<th>controls (normal serum)</th>
<th>uraemic serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA polymerase (pmoles/hr·mg prot.)</td>
<td>9.0 ± 8.39 (n = 6)</td>
<td>7.94 ± 6.44 (n = 12)</td>
</tr>
<tr>
<td>Thymidine kinase (pmoles/hr·mg prot.)</td>
<td>30.4 ± 11.3 (n = 8)</td>
<td>19.9 ± 10.8 (n = 16)</td>
</tr>
</tbody>
</table>

inhibition, confirmed by block-variance-analysis (Table I).

A significant correlation of the thymidine kinase levels with the haemoglobin concentration in the blood of the patients was found (Figure 1), but no coherence with other parameters of chronic renal insufficiency (urea, creatinine and inorganic phosphate in the serum).

![Graph](image)

Figure 1. Correlation of thymidine kinase levels from healthy lymphocytes incubated in uraemic sera, and blood haemoglobin concentration

In lymphocytes of chronic uraemic patients identical enzyme distribution was found (Table II); significantly diminished thymidine kinase activity, unaltered DNA polymerase levels (T-test).
### TABLE II. Enzymes of DNA-synthesis in the Lymphocytes

<table>
<thead>
<tr>
<th>DNA polymerase (pmoles/hr-mg prot.)</th>
<th>healthy</th>
<th>chronic renal insufficiency</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>9.22 ± 5.89 (n = 6)</td>
<td>8.64 ± 1.28 (n = 8)</td>
</tr>
</tbody>
</table>

| Thymidine kinase (pmoles/hr-mg prot.) | 23.8 ± 12.3 (n = 8) | 10.8 ± 3.14 (n = 8) |

Figure 2. Thymidine and DNA polymerase activity in healthy lymphocytes incubated with increasing quantities of higher molecular urine fraction.

Incubation of healthy lymphocytes with increasing quantities of higher molecular urine fraction induced no significant alteration or dose dependence of either enzyme (Friedman-test); merely a trend towards slight increase was observed (Figure 2).

### DISCUSSION

The importance of thymidine kinase is the regulating function in DNA metabolism; the resulting end-product of the enzyme-reaction, thymidine triphosphate, has a restraining effect upon the thymidine kinase itself, the enzyme activity correlating with the DNA synthesis estimated by $^3$H-thymidine uptake (Hartje & Wilmanns, 1970). Therefore the depression of this enzyme may be one of the main reasons responsible for the inhibition of lymphocyte transformation in chronic renal insufficiency, the more so since the spontaneous $^3$H-thymidine uptake of cultured lymphocytes shows identical behaviour (Korz et al. 1975). The correlation with blood haemoglobin content in both cases may reflect the retention of a common uraemic toxin.

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Phytohaemagglutinin stimulation enhances $^{3}$H-thymidine uptake by both investigated enzymes simultaneously (Loeb et al, 1970); as spontaneous lymphocyte transformation shows parallel proportions compared with results after PHA stimulation (Korz et al, 1975), it can be concluded indirectly, that in this case too the inhibition of thymidine kinase activity may be responsible for the disturbed lymphocyte proliferation.

Higher molecular urine metabolites had no influence upon either enzyme activities. These toxins may merely induce a separate cytotoxic impairment of cell viability in renal failure, if the retention of these metabolites in chronic uraemia is assumed.

References

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