RIBONUCLEASE ACTIVITY IN RENAL FAILURE:
EVIDENCE FOR TOXICITY

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Summary

The normal level of serum or plasma poly C-avid ribonuclease activity is
1047±247 U/mL. Serum levels increase proportionately with elevations in
serum creatinine, reaching levels of 9,500-35,000 in patients undergoing
dialysis. The levels can be normalised by successful renal transplantation but
not by dialysis. Purified human urinary ribonuclease, a glycoprotein enzyme
similar to the serum ribonuclease, was capable of: 1) inhibiting the incorpora-
ton of 3H-thymidine into mitogen-stimulated lymphocytes; 2) inhibiting the
proliferation and growth of bone marrow red cell colonies; and 3) adversely
affecting the growth and viability of precursor fat cells.

Introduction

In 1975 we reported on the isolation, purification and properties of a ribonuclease
from normal human urine.1 The enzyme, a glycoprotein, has a molecular weight
of 33,000 and appears to consist of one polypeptide chain with internal
disulphide bridges. The enzyme preferentially degrades the synthetic RNA
polymer Poly C. Poly G, A, and I are refractory to its action. Similar type
enzymes have been purified by Schmukler2 (serum-mol wt 32,000), Reddi3
(urine; mol wt 21,000), and Bardon4 (serum and urine; mol wt 14,000).
Furthermore Akagi et al5 have shown that in normal serum, poly C-avid
ribonucleases can be found with variable molecular weights; 45,000, 32,000,
20,000 and 13,000. This paper documents the importance of ribonuclease levels
in renal failure.

Methods

Ribonuclease activity was measured according to the method of Zimmerman
and Sandeen6 as modified by Rabin and Weinberger1. Creatinine clearances
were measured in patients under maximal hydration using standard techniques.
Molecular weight of the ribonuclease activity was measured in uraemic serum using Sephadex G-100 columns (2.5 x 90 cm) according to the method of Andrews7.

Toxicity studies using purified human urinary ribonuclease were carried out in the following cell systems: mitogen-stimulated lymphocytes, according to the method of Boyum8; bone marrow red cells, according to the method of Freedman et al9; fat cell cultures according to the method of Roncari et a10. Ribonuclease concentration in cell culture systems was 20,000 U/mL unless otherwise specified.

Results

**Serum or Plasma Ribonuclease**

In normal people the serum or plasma ribonuclease level was found to be 1,041 ± 247 U/ml. In patients undergoing haemodialysis or peritoneal dialysis the levels ranged from 9,500 - 35,000 U/mL (Table I). Dialysis does not affect these levels. In between these two extremes, patients with increasing degrees of renal insufficiency were found to have increasing levels of serum ribonuclease; there was good correlation between levels of serum creatinine and ribonuclease (Figure 1). Furthermore the relationship between the creatinine clearance and the serum ribonuclease emphasised the fact that as renal function deteriorates the serum ribonuclease increases (Figure 2). Patients with other diseases and normal renal function have normal serum ribonuclease levels.

When uraemic serum is chromatographed on Sephadex G-100 two major peaks of ribonuclease activity are seen: one of molecular weight 33,000 and the other of molecular weight 18,000 (Figure 3). Both of these peaks of activity can be inhibited by rabbit-anti-human-urinary ribonuclease antibody. When a patient with renal failure is successfully transplanted, the serum ribonuclease level falls to within normal levels within 24-48 hours; and when rejection supervenes the serum ribonuclease activity begins to increase (Figure 4).

**Urine Ribonuclease**

Normal people excrete up to 6.1 x 10^6 U/day. Patients with renal insufficiency
Figure 1. Relation between serum ribonuclease and serum creatinine

Figure 2. Relation between serum ribonuclease and creatinine clearance
Figure 3. Sephadex G-100 chromatography of uraemic serum. Peaks of ribonuclease activity, a) 33,000, and b) 18,000

Figure 4. Changes in serum ribonuclease activity with successful transplantation, and with rejection

(whether primary tubular or glomerular) generally excrete increased amounts of poly C-avid ribonuclease activity (Table II). It can be seen that there is a greater mean excretion in the primary tubular group but the overlap between the glomerular and tubular groups is such that definitive diagnoses cannot be made. It is also clear from the data that patients undergoing dialysis, and particularly patients with polycystic kidney disease, excrete very large amounts of enzyme activity.
TABLE II. Mean Urinary Excretion of RNase

<table>
<thead>
<tr>
<th>Condition</th>
<th>U/day x 10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3.45 ± 2.86</td>
</tr>
<tr>
<td>Primary glomerular</td>
<td>5.42 ± 10.26</td>
</tr>
<tr>
<td>Primary tubular</td>
<td>10.00 ± 18.26</td>
</tr>
<tr>
<td>Dialysis</td>
<td>13.57 ± 44.20</td>
</tr>
<tr>
<td>Polycystic kidneys (on dialysis)</td>
<td>22.70 ± 20.40</td>
</tr>
</tbody>
</table>

Toxicity of Ribonuclease

The addition of purified human urinary ribonuclease (mol wt 33,000) to cultures of lymphocytes, led to a significant inhibition of the uptake of titrated \(^3\)H-thymidine. The inhibition occurred even when the enzyme was added after the addition of mitogens to the culture system (Figure 5).

![Graph showing the effect of ribonuclease on the incorporation of \(^3\)H-thymidine into lymphocytes, stimulated by mitogens, PHA, Con A and pokeweed](image)

Figure 5. Effect of ribonuclease on the incorporation of \(^3\)H-thymidine into lymphocytes, stimulated by mitogens, PHA, Con A and pokeweed.

Similarly, the results depicted in Figure 6 indicate that the enzyme is capable of inhibiting the normal growth or proliferation of bone marrow cultures. When the purified enzyme was added to precursor fat cell cultures the incorporation of DNA appeared to be within the low normal range but the via-
bility of fat cells produced in culture was adversely affected.

Approximately 25% of the cells were non-viable as determined by trypan blue staining methods and by morphological studies.

Discussion

The data presented clearly indicate that in renal failure, serum Poly C-avid ribonuclease activity increases, no matter what the aetiology of the renal disease. Successful transplantation restores the serum ribonuclease to normal.

Patients requiring haemo- or peritoneal dialysis have levels of serum ribonuclease activity greater than 9,500 U/mL and generally in excess of 15,000 U/mL.

The toxicity studies indicate that when purified human urinary ribonuclease is added to cell cultures at concentrations similar to the activity found in patients undergoing dialysis, inhibition of normal cell proliferation and cell growth is observed. The inhibitions described are not surprising in view of the effects produced by high levels of ribonuclease in previous 'in vitro' studies involving the growth of viruses and tumour cells.

These observations can help to explain the immunodeficiency, anaemia and hyperlipidaemia seen in renal failure. Indeed the poly C-avid ribonucleases represent a class of high molecular weight, non-dialysable 'toxins' which may be having widespread adverse effects on cell growth and protein synthesis.
References

1 Rabin, EZ and Weinberger, V (1975) *Biochem. Med.* 14, 1
14 Le Clerk, J (1956) *Nature*, 177, 578
15 Vasilev, JM, Gelfand, IM, Guelstein, VI and Fetisova, EK (1970) *J. Cell Physiol.*, 75, 305

Open Discussion

ULDALL (Toronto) You seem to be suggesting that the higher the degree of uraemia the greater the blood levels of ribonuclease, perhaps due to over-production. Have you any evidence that by dialysing the patient better you can reduce production and blood levels?

RABIN No, we have not studied that directly, but we find that the longer the patient is on dialysis the more the levels continue to increase.

GREGERSEN (Esbjerg, Denmark) You state that ribonuclease could contribute to the development of anaemia in uraemic patients. Have you some information about the ribonuclease levels in patients with polycystic kidneys, in whom anaemia is less common?

RABIN The levels of ribonuclease reflect renal function; as the creatinine goes up, the ribonuclease levels go up. Basically it is directly related to filtration. We have one patient with polycystic kidney disease who has high ribonuclease levels, who is not anaemic. We have four other polycystics who are anaemic, who have these high levels. The problem in interpretation is that ribonucleases are glyco-proteins, and the actual mechanism by which the ribonuclease acts may be twofold. One is the effect on protein synthesis and second, because it is a glyco-protein it can be a non-specific competitor for cell-membranes with other glyco-proteins, such as erythropoietin. But if I say erythropoietin acts through a DNA/RNA synthetic mechanism, in fact you may have some patients (e.g. polycystics) in whom very high erythropoietin levels may overcome the effects of ribonuclease. You may have a push-pull system.