Biological Half-Life, Organ Distribution and Excretion of $^{125}$I-labelled Toxic Peptide from the Blue-green Alga *Microcystis aeruginosa*

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**Abstract**

*M. aeruginosa* is a bloom-forming cyanobacterium which is common in fresh-water lakes. It contains a potent hepatotoxin which when purified has been shown to be a heptapeptide of molecular weight 1019. The toxin was iodinated with $^{125}$I using the lactoperoxidase method, the labelled toxin administered intravenously to adult female rats and the half-life and organ distribution measured. The blood half-life after redistribution into extracellular pools was 42 min. The liver and kidneys showed accumulation of $21.7 \pm 1.1$ and $5.6 \pm 0.2\%$ of the dose respectively after 30 min. Little accumulation was observed in other organs and tissues. Small-intestinal contents and urine contained $9.4 \pm 6.1$ and $2.9 \pm 1.2\%$ of the dose respectively after 120 min. It was concluded that the liver is the main target organ for both accumulation and excretion of the toxin.

**Introduction**

Toxic water-blooms of *Microcystis aeruginosa* have been shown to cause livestock deaths in most continents of the world (Carmichael 1981). Toxic injury to human populations has also been reported following occurrence of this cyanobacterium in municipal drinking water supplies (Zilberg 1966; Falconer *et al.* 1983). Studies of the pathological changes in animals following oral or intraperitoneal administration of toxic bloom have consistently shown liver injury. The acute response in mice is hepatocyte necrosis with destruction of sinusoidal endothelium and extensive haemorrhage into the liver (Falconer *et al.* 1981). In domestic animals the liver is also the target organ, with extensive damage to hepatocytes (Jackson *et al.* 1984).

Extraction and partial purification of the toxic material from naturally occurring blooms demonstrated a low molecular weight cyclic peptide (Elleman *et al.* 1978), which has subsequently been fully characterized (Botes *et al.* 1984). The toxin is very stable and not susceptible to common proteolytic degradation. The peptide present in material collected from a water-bloom on Malpas Dam near Armidale, N.S.W., has a cyclic ring structure containing the amino acids L-tyrosine, D-alanine, D-isoglutamic acid, $\beta$-methyl-D-aspartic acid, N-methyl-dehydroalanine, L-methionine and a new hydrophobic $\beta$-amino acid with an unsaturated side-chain (Botes *et al.* 1984).

The peptide can be iodinated by the lactoperoxidase method without loss of toxicity (Runnegar *et al.* 1986). As part of our continuing study of the mode of action of *M. aeruginosa* hepatotoxin we describe here the clearance from circulation, the tissue uptake and distribution of $^{125}$I-labelled toxin in the rat.
Methods

All experiments were carried out on adult female albino rats from the University of New England animal colony, provided with food and water up to the time of experiment. Body weights ranged from 207 to 249 g.

Labelled toxic peptide from *M. aeruginosa* was prepared after reversed-phase HPLC separation of semi-purified material, using a gradient from 15 to 25% acetonitrile in 0·007 M ammonium acetate (Runnegar et al. 1986). The toxic peptide chosen for iodination was the unoxidized, methionine-containing heptapeptide. Iodination of 2 μg of peptide was carried out as previously described, using 7·4 MBq of $^{125}$I, without carrier $^{127}$I present; the product was re-purified by use of Sep Pak reversed-phase cartridge (Millipore Waters Associates, Lane Cove, N.S.W.) (Runnegar et al. 1986). The resulting $^{125}$I-labelled toxic peptide was stored at 4°C prior to use within 7 days of preparation. Analytical separation of a portion of the $^{125}$I-labelled peptide product by the same HPLC technique described above showed two peaks of about equal radioactivity, one corresponding to iodinated methionine-containing peptide, and the other to iodinated methionine sulfoxide-containing peptide. Both these peptides are of similar toxicity and result in the same histopathological changes on poisoning (Runnegar et al. 1986).

Labelled peptide containing $4·4·6 \times 10^{6}$ c.p.m. was made up to 0·5 ml in 0·9% (w/v) NaCl for intravenous injection into the femoral vein. Prior to injection the rats were sedated with 0·1 ml Stresnil (Smith, Kline and French Laboratories, French's Forest, N.S.W.) allowed 10 min to relax, then anaesthetized with approximately 0·2 ml intraperitoneal Nembutal (Ceva Chemicals Australia Pty Ltd, Hornsby, N.S.W.) When the rats were fully unconscious the labelled peptide was administered and eight blood samples were collected at intervals from the tail. At either 30 or 120 min the rats were killed by opening the thorax while still fully anaesthetized. Tissue and blood samples were collected. In the rats killed at 120 min, duodenal and upper, small-intestinal contents were collected and divided into contents from 1–30 cm from the pyloric sphincter, and 30–60 cm. No radioactivity was detected beyond 60 cm down the intestine.

Radioactivity was measured on an automatic well-type scintillation counter (Packard Instrument Co., Illinois, U.S.A.).

![Graph](image)

Fig. 1. Disappearance curve of $^{125}$I in blood following intravenous injection of $^{125}$I-labelled toxic peptide from *M. aeruginosa*. Values given are log$_{10}$ mean radioactivity ± s.e.m. Inset graph shows the curve obtained by subtracting the slow phase disappearance curve ($t_{42} = 42$ min) from the blood radioactivity measured, to obtain the fast-phase disappearance curve ($t_{21} = 2·1$ min).
Results

Fig. 1 shows the biphasic disappearance curve of blood radioactivity, the first component having a half-life of 2.1 min and the second a half-life of 42 min, calculated from a logarithmic plot of the radioactivity with deduction of the slow phase prior to calculation of the half-life of the first phase (Janaky et al. 1982).

Table 1. Tissue concentration of $^{125}$I radioactivity in female rats at 30 or 120 min after intravenous injection of $^{125}$I-labelled toxic peptide purified from a water-bloom of *M. aeruginosa*

Results ± s.e.m. expressed as the ratio of tissue radioactivity/g + blood radioactivity/g. Five rats per group

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Ratio 30 min</th>
<th>Ratio 120 min</th>
<th>Tissue</th>
<th>Ratio 30 min</th>
<th>Ratio 120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>3.0±0.1</td>
<td>4.7±0.7*</td>
<td>Ovaries</td>
<td>0.5±0.0</td>
<td>0.6±0.01</td>
</tr>
<tr>
<td>Gut contents</td>
<td></td>
<td></td>
<td>Spleen</td>
<td>0.5±0.02</td>
<td>0.8±0.1*</td>
</tr>
<tr>
<td>0-30 cm duodenum</td>
<td>28.7±3.4</td>
<td>14.6±3.5*</td>
<td>Brain</td>
<td>0.04±0.01</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>30-60 cm duodenum</td>
<td>2.0±2.5*</td>
<td>0.5±0.4</td>
<td>Adrenals</td>
<td>0.4±0.02</td>
<td>0.5±0.03</td>
</tr>
<tr>
<td>Duodenum</td>
<td>0.6±0.4</td>
<td>1.6±0.4</td>
<td>Fat (intra-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>3.5±0.2</td>
<td>6.2±0.7*</td>
<td>abdominal</td>
<td>&lt;0.1</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>Urine (n=4)</td>
<td>9.3±4.3</td>
<td>41.4±18.2</td>
<td>Muscle (leg)</td>
<td>0.13±0.01</td>
<td>0.15±0.01</td>
</tr>
</tbody>
</table>

*Significantly different from 30 min, *P*<0.01 by *t*-test.

Organ and tissue distribution of radioactivity are shown in Table 1 as a concentration ratio compared with blood radioactivity at the time of death. It is apparent that the liver and kidneys show the highest tissue concentration of radioactivity, which increased when compared to blood between 30 and 120 min after injection. That both organs are on excretory pathways is clear from the concentration of radioactivity in gut contents and urine. The proportion of injected radioactivity in the major compartments of the body is shown in Table 2.

Table 2. Distribution of $^{125}$I radioactivity from $^{125}$I-labelled toxic *M. aeruginosa* peptide at 30 and 120 min after intravenous injection in female rats

Results ± s.e.m. expressed as percentage of dose injected, located in organ or excretion pathway. Five rats per group

<table>
<thead>
<tr>
<th>Time after injection</th>
<th>Liver</th>
<th>Gut contents (total)</th>
<th>Kidney</th>
<th>Urine (total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td>21.7±1.1</td>
<td>7.0±0.3</td>
<td>5.6±0.2</td>
<td>0.9±0.5</td>
</tr>
<tr>
<td>120 min</td>
<td>19.2±0.3</td>
<td>9.4±1.1</td>
<td>5.3±0.4</td>
<td>1.9±1.2</td>
</tr>
</tbody>
</table>

From this it can be seen that the liver and kidney content of $^{125}$I has not changed between 30 and 120 min, and therefore the ratio increase in Table 1 is due to decreased blood radioactivity (from 44.8±1.9 to 28.0±1.9 c.p.m./mg at 120 min). The increases seen in radioactivity of gut contents and urine between 30 and 120 min are not statistically significant due to the wide range of values in both compartments.

Discussion

From histopathological studies it has been made clear that the major target organ for the toxic action of *M. aeruginosa* is the liver (Falconer et al. 1981). In the study
presented here the liver is identified as accumulating about 20% of an intravenous dose of toxic peptide within 30 min. The rapidity of this accumulation reflects the large blood flow through the liver.

The half-life of the labelled toxic peptide in the blood resembles that of peptide hormones. The rapid initial rate of clearance from the blood is interpreted as the redistribution from circulation into extracellular fluid and tissue binding sites. For the peptide hormone \([\text{H}]\text{vasopressin}\) this rapid phase in the rat had a half-life of 1·74 min for intact peptide and 2·5 min for blood radioactivity, in close agreement with the value of 2·1 min found here for the toxic peptide (Janaky et al. 1982). Vasopressin is a linear peptide of molecular weight 1100, compared with the toxic cyclic peptide of molecular weight 1019. This first redistribution phase is a reversible relocation in large part, with an equilibrium between blood extracellular fluid and dissociable binding sites.

The second phase of extraction of labelled peptide from the blood is an irreversible loss as cell uptake and organ excretion occur. In these experiments the toxic peptide radioactivity had a second half-life of 42 min, compared with 28 min for vasopressin (Janaky et al. 1982), and 6·8 min for prolactin (Falconer and Vacek 1983). It is interesting to compare the role of the kidneys in the breakdown of the toxic peptide and peptide hormones. In the case of vasopressin most of the hormone is removed from circulation by the kidney, as also occurs with prolactin (Emmanouel et al. 1981). In these experiments only one-quarter of the radioactivity of liver was present in kidney, and gut contents contained nearly seven times as much radioactivity as urine after 120 min. Kidney and urine radioactivity will include any free \(^{125}\text{I}-\text{iodide}\), either contaminating the labelled peptide or generated within the tissue from hydrolysis or deiodination or both of the toxic peptide. Thus the radioactivity of kidney tissue and urine must be regarded as an overestimate of the actual \(^{125}\text{I}-\text{labelled toxic peptide}\). From this comparison it can be concluded that the liver plays the major role in uptake and excretion of the toxic peptide.

Unlike peptides such as arginine-vasopressin and prolactin, the cyclic peptide is resistant to enzymic hydrolysis (Runnegar and Falconer 1981) and has no cleavage point for tryptic hydrolysis. It will therefore be more resistant to degradation in tissues and may require to be excreted in bile in either its original form or after conjugation. The toxic peptide enters the hepatocytes via the bile-acid transporter (Runnegar et al. 1981) and presumably leaves via the bile since it rapidly appeared in the duodenum.

Whether there is any entero-hepatic circulation remains to be demonstrated. Earlier work (Runnegar et al. 1986) using mice showed an almost complete clearance from undamaged liver of radioactivity from \(^{125}\text{I}-\text{labelled toxic peptide}\) after 24 h. Only in mice showing liver damage was radioactivity retained, up to 40–60% of administered dose in mice with marked hepatocyte necrosis.

It is concluded that the liver is both the target organ of \(M.\ aquuginosa\) toxin and the major excretory route for removal of toxin from the circulation.

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References


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