Acrolein produced from polyamines as one of the uraemic toxins

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Abstract

It is well known that the addition of spermine or spermidine to culture medium containing ruminant serum inhibits cellular proliferation. This effect is caused by the products of oxidation of polyamines that are generated by serum amine oxidase. Among the products, we found that acrolein is a major toxic compound produced from spermine and spermidine by amine oxidase. We then analysed the level of polyamines (putrescine, spermidine and spermine) and amine oxidase activity in plasma of patients with chronic renal failure. It was found that the levels of putrescine and the amine oxidase activity were increased, whereas spermidine and spermine were decreased in plasma of patients with chronic renal failure. The levels of free and protein-conjugated acrolein were also increased in plasma of patients with chronic renal failure. An increase in putrescine, amine oxidase and acrolein in plasma was observed in all cases such as diabetic nephropathy, chronic glomerulonephritis and nephrosclerosis. These results suggest that acrolein is produced during the early stage of nephritis through kidney damage and also during uraemia through accumulation of polyamines in blood due to the decrease in their excretion into urine.

Introduction

It is well established that polyamines (putrescine, spermidine and spermine) are necessary for cell growth [1,2]. However, the addition of spermidine or spermine to culture medium containing ruminant serum inhibits cellular proliferation [3,4]. This effect is caused by the products of oxidation of polyamines that are generated by serum amine oxidase [5]. Ruminant serum amine oxidase catalyses the oxidative deamination of spermidine and spermine to produce, respectively, an aminoaldehyde [\(N'-(4-aminobutyl)-aminopropionaldehyde\)] or an aminodialdehyde [\(N,N'\)-bis(3-propionaldehyde)-1,4-butanediamine], with \(H_2O_2\) and ammonia [6]. Acrolein (\(CH_2=CHCHO\)) is then spontaneously formed from these two aminoaldehydes [7]. Spermine is oxidized as follows:

\[
\begin{align*}
NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2 + 2O_2 + 2H_2O & \rightarrow CHO(CH_2)_2NH(CH_2)_2NH_2 + 2H_2O_2 \\
& + 2NH_3 \\
& \rightarrow NH_2(CH_2)_2NH_2 + 2CH_2=CHCHO + 2NH_3 \\
& + 2H_2O_2
\end{align*}
\] (1)

On the other hand, polyamine oxidase produces 3-aminopropanal from spermine, which also forms acrolein spontaneously [8].

\[
\begin{align*}
NH_2(CH_2)_2NH(CH_2)_2NH(CH_2)_2NH_2 + O_2 + H_2O & \rightarrow NH_2(CH_2)_2NH(CH_2)_2NH_2 + NH_3 + H_2O_2 \\
& + NH_3 \\
& \rightarrow NH_2(CH_2)_2NH(CH_2)_2CHO + H_2O_2 \\
& \rightarrow NH_2(CH_2)_2NH(CH_2)_2CHO
\end{align*}
\] (2)

Polyamines have been suggested to be one of the uraemic toxins [9]. However, this idea has not been carefully explored. In a previous report dealing with uraemia, the total polyamine levels in serum, estimated using an antibody against polyamines, were higher in patients with advanced adult uraemia than in ambulatory uraemic children [9]. In the present study, we determined the levels of each polyamine, i.e. putrescine, spermidine and spermine, together with amine oxidase activity and the level of acrolein in plasma of patients with renal failure. We found that acrolein is a major toxic compound produced from spermine and spermidine by amine oxidase, and that acrolein is accumulated in plasma of patients with chronic renal failure. Furthermore, it was found that the main amine oxidase producing acrolein from spermine and spermidine is polyamine oxidase.

Results and discussion

Correlation between cytotoxicity and acrolein produced from spermine and 3-aminopropanal

As shown in Figure 1(A), the inhibitory effect of spermine was seen with FM3A cells exposed to spermine in the presence of fetal bovine serum (FBS). The toxicity is caused...
by factors produced from spermine by amine oxidase. The evidence for this is that aminoguanidine, an inhibitor of amine oxidase, blocked the effects of spermine (Figure 1A). Polyamines are converted to aminoaaldehydes and \( \text{H}_2\text{O}_2 \) by amine oxidase [6]. Then acrolein is spontaneously formed from the aminoaaldehydes [7]. The concentration of spermine (15–30 µM) that inhibited cell growth was slightly higher than that of acrolein (7.5–15 µM; Figure 1B), but was much lower than that of \( \text{H}_2\text{O}_2 \) (0.2–0.4 mM; results not shown). In addition, aldehyde dehydrogenase, but not catalase, could prevent the effects of spermine on cell growth (Figure 1C). These results suggest that acrolein is more strongly involved than \( \text{H}_2\text{O}_2 \) in the inhibition of cell growth by spermine.

Next we examined the effects of various aldehydes on cell growth to determine whether acrolein is a major inhibitory factor of cell growth. The concentrations of formaldehyde (HCHO), acetaldehyde (CH\(_3\)CHO) and propionaldehyde (CH\(_3\)CH\(_2\)CHO; 0.25–5 mM) required to inhibit cell growth were much higher than that of spermine. However, 3-aminopropanal inhibited cell growth at concentrations (25–50 µM) comparable with that of spermine (Figure 1D). The addition of aldehyde dehydrogenase recovered cell growth. Spermidine also inhibited cell growth at concentrations of 0.1–0.15 mM. Acrolein produced from spermine, 3-aminopropanal and spermidine in the presence of FBS was then measured by the method of Alarcon [10].
The acrolein produced was in the order spermine > 3-aminopropanal > spermidine, which paralleled the order of cytotoxicity. These results strongly suggest that acrolein, but not aminopropanal itself, is a major inhibitory factor of cell growth produced from spermine.

Increase in putrescine, amine oxidase and acrolein in plasma of patients with chronic renal failure

The polyamine content and amine oxidase activity in plasma of patients with renal failure were measured. Plasma was divided into moderate (<8 mg/dl) and severe (>8 mg/dl) classes according to the value of serum creatinine. As shown in Table 1, the level of putrescine in plasma of patients with renal failure was higher than that in normal subjects, whereas spermidine and spermine levels were lower than those in normal subjects. We next determined the activity of amine oxidase in plasma as the ability of the enzyme to degrade spermine (Table 1). Amine oxidase activity in plasma of patients with renal failure was higher than that in normal subjects. In general, the change of polyamine levels and the increase in amine oxidase were greater in patients with severe renal failure than in those with moderate failure. The results suggest that acrolein, a toxic compound, is produced from spermidine and spermine by amine oxidase in the plasma of patients with renal failure.

Free and protein-conjugated acrolein in plasma of patients with renal failure were determined by HPLC and ELISA, respectively. As shown in Table 2, both types of acrolein were increased in plasma of patients with renal failure. The concentration of acrolein that causes 50% inhibition of cell growth (IC50) is 5–10 μM in a cell-culture system [11]. Free acrolein in plasma of uraemic patients was 1–1.4 μM, whereas that in normal subjects was 0.5 μM. The acrolein found as protein conjugates in plasma of uraemic patients was equivalent to 140–170 μM, which is about 5-fold higher than in plasma of normal subjects. The results suggest that the levels of free and conjugated acrolein in plasma of patients may be sufficient to cause cell damage.

The uraemic patients had diseases with different primary aetiologies. Thus we compared polyamine levels, amine oxidase activity and acrolein levels between patients with different diseases. In all cases (diabetic nephropathy, chronic glomerulonephritis and nephrosclerosis), the changes in polyamine content, amine oxidase activity and protein-conjugated acrolein were very similar (results not shown). The results suggest that production of acrolein is a common

### Table 1 | Polyamine contents and amine oxidase activity in plasma of normal subjects and patients with chronic renal failure

<table>
<thead>
<tr>
<th>Normal subject or patient with chronic renal failure</th>
<th>Polyamine content (pmol/ml of plasma)</th>
<th>Amine oxidase (degradation of Spm, nmol/ml of plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 19)</td>
<td>Put 49.5 ± 31.2</td>
<td>30.7 ± 39.5</td>
</tr>
<tr>
<td>Moderate (n = 13)</td>
<td>Spd 72.9 ± 34.9</td>
<td>9.22 ± 7.58</td>
</tr>
<tr>
<td>Severe (n = 9)</td>
<td>Spm 30.7 ± 34.9</td>
<td>5.55 ± 6.56</td>
</tr>
</tbody>
</table>

### Table 2 | Free and protein-conjugated acrolein in plasma of normal subjects and patients with chronic renal failure

<table>
<thead>
<tr>
<th>Normal subject or patient with chronic renal failure</th>
<th>Acrolein content (nmol/ml of plasma)</th>
<th>FDP-Lys (nmol/ml of plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 19)</td>
<td>0.53 ± 0.18</td>
<td>31.2 ± 8.8</td>
</tr>
<tr>
<td>Moderate (n = 13)</td>
<td>1.02 ± 0.98</td>
<td>138 ± 51.1***</td>
</tr>
<tr>
<td>Severe (n = 19)</td>
<td>1.42 ± 0.84**</td>
<td>170 ± 85.8***</td>
</tr>
</tbody>
</table>
feature of patients with chronic renal failure, irrespective of the original disease that leads to renal failure.

We next examined the properties of amine oxidase in plasma of patients with severe renal failure. For this purpose, we studied the effects of an inhibitor of monoamine oxidase, pargyline, an inhibitor of monoamine and diamine oxidases, semicarbazide, and an inhibitor of polyamine oxidase, MDL72527. The activity of amine oxidase in plasma of all eight patients examined was inhibited by MDL72527, and activity in four patients was inhibited by semicarbazide. Pargyline only inhibited activity in plasma of one patient. Inhibition by MDL72527 was greater than that by semicarbazide in plasma of four patients, whose activities were inhibited by semicarbazide. The results indicate that acrolein is produced mainly by polyamine oxidase and partly by diamine oxidase in plasma of these patients.

There are also reports that 3-aminopropanal produced from spermine is strongly involved in cell damage during ischaemia [12,13]. T-cell proliferation is also repressed by polyamine oxidation in peripheral blood mononuclear cells [14]. In this case, it has been reported that both acrolein and H$_2$O$_2$ produced from spermine are involved in the cell damage. Acrolein has been reported to cause lipid peroxidation of erythrocytes and then haemolysis [15]. Thus acrolein may be involved in various kinds of cell damage. It should be noted that other candidates as uraemic toxins, such as urea, methylguanidine and indoxyl sulphate [16,17], did not cause significant cell damage using NIH 3T3 cells in the present study (results not shown).

Our results, taken together, suggest that acrolein is produced from spermine and spermidine during the early stage of nephritis due to kidney damage and also during uraemia through accumulation of polyamines in blood due to the decrease in their excretion into urine.

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**References**


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