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Polyamines in renal failure

Review Article

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Summary. The levels of polyamines (putrescine, spermidine and spermine) and polyamine oxidase in plasma of patients with chronic renal failure were determined. The level of putrescine was increased but the level of spermine was decreased in the plasma of these patients. The patients also had increased plasma polyamine oxidase activity leading to increased degradation of spermine. As acrolein was a major toxic compound produced from spermine by polyamine oxidase, the levels of free and protein-conjugated acrolein in plasma were also measured. Acrolein levels were enhanced in plasma of patients with chronic renal failure. The accumulated acrolein found as protein conjugates was equivalent to 170 μM, which was about 5-fold higher than in plasma of normal subjects. It was found that acrolein is mainly produced by spermine oxidase in plasma. An increase in putrescine, spermine oxidase and acrolein in plasma was observed in all cases such as diabetic nephropathy, chronic glomerulonephritis and nephrosclerosis. After patients with chronic renal failure had undergone hemodialysis, their levels of plasma polyamines, spermine oxidase and acrolein returned towards normal. It is likely that acrolein produced from spermine accumulates in the blood due to decreased excretion into urine and may function as a uremic "toxin".

Keywords: Polyamine – Spermine oxidase – Acrolein – Renal failure – Hemodialysis – Uremic toxin

Abbreviations: AcPAO, acetylpolyamine oxidase; FDP-Lys, N^{ε}-(3-formyl-3,4-dehydropiperidino)-lysine; HD, hemodialysis; MDL72527, N^1,N^4-bis(2,3-butadienyl)-1,4-butanediamine; SMO, spermine oxidase; SPM, spermine; SSAT, spermidine/spermine N^1-acetyltransferase.

Introduction

Polyamines (putrescine, spermidine and spermine) are present at millimolar concentrations in both prokaryotic and eukaryotic cells and play regulatory roles in cell growth (Cohen, 1998; Igarashi and Kashiwagi, 2000). Polyamines exist mostly as polyamine-RNA complexes (Watanabe et al., 1991) and thus affect translation at various steps (Igarashi et al., 1979a, b; Igarashi and Morris, 1984; Yoshida et al., 2004). However, the addition of spermidine or spermine to culture medium containing ruminant serum inhibits cellular proliferation (Higgins et al., 1969; Gaugas and Dewey, 1979). This effect is caused by the products of oxidation of polyamines that are generated by serum amine oxidase (Bachrach, 1970). Ruminant serum amine oxidase catalyzes 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 (Fig. 1) (Tabor et al., 1964). Acrolein (CH_2=CHCHO) is then spontaneously formed from these two aminoaldehydes (Fig. 1) (Kimes and Morris, 1971). On the other hand, spermine oxidase produces 3-aminopropanal together with H_2O_2 and ammonia from spermine, which also forms acrolein spontaneously (Fig. 1) (Morgan et al., 1986).

Polyamines have been suggested to be one of the uremic "toxins" which accelerate the progression of uremia (Cambell et al., 1978). However, this idea has not been carefully explored. In a previous report dealing with uremia, the total polyamine levels in serum, estimated with an antibody against polyamines (Cambell et al., 1978), were higher in patients with advanced adult uremia than those in ambulatory uremic children. In this review, we provide information supporting the possibility of polyamines being one of the uremic toxins. In addition, the question of which is the main toxic compound produced from polyamines will be discussed.

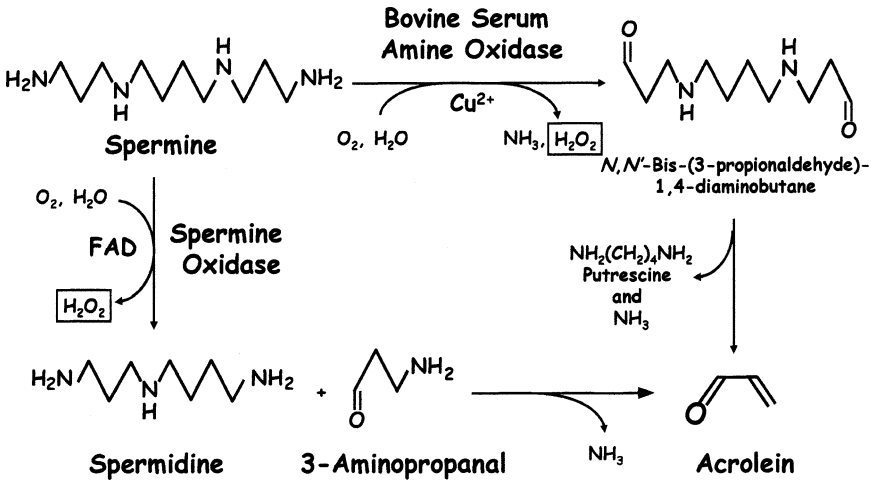

Fig. 1. Production of acrolein from spermine by spermine oxidase and bovine serum amine oxidase

Correlation between cytotoxicity and acrolein produced from spermine

As toxic compounds, acrolein and H_2O_2 are produced from spermine by amine oxidase (Fig. 1). Cell growth of mouse mammary carcinoma FM3A cells was inhibited in the presence of 30 μM spermine and 2% fetal bovine serum containing amine oxidase (Fig. 2A). Addition of aldehyde dehydrogenase, but not catalase, could prevent the inhibitory effect of spermine on cell growth (Fig. 2A). These results suggest that acrolein is more strongly involved than H_2O_2 in the inhibition of cell growth by spermine.

The inhibitory effect of acrolein and H_2O_2 on cell growth was tested. Cell growth was inhibited by 15 μM acrolein and 0.4 mM H_2O_2 to almost the same degree (Fig. 2B, C). The inhibitory effect of various aldehydes on cell growth was also tested. The concentration of HCHO and CH_3CH_2CHO required to inhibit cell growth was much higher than that of spermine (Fig. 2E, F). However, 3-aminopropanal, which spontaneously produces acrolein, inhibited cell growth at a concentration of 25 to 50 μM, comparable with that of spermine (Fig. 2D). These results suggest that acrolein produced from spermine is a major inhibitory factor of cell growth (Sharmin et al., 2001).

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

The polyamine content and polyamine 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. We subsequently determined the activity of polyamine oxidase in plasma as the ability of the enzyme to degrade spermine (Table 1). Polyamine oxidase activity in the plasma of patients with renal failure was higher than that in normal subjects. In general, the change of polyamine levels and the increase in polyamine oxidase in patients with severe renal failure were greater than those in patients with moderate failure. The results suggest that acrolein, a toxic compound, is produced from spermidine and spermine by polyamine oxidase in the plasma of patients with renal failure.

Free and protein-conjugated acrolein in plasma were determined by HPLC and ELISA, respectively. As shown in Table 1, protein-conjugated acrolein was increased in the plasma of patients with renal failure. Although the level of free acrolein was low, it also increased in the plasma of patients with renal failure. Free acrolein in plasma of uremic patients was 1–1.4 μM, whereas that in normal subjects was 0.5 μM. The acrolein found as protein conjugates in plasma of uremic patients was equivalent to 140–170 μM, which is about 5-fold higher than its concentration in the plasma of normal subjects. The results suggest that the level of acrolein in the plasma of patients may be sufficient to cause cell damage.

The uremic patients had diseases with different primary etiologies. 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, polyamine oxidase activity and protein-conjugated acrolein were very similar (results

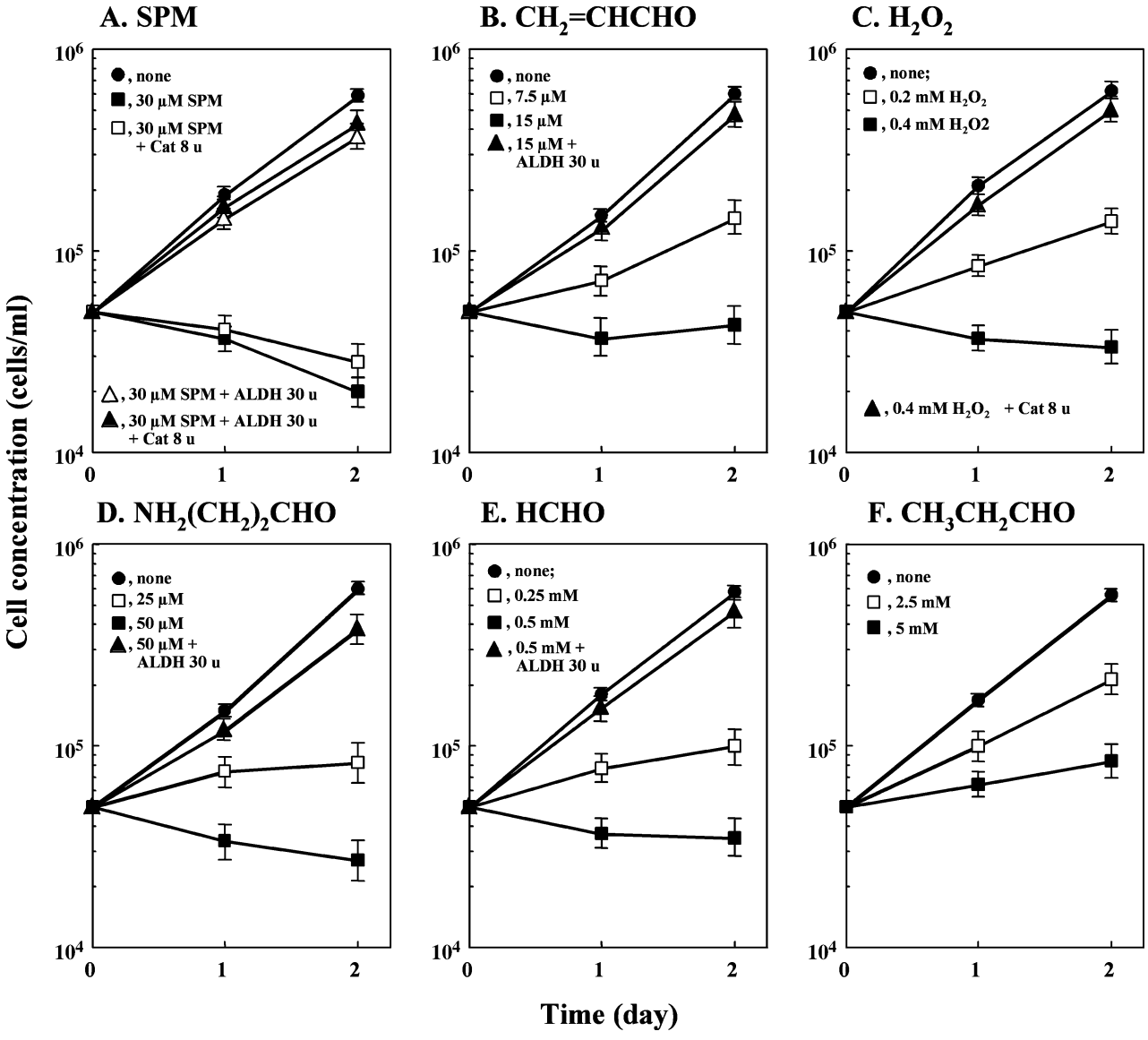

Fig. 2. Effect of spermine (**A**), acrolein (**B**), hydrogen peroxide (**C**), 3-aminopropanal (**D**), formaldehyde (**E**), and acetaldehyde (**F**) on cell growth of FM3A cells cultured in the presence of 2% fetal calf serum. Cells were cultured under standard conditions (Nishimura et al., 2005) except that the indicated chemicals and enzymes were added to the medium together with 2% fetal calf serum. Cat, catalase; ALDH, aldehyde dehydrogenase. Each point is the mean with standard deviation of triplicate determinations

not shown). The results suggest that production of acrolein is a common feature of patients with chronic renal failure, irrespective of the original disease that leads to renal failure (Sakata et al., 2003).

The severity of renal failure is judged by the level of creatinine in blood. Serum creatinine levels were correlated with the reduction in spermine and increase in polyamine oxidase and acrolein in plasma as mentioned above, suggesting that acrolein produced from spermine may function as the uremic ''toxin'' which accelerates the progression of uremia. In addition to chronic renal failure, there are also reports that 3-aminopropanal produced from spermine is strongly involved in cell damage during ischemia (Ivanova et al., 1998; 2002).

It has been reported that acrolein can be produced from membrane phospholipid (Uchida et al., 1998), although the major aldehydes produced during lipid peroxidation are 4-hydroxy-2-nonenal and malonaldehyde (Uchida, 1999). When we measured acrolein production from phospholipids, it was very low. Thus, our results suggest that acrolein is produced mainly from spermine.

Table 1. Polyamine and acrolein contents and spermine oxidase activity in plasma of normal subjects and patients with chronic renal failure[a,b]

Category of subjects[c]	Level (pmol/ml of plasma) of polyamine[d]			Spermine oxidase activity (nmol/ml of plasma)[e]	Acrolein (nmol/ml of plasma)[f]	FDP-Lys (nmol/ml of plasma)[g]
	Putrescine	Spermidine	Spermine			
Normal (n = 19)	49.5 ± 31.2	72.9 ± 34.9	30.7 ± 39.5	1.66 ± 0.97	0.53 ± 0.18	31.2 ± 8.80
Moderate (n = 13)	107 ± 86.2*	68.9 ± 53.4	9.22 ± 7.58	3.56 ± 2.10**	1.02 ± 0.98	138 ± 51.1***
Severe (n = 9)	91.0 ± 29.8***	46.1 ± 15.4**	7.55 ± 6.56*	3.96 ± 3.19**	1.42 ± 0.84**	170 ± 85.8***

[a] Amino acids in plasma were removed by cellulose phosphate column chromatography before polyamine analysis. To 1.8 ml of plasma, 0.2 ml of 50% trichloroacetic acid was added and centrifuged for 10 min at 12,000 × *g*. The supernatant thus obtained was neutralized with 6 N KOH, and applied to a cellulose phosphate column (1 ml) previously equilibrated with a buffer containing 0.1 M boric acid–Na_2CO_3 and 0.025 M NaCl (pH 8.0). Amino acids were eluted with 10 ml of the same buffer, and polyamines were then eluted with 3 ml of a buffer containing 0.2 M boric acid–Na_2CO_3 and 0.8 M NaCl (pH 8.0)

[b] Results are means with standard deviations; * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ versus normal subjects

[c] Moderate, <8 mg of serum creatinine per dl; severe, >8 mg of serum creatinine per dl. Creatinine in plasma was determined by a standard method for blood chemistry, the creatinase-peroxidase (CRTNas-POD) method

[d] Polyamine contents were measured by HPLC as described previously (Igarashi et al., 1986)

[e] Degradation of spermine. The reaction mixture for spermine oxidase activity (0.075 ml) contained 10 mM Tris-HCl (pH 7.5), 0.2 mM spermine and 0.065 ml of plasma. After incubation at 37 °C for 48 h, 0.55 ml of 5% trichloroacetic acid was added to 0.02 ml of the reaction mixture and the mixture was centrifuged for 10 min at 12,000 × *g*. A 10 µl aliquot of the supernatant was used for the polyamine measurement by HPLC

[f] Acrolein was determined by HPLC (Bohnenstengel et al., 1997) as described previously (Sakata et al., 2003)

[g] Protein-conjugated acrolein (FDP-Lys; N^{ε}-(3-formyl-3,4-dehydropiperidino)-lysine) was determined by the method of Uchida et al. (1998) using ACR-Lysine adduct ELISA system (NOF Corporation, Tokyo) and 0.05 ml of plasma. After the reaction was terminated, absorbance at 450 nm was measured by a microplate reader (Bio-Rad Model 550)

Properties of polyamine oxidase in plasma of patients with chronic renal failure

To confirm that acrolein is produced by polyamine oxidase, the effects of an inhibitor of monoamine oxidase, pargyline, an inhibitor of monoamine and diamine oxidases, semicarbazide, and an inhibitor of polyamine oxidase, MDL72527 [N^1,N^4-bis(2,3-butadienyl)-1,4-butanediamine] (Wang et al., 2001), were examined. The activity of polyamine oxidase in the plasma of all eight patients was inhibited by MDL72527, and that in four patients was inhibited by semicarbazide. Pargyline inhibited the activity in the plasma of only one patient. Inhibition by MDL72527 was greater than that by semicarbazide in the plasma of the four patients in which the activity was inhibited by semicarbazide. The results confirmed that acrolein is produced mainly by polyamine oxidase. There are two kinds of polyamine oxidase: one is spermine oxidase (SMO) and the other is acetylpolyamine oxidase (AcPAO). Table 2 summarizes the K_m values of various substrates for SMO and AcPAO together with spermidine/spermine N^1-acetyltransferase (SSAT) (Libby et al., 1991; Wang et al., 2003; Wu et al., 2003). These data indicate that SMO is involved mainly in the production of acrolein. Indeed, more acrolein was produced from spermine than from acetylspermine. There are reports that SMO and SSAT are induced during kidney ischemia–reperfusion injury in rats (Zahedi et al., 2003), and spermine and spermidine decreased following transient focal cerebral ischemia in spontaneously hypertensive rats (Adibhatla et al., 2002). Thus, production of acrolein from spermine is proposed as shown in Fig. 1.

Table 2. K_m of polyamine-metabolizing enzymes for various substrates

Enzyme	K_m (µM)			
	Spermine	Spermidine	Acetylspermine	Acetylspermidine
Human SSAT	5.0	55	36	–[a]
Human SMO	1.63	–	51	–
Mouse AcPAO	716	–	1.78	36.8

[a] No significant activity

Decrease in polyamines from plasma in patients with chronic renal failure by hemodialysis

Patients with severe renal failure undergo hemodialysis (HD). Thus, we tested with bovine blood whether spermine is removed by HD. We used three kinds of tubes which are used for HD and exactly the same apparatus as that used for the treatment of patients with chronic renal failure. Both spermine and spermidine were effectively removed from blood within 1 h by HD (Fig. 3). Next, the levels of polyamines, SMO and acrolein in plasma of patients undergoing HD (HD patients) were compared with those of patients without HD. The levels of spermine and spermidine were slightly higher for HD patients. However, there were no statistically significant differences. The levels of SMO, free acrolein and protein-conjugated acrolein decreased greatly in HD patients (Fig. 4). The levels of SMO and free acrolein in HD patients were similar to that in normal subjects, but the level of protein-conjugated acrolein was slightly but significantly higher than that in normal subjects. Once acrolein reacted with protein,

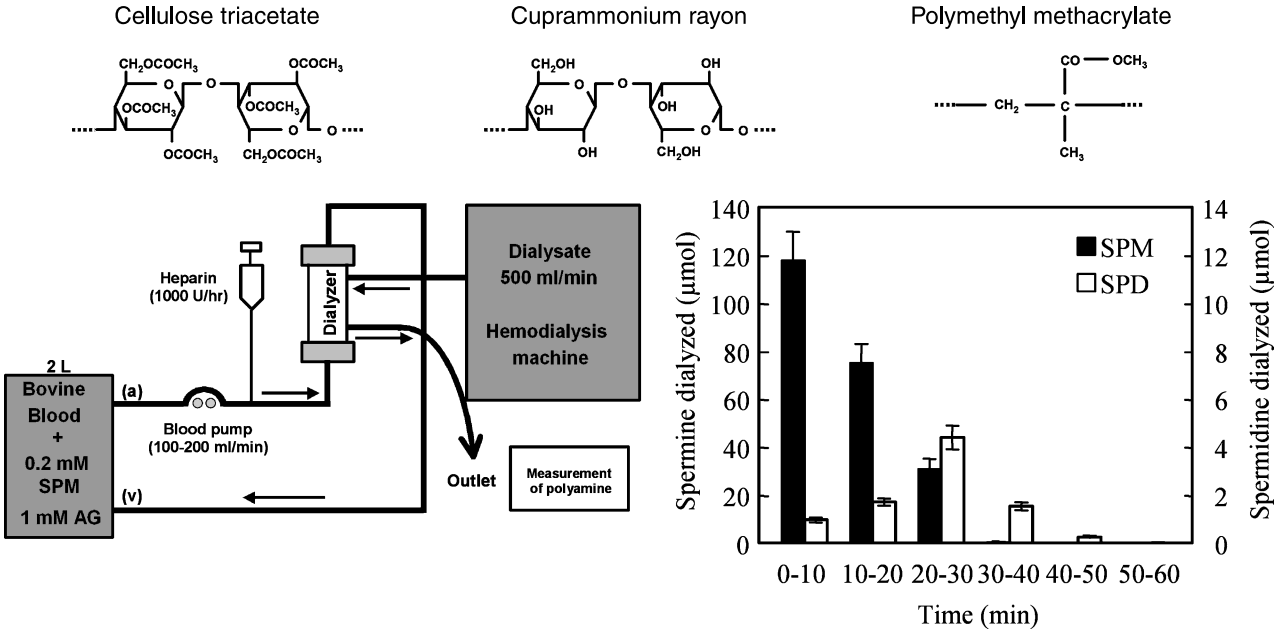

Fig. 3. Removal of spermine from blood by HD with three different kinds of dialysis membranes. Structure of HD tubes used was shown. Essentially the same results were obtained with these three different dialysis membranes. Diagrams of the method used for HD and time course of the level of spermine dialyzed from blood are shown

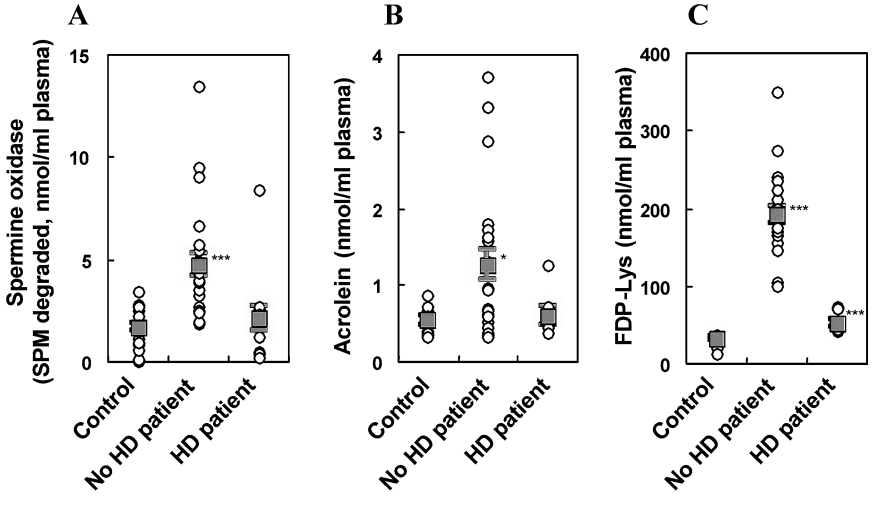

Fig. 4. Comparison of spermine oxidase (**A**), free acrolein (**B**) and protein-conjugated acrolein (**C**) in plasma of control subjects and patients with chronic renal failure. SMO and free and protein-conjugated acrolein were measured as described in footnotes e–g of Table 1. Numbers of samples: control, n = 9; no HD patients, n = 23; HD patients, n = 7. $^{*}P < 0.05$, $^{***}P < 0.001$ versus control subjects

protein-conjugated acrolein was not removed by HD. Taken together, these results indicate that HD is effective in reducing the levels of polyamines, SMO and protein-conjugated acrolein through removal of free acrolein.

Concluding remarks and future perspectives

We have found a decrease in spermine and an increase in putrescine, polyamine oxidase and acrolein in the plasma of patients with chronic renal failure. In these patients, acrolein was mainly produced from spermine by SMO. We have also found that such changes were small in plasma from patients with renal failure undergoing routine HD. Our results suggest that acrolein may function as a uremic ''toxin'' which accelerates the progression of uremia. It should be noted that other candidate of the uremic ''toxins'' including urea, methylguanidine, and indoxyl sulfate (Muting, 1965; Niwa et al., 1998) did not cause significant cell damage in NIH3T3 cells.

Our results suggest that an inhibitor of polyamine oxidase may be helpful for improving the symptoms of chronic renal failure. The inhibitor may delay the commencement of HD and also decrease the frequency of required HD. Since MDL72527 is a potent inhibitor of polyamine oxidase, it may be possible to develop a therapeutically useful drug with MDL72527 as a lead compound.

Creatinine in blood is used as a marker of chronic renal failure, but it is not toxic. Since acrolein is toxic and reflects cell damage, it is probably useful as a marker of chronic renal failure together with creatinine.

Note added in proof

We have recently found that increased levels of polyamide oxidase and acrolein are also good markers of stroke. (Tomitori H, Usui T, Saeki N, Ueda S, Kase H, Nishimura K, Kashiwagi K, Igarashi K (2005) Polyamine oxidase and acrolein as novel biochemical markers for diagnosis of cerebral stroke. Stroke 36: 2609–2613)

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