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Polyamine Cytotoxicity in the Presence of Bovine Serum Amine Oxidase

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The toxicity of extracellular spermine, determined in the presence of fetal calf serum, was studied using three cell lines: FM3A, L1210, and NIH3T3 cells. Amine oxidase in fetal calf serum produces aminodialdehyde generating acrolein spontaneously, H_2O_2, and ammonia from spermine. Spermine toxicity was prevented by aldehyde dehydrogenase, but not by catalase. Similar concentrations of spermine and acrolein were needed to produce toxicity. Other aldehydes (formaldehyde, acetaldehyde, and propionaldehyde) and hydrogen peroxide were less toxic than acrolein. Spermidine and 3-aminopropanal, which produces acrolein, also exhibited severe cytotoxicity. The degree of cytotoxicity of spermine, spermidine, and 3-aminopropanal was nearly parallel with the amount of acrolein produced from each compound. Thus, it was deduced that acrolein is a major toxic compound produced from polyamines (spermine and spermidine) by amine oxidase.

Key Words: **cytotoxicity; spermine; spermidine; acrolein; amine oxidase.**

It is well established that polyamines are necessary for normal cell growth (1, 2). However, the addition of polyamines (spermidine and spermine) to culture medium containing ruminant serum is known to inhibit cellular proliferation (3, 4). This effect is caused by the products of oxidation of polyamines that are generated by serum amine oxidase (5). 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 (6). There is another report that spermine is cleaved into spermidine and 3-aminopropanal [$NH_2(CH_2)_2CHO$] by serum amine oxidase (7), although this was denied recently (8). It has been also reported that acrolein [$CH_2 = CHCHO$] is spontaneously formed from the above aminoaldehydes (9) and 3-aminopropanal (7). Accordingly, the principal candidates for mediating cytotoxicity include acrolein (10), aminoaldehydes (11, 12), 3-aminopropanal (7, 13), and hydrogen peroxide (14, 15). There is also a report that both acrolein and hydrogen peroxide are involved in cytotoxicity by spermine since a combination of aldehyde dehydrogenase and catalase completely prevented the cytotoxicity (16).

In *Escherichia coli*, the toxicity of aminoaldehydes is much lower than that of acrolein (17). However, in animal cells, there is no report on a direct comparison of the cytotoxicity of polyamines (spermidine and spermine), acrolein, aminoaldehydes, 3-aminopropanal, and H_2O_2 in the presence of serum. In this study, we tried to identify the degradation product of polyamines, produced by amine oxidase, that is the main inhibitory factor for cell growth. We have found that the major inhibitory factor produced from polyamines by serum amine oxidase is acrolein.

MATERIALS AND METHODS

Cell culture. Mouse mammary carcinoma FM3A cells (5×10^4 cells/ml) were cultured in ES medium (Nissui Pharmaceutical Co.) supplemented with 50 U/ml streptomycin, 100 U/ml penicillin G, and 2% heat-inactivated fetal calf serum (FCS) at 37°C in an atmosphere of 5% CO_2, according to the method of Ayusawa *et al.* (18). Where indicated, 1 mM aminoguanidine (AG) was added to inhibit amine oxidase in serum. Mouse L1210 leukemia cells (5×10^4 cells/ml) were cultured in RPMI1640 medium supplemented with 50 U/ml streptomycin, 100 U/ml penicillin G, and 10% heat-inactivated FCS. NIH3T3 cells (5×10^4 cells/ml) were cultured in DMEM, supplemented with 2 mM glutamine, 50 μg/ml gentamicin, and 10% FCS. The viable cell number was counted in the presence of 0.25% trypan blue.

Measurement of polyamines. Cells (6×10^6) were homogenized with 0.3 ml of 0.2 N $HClO_4$ and centrifuged for 10 min at 12,000*g*. Polyamine contents were measured as described previously (19) using the supernatant. The retention times for N^1- (or N^8-)acetylspermidine, putrescine, spermidine, and spermine were 6, 9, 15, and 25 min, respectively. Protein was determined by the method of

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Lowry *et al.* (20) using the precipitate. The amount of polyamines was expressed as nmol/mg protein.

Assay for amine oxidase. The reaction mixture (0.15 ml) containing 10 mM Tris–HCl, pH 7.5, 0.2 mM substrate (spermine, spermidine, putrescine, N^1-acetylspermidine, and N^8-acetylspermidine) and 0.03 ml FCS was incubated at 37°C for 1, 2, or 4 h. To 0.02 ml of the reaction mixture, 0.55 ml of 5% trichloroacetic acid (TCA) was added and centrifuged as described above. A 10-μl aliquot of the supernatant was used for the polyamine analysis. The substrate degraded was linear during the incubation time.

Measurement of acrolein. Acrolein was measured according to the method of Alarcon (21). The reaction mixture (0.4 ml) containing 30 mM potassium phosphate buffer, pH 7.0, 0.2 mM substrate (spermine, spermidine, putrescine, N^1-acetylspermidine, N^8-acetylspermidine, and 3-aminopropanal) and 0.05 ml FCS was incubated at 37°C for 2, 4, or 6 h. The reaction was terminated by the addition of 2.6 ml mixed reagent containing 18 mM *m*-aminophenol, 33 mM hydroxylamine hydrochloride, and 1.15 N HCl. After boiling for 10 min, the precipitate was removed by centrifugation. Fluorescence was measured at an excitation wavelength of 350 nm and an emission wavelength of 510 nm.

Assay for DNA synthesis. FM3A cells were cultured for 24 h as described above and harvested by centrifugation at 300*g* for 5 min. DNA synthesis was measured by incorporation of [^{3}H]thymidine into DNA by incubation of cells (5×10^5/ml in 2 ml ES medium) with 185 kBq [^{3}H]thymidine (248 GBq/mmol) for 2 h. Radioactivity was measured by the method of Seyfried and Morris (22).

Materials. Catalase from bovine liver (17,300 units/mg protein), aldehyde dehydrogenase from bakers yeast (4.3 units/mg protein), N^1-acetylspermidine and N^8-acetylspermidine were obtained from Sigma. Formaldehyde, acetaldehyde, propionaldehyde, and hydrogen peroxide were purchased from Nacalai Tesque Inc. Acrolein and 3-aminopropanal diethylacetal were purchased from Tokyo Kasei Kogyo Co. 3-Aminopropanal was prepared from 3-aminopropanal diethylacetal by the method of Ivanova *et al.* (13), and it was immediately used after neutralization without purification by ion exchange column chromatography. Other reagents used were of analytical grade.

RESULTS

Comparison of Cytotoxicity of Various Polyamines, H_2O_2 and Various Aldehydes in the Presence of FCS

It is known that polyamines (spermine and spermidine) inhibit cell growth in the presence of FCS. In the present study, this inhibitory effect of spermine was seen with FM3A cells exposed to spermine in the presence of FCS (Fig. 1A). The toxicity is caused by factors produced from polyamines by amine oxidase. The evidence for this is that aminoguanidine (AG), an inhibitor of amine oxidase, blocked the effects of spermine (Fig. 1A). Polyamines are converted to aminoaldehydes and H_2O_2 by amine oxidase (6). Then, acrolein is spontaneously formed from the aminoaldehydes (9). Both H_2O_2 and acrolein inhibited cell growth. The addition of catalase prevented the effects of H_2O_2, and the addition of aldehyde dehydrogenase prevented the effects of acrolein (Figs. 1B and 1C). The concentration of spermine (15 to 30 μM) that inhibited cell growth was slightly higher than that of acrolein (7.5 to 15 μM), but was much lower than that of H_2O_2 (0.2 to 0.4 mM). In addition, aldehyde dehydrogenase, but not catalase, could prevent the effects of spermine on cell growth (Fig. 1D). These results suggest that acrolein is more strongly involved than H_2O_2 in the inhibition of cell growth by spermine.

Next, we examined the effect of various aldehydes on cell growth to determine whether acrolein is a major inhibitory factor of cell growth. As shown in Fig. 2, the concentrations of formaldehyde (HCHO), acetaldehyde (CH_3CHO), and propionaldehyde (CH_3CH_2CHO) (0.25 to 5 mM) required to inhibit cell growth were much higher than that of spermine. However, 3-aminopropanal inhibited cell growth at concentrations (25 to 50 μM) comparable to that of spermine. The addition of aldehyde dehydrogenase with these aldehydes recovered cell growth. Among these aldehydes, only 3-aminopropanal spontaneously forms acrolein (7). These results strongly suggest that acrolein, but not aminoaldehyde itself, is a major inhibitory factor of cell growth produced from spermine.

The inhibitory effect of the other major polyamine, spermidine, was then examined. As shown in Fig. 3A, the concentration of spermidine necessary to inhibit cell growth was 0.1 to 0.15 mM, and the effect of spermidine was prevented by AG. Aldehyde dehydrogenase was also able to prevent the toxic effects of spermidine (Fig. 3B). However, catalase provided only modest protection against spermidine toxicity. It has been reported that an aminoaldehyde is formed from N^8-acetylspermidine by serum amine oxidase in small amounts, but not from N^1-acetylspermidine (23). This was confirmed by our assay method measuring the decrease in substrate by amine oxidase (data not shown). Cell growth was slightly inhibited by 0.3 mM N^8-acetylspermidine, but not by 0.3 mM N^1-acetylspermidine (Fig. 3C). These results support the idea that the cytotoxic effect of spermidine, like that of spermine, is mainly due to acrolein produced from aminoaldehyde by amine oxidase. Putrescine (1 to 3 mM) did not exhibit cytotoxicity and was not degraded by serum amine oxidase (data not shown).

The polyamine contents of cells were determined (Table 1). When AG was added to the medium together with spermine, spermine accumulated in the cells. However, when spermine was added to the medium without AG, accumulation of spermine was not observed. Addition of AG alone did not alter cellular polyamine content. The results suggest that most of the exogenously added spermine (30 μM) was degraded by amine oxidase. On the other hand, spermidine accumulated in the cells cultured with or without AG, suggesting that only a small portion of the exogenously added spermidine (0.15 mM) was degraded by amine oxidase. The results also suggest that accumulation of spermine or spermidine in cells does not directly inhibit cell growth, since inhibition of cell growth by spermine or spermidine was not observed in the presence of AG (Figs. 1A and 3A). DNA synthesis, another indicator of cell growth, was also measured in treated

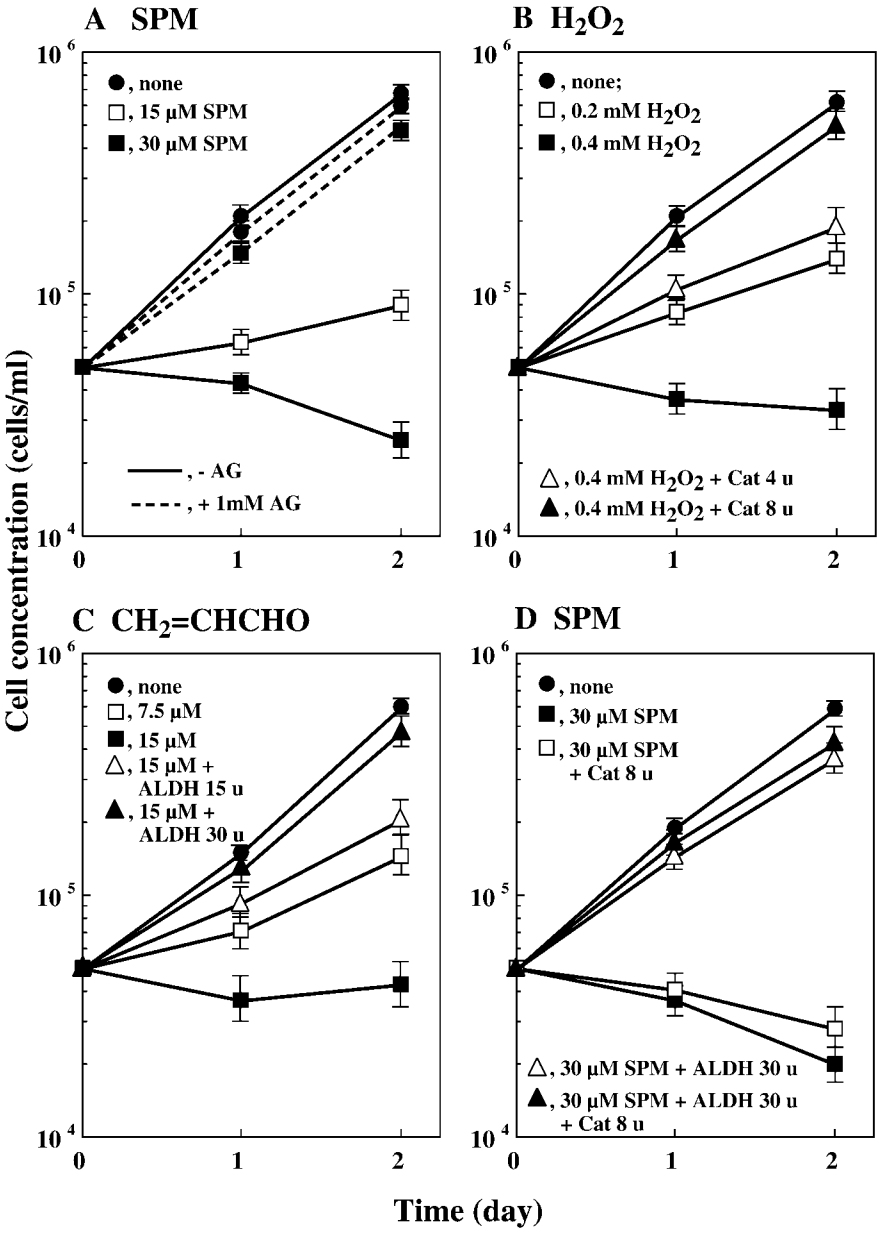

FIG. 1. Effect of spermine, H_2O_2 and acrolein on cell growth of FM3A cells cultured in the presence of 2% FCS. Cells were cultured under standard conditions except that chemicals and enzymes shown in the figure were added to the medium together with 2% FCS. Cat, catalase; ALDH, aldehyde dehydrogenase. Each point is the mean ± S. D. of triplicate determinations.

and untreated cells. As shown in Table 1, inhibition of DNA synthesis was parallel with inhibition of cell growth: The addition of 30 μM spermine or 0.15 mM spermidine to the medium without AG greatly inhibited DNA synthesis.

Spermine toxicity in the presence of FCS was examined by using two other cell lines. As shown in Fig. 4, growth of L1210 and NIH3T3 cells was inhibited by spermine (7.5 to 15 μM). These effects were reversed by the addition of aldehyde dehydrogenase, but not catalase. In these experiments, lower concentrations of spermine were required to inhibit growth of L1210 and NIH3T3 cells than to inhibit growth of FM3A cells. This may reflect the use of 10% FCS during culture of L1210 and NIH3T3 cells rather than 2% FCS used for culture of FM3A cells. The results suggest that the mechanism of toxicity of spermine (involving acrolein production) is similar in most cells.

Correlation between Cytotoxicity and Acrolein Produced from Polyamines and 3-Aminopropanal

To clarify that acrolein is the major toxic compound produced from polyamines by serum amine oxidase,

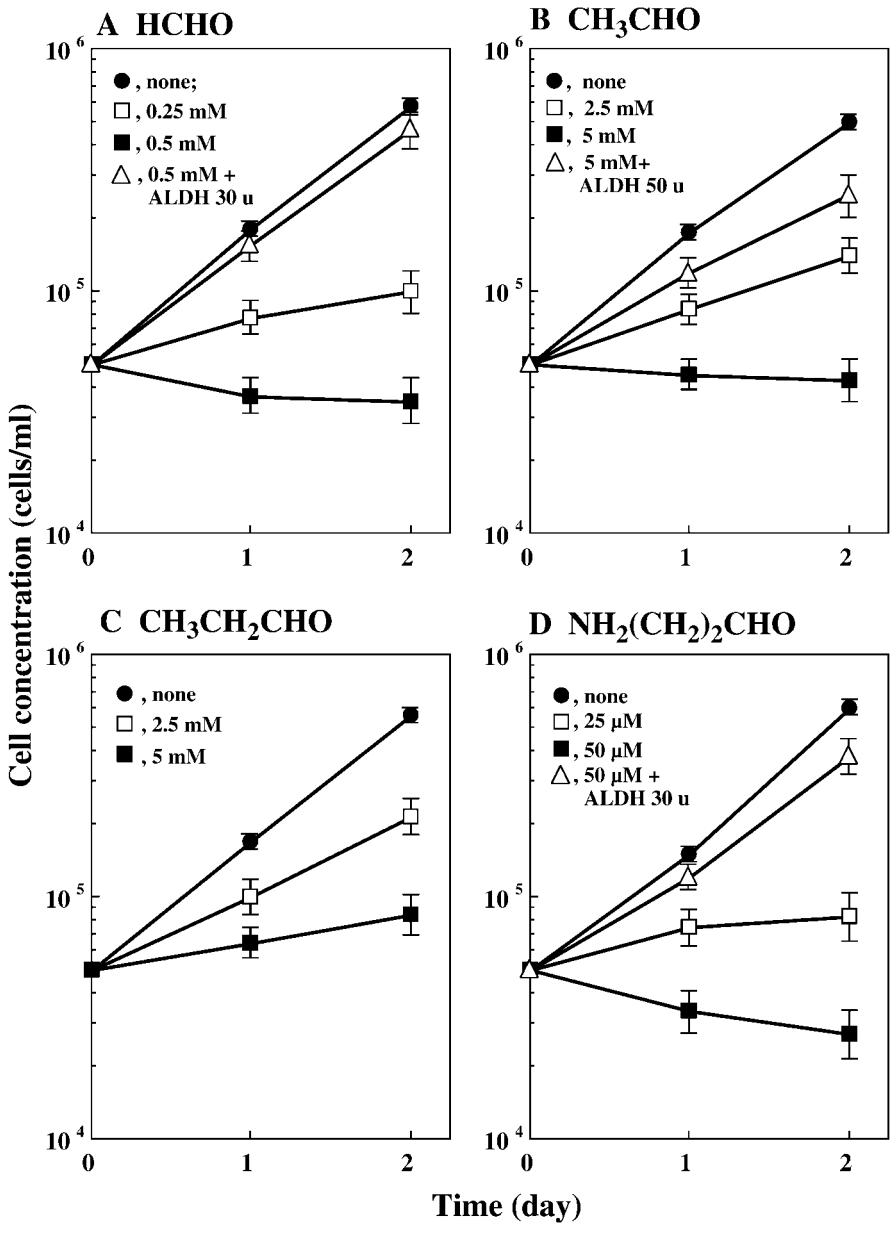

FIG. 2. Effect of various aldehydes on cell growth of FM3A cells. Cells were cultured under standard conditions except that chemicals and aldehyde dehydrogenase (ALDH) shown in the figure were added to the medium together with 2% FCS. Each point is the mean ± S. D. of triplicate determinations.

acrolein produced from polyamine analogues and 3-aminopropanal was measured in the presence of FCS. As shown in Fig. 5A, acrolein was generated from spermine, spermidine and N^8-acetylspermidine, but not from N^1-acetylspermidine and putrescine, after the short lag. During the lag period, aminodialdehyde or aminoaldehyde may be produced by amine oxidase. Acrolein was produced from 3-aminopropanal without the lag regardless of the presence and absence of FCS, confirming that acrolein was spontaneously generated from 3-aminopropanal. These results indicate that the degree of cytotoxicity of spermine, spermidine, and N^8-acetylspermidine was parallel with the amount of acrolein formed from the compound. 3-Aminopropanal may damage cells in a relatively short time because acrolein is produced without the lag.

It has been reported that the toxicity of aminoaldehyde is much lower than that of acrolein in *Escherichia coli* (17). In animal cells, it was also expected that the concentration of spermine necessary to inhibit cell growth in the presence of amine oxidase would be much higher than that of acrolein because acrolein was gradually generated with time from spermine or 3-aminopropanal (Fig. 5A). However, acrolein concen-

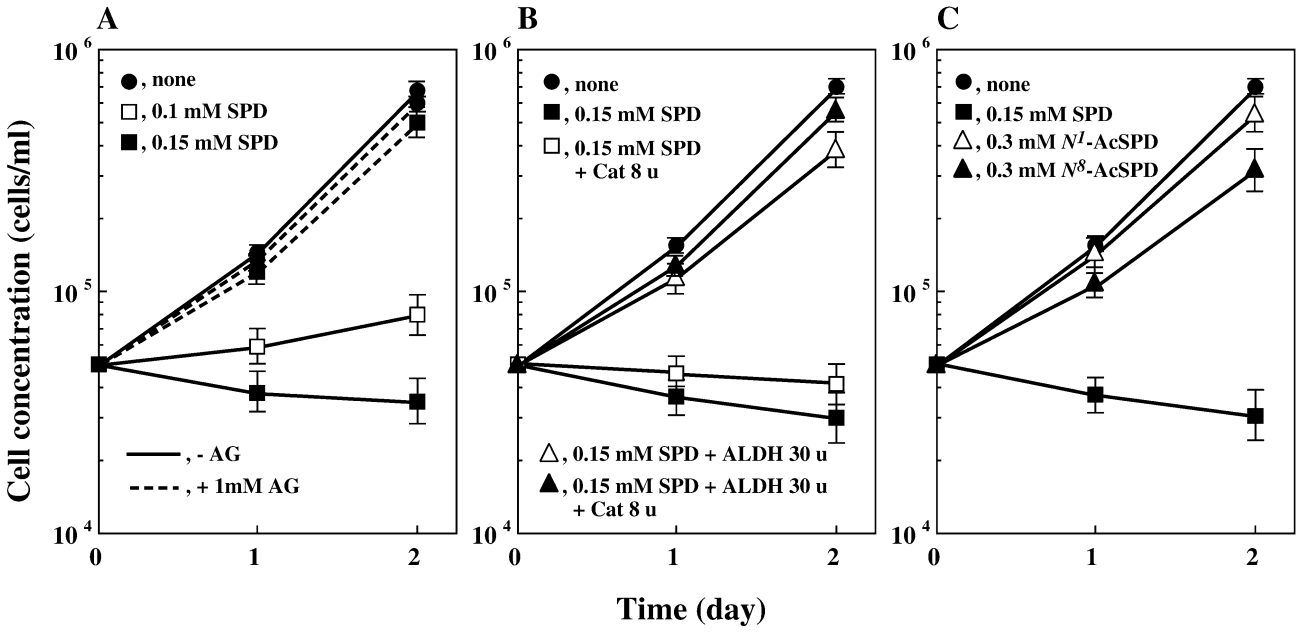

FIG. 3. Effect of spermidine and acetylspermidine on cell growth of FM3A cells. Cells were cultured under standard conditions except that chemicals and enzymes shown in the figure were added to the medium together with 2% FCS. Cat, catalase; ALDH, aldehyde dehydrogenase. Each point is the mean ± S. D. of triplicate determinations.

tration (7.5 to 15 μM) necessary to inhibit cell growth was close to that of spermine (15 to 30 μM). Therefore, the stability of acrolein in the presence of FCS was examined. As shown in Fig. 5B, the amount of acrolein was decreased with time as well as the increase in FCS in the medium. When cells were cultured in the medium preincubated with 15 μM acrolein for 2 h at 37°C, acrolein cytotoxicity decreased greatly due to the degradation of acrolein (data not shown). These results suggest that the degree of the inhibition of cell growth depends on the actual amount of acrolein in the medium.

DISCUSSION

Since many factors generated from polyamines (spermine and spermidine) by amine oxidase have been reported as candidates to inhibit cell growth, we tried to clarify which is the major inhibitory factor. Our results indicate that acrolein is the most potent inhibitor. Other aldehydes, which do not produce acrolein, and H_2O_2 were weak inhibitors compared to acrolein.

We confirmed previous results showing that serum amine oxidase degrades spermine and spermidine with

TABLE 1

Polyamine Contents and DNA Synthesis in FM3A Cells Cultured with Spermine and Spermidine in the Presence and Absence of Aminoguanidine

Culture	Time (day)	Polyamine content spermidine (nmol/mg protein) Putrescine		Spermine	[^{3}H]Thymidine incorporated (nmol/μg DNA)
Control	1	3.91 ± 0.70	12.8 ± 2.57	5.95 ± 0.66	2460 ± 159
	2	3.95 ± 0.48	11.4 ± 1.64	6.77 ± 0.51	n.d.[a]
AG 1 mM	1	2.15 ± 0.37	12.9 ± 1.21	7.51 ± 0.83	2386 ± 173
	2	1.71 ± 0.28	10.8 ± 1.19	6.46 ± 0.72	n.d.
Spermine 30 μM	1	3.95 ± 0.33	10.6 ± 1.41	5.92 ± 0.77	212 ± 58
	2	5.16 ± 0.45	8.81 ± 0.89	5.61 ± 0.65	n.d.
Spermine 30 μM + AG 1 mM	1	0.31 ± 0.12	3.59 ± 0.43	19.6 ± 1.06	2148 ± 192
	2	0.23 ± 0.11	1.69 ± 0.29	14.5 ± 1.26	n.d.
Spermidine 0.15 mM	1	2.90 ± 0.45	20.1 ± 2.78	6.84 ± 1.49	185 ± 54
	2	3.15 ± 0.43	21.0 ± 2.24	7.88 ± 1.12	n.d.
Spermidine 0.15 mM + AG 1 mM	1	1.37 ± 0.32	22.8 ± 3.30	7.54 ± 1.48	2158 ± 185
	2	1.18 ± 0.28	23.7 ± 3.12	8.42 ± 1.75	n.d.

Note. Cells were cultured in the presence and absence of polyamines with and without aminoguanidine (AG). The values are expressed as the mean ± S.D. for three determinations. Control culture was performed in the absence of polyamines and AG.

[a] Not determined.

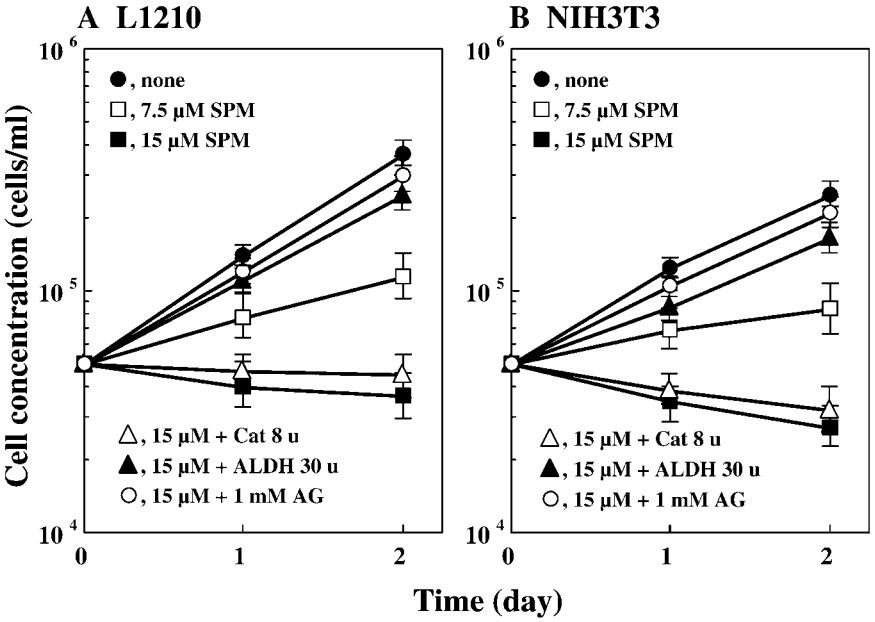

FIG. 4. Effect of spermine on cell growth of L1210 and NIH3T3 cells. Cells were cultured under standard conditions except that chemicals and enzymes were added to the medium together with 10% FCS. Cat, catalase; ALDH, aldehyde dehydrogenase. Each point is the mean ± S. D. of triplicate determinations.

similar efficiency (21). In light of this, it was difficult to explain why the concentration of spermine necessary to inhibit cell growth (15 to 30 μM) was much lower than that of spermidine (0.1 to 0.15 mM). A concentration of spermidine twice that of spermine should exhibit the same inhibitory effect, because two molecules of acrolein can be formed from spermine and one from spermidine. It is now believed that oxidation of spermine and spermidine by amine oxidase occurs at the terminal primary amines rather than at the internal secondary amines (6, 8). Accordingly, dioxidized spermine (aminodialdehyde) is produced from spermine. Since it has been reported that acrolein is more easily generated from aminodialdehyde than aminoaldehyde (9), acrolein may be more efficiently produced from spermine than from spermidine (Fig. 5A). Our

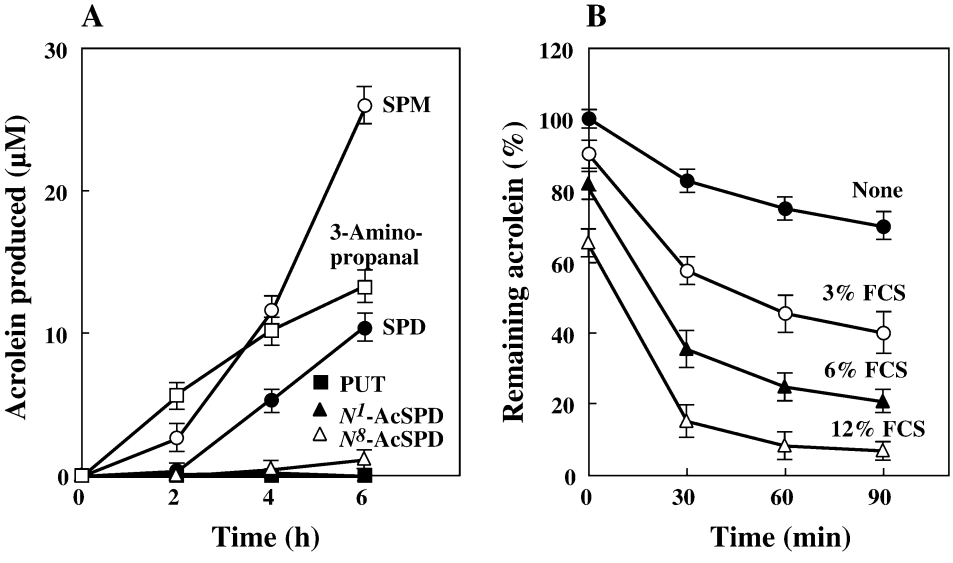

FIG. 5. Acrolein produced from various polyamine analogues and 3-aminopropanal (A) and effect of FCS on the stability of acrolein (B). Acrolein was measured as described under Materials and Methods. 1 mM *m*-aminophenol was added to the reaction mixture of A, but not B. Measurable acrolein increased greatly in the presence of 1 mM *m*-aminophenol in the reaction mixture. In A, the substrate concentration was 0.2 mM. In B, acrolein concentration in the reaction mixture was 15 μM.

results clearly show that the degree of cytotoxicity of polyamines was parallel with the amount of acrolein formed from each polyamine.

As for the mechanism of acrolein cytotoxicity, two modes of actions are postulated: (i) cytotoxicity is caused by acrolein effecting changes in the expression of one or more growth- or stress-related genes or transcription factors secondary to a reduction of glutathione levels; and (ii) activation of the transcription factors NF-κB and AP1 can be inhibited by acrolein (24).

When cytotoxicity was induced by 0.15 mM spermidine (Fig. 3B), H_2O_2 was partially involved in cell toxicity because the addition of catalase slightly recovered cell growth. This is reasonable because one molecule of H_2O_2 is formed during the degradation of spermidine by amine oxidase and 0.2 mM H_2O_2 significantly inhibited cell growth (Fig. 1B). However, our results could not explain the finding by Averill-Bates *et al.* (15) that cell toxicity caused by 3.8 μM spermine was recovered by catalase. In the current study, concentrations of H_2O_2 less than 10 μM had no effect on cell growth.

Addition of high concentrations of polyamine (more than 1 mM spermine or 5 mM spermidine) together with FCS and AG to medium also inhibited cell growth (25, 26). Under these conditions, high concentrations of polyamines accumulated in these cells (25, 26). During the conversion of spermine to spermidine, or spermidine to putrescine in cells, 3-acetamidopropanal is formed by polyamine oxidase (27). Since acrolein is hardly formed from 3-acetamidopropanal, cells are not damaged during polyamine metabolism. The inhibition of cell growth by overaccumulation of polyamines was mainly due to the inactivation of ribosomes through replacement of Mg^{2+} on magnesium-binding sites by polyamines (26, 28).

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REFERENCES

1. Tabor, C. W., and Tabor, H. (1984) Polyamines. *Annu. Rev. Biochem.* **53,** 749–790.
2. Cohen, S. S. (1998) A Guide to the Polyamines. Oxford University Press, Oxford.
3. Higgins, M. L., Tillman, M. C., Rupp, J. P., and Leach, F. R. (1969) The effect of polyamines on cell culture cells. *J. Cell. Physiol.* **74,** 149–154.
4. Gaugas, J. M., and Dewey, D. L. (1978) Evidence for serum binding of oxidized spermine and its potent G_1-phase inhibition of cell proliferation. *Br. J. Cancer* **39,** 548–557.
5. Bachrach, U. (1970) Oxidized polyamines. *Annu. N. Y. Acad. Sci.* **171,** 939–956.
6. Tabor, C. W., Tabor, H., and Bachrach, U. (1964) Identification of the aminoaldehyde produced by the oxidation of spermine with purified plasma amine oxidase. *J. Biol. Chem.* **239,** 2194–2203.
7. Houen, G., Bock, K., and Jensen, A. L. (1994) HPLC and NMR investigation of the serum amine oxidase catalyzed oxidation of polyamines. *Acta Chem. Scand.* **48,** 52–60.
8. Lee, Y., and Sayre, L. M. (1998) Reaffirmation that metabolism of polyamines by bovine plasma amine oxidase occurs strictly at the primary amino termini. *J. Biol. Chem.* **273,** 19490–19494.
9. Kimes, B. W., and Morris, D. R. (1971) Preparation and stability of oxidized polyamines. *Biochim. Biophys. Acta* **228,** 223–234.
10. Alarcon, R. A. (1970) Acrolein. IV. Evidence for the formation of the cytotoxic aldehyde acrolein from enzymatically oxidized spermine or spermidine. *Arch. Biochem. Biophys.* **137,** 365–372.
11. Kazmi, S. M. I., Li, D., Koop, K., Conant, J., and Lau, C. Y. (1992) Role of aldehyde dehydrogenase in the biological activity of spermine dialdehyde, a novel immunosuppressive/purging agent. *Pharmacol. Res.* **25,** 383–392.
12. Bonneau, M.-J., and Poulin, R. (2000) Spermine oxidation leads to necrosis with plasma membrane phosphatidylserine redistribution in mouse leukemia cells. *Exp. Cell Res.* **259,** 23–34.
13. Ivanova, S., Botchkina, G. I., Al-Abed, Y., Meistrell, M., III, Batliwalla, F., Dubinsky, J. M., Iadecola, C., Wang, H., Gregersen, P. K., Eaton, J. W., and Tracey, K. J. (1998) Cerebral ischemia enhances polyamine oxidation: identification of enzymatically formed 3-aminopropanal as an endogenous mediator of neuronal and glial cell death. *J. Exp. Med.* **188,** 327–340.
14. Henle, K. J., Moss, A. J., and Nagle, W. A. (1986) Mechanism of spermidine cytotoxicity at 37°C and 43°C in Chinese hamster ovary cells. *Cancer Res.* **46,** 175–182.
15. Averill-Bates, D. A., Agostinelli, E., Przybytkowski, E., Mateescu, M. A., and Mondovi, B. (1993) Cytotoxicity and kinetic analysis of purified bovine serum amine oxidase in the presence of spermine in Chinese hamster ovary cells. *Arch. Biochem. Biophys.* **300,** 75–79.
16. Averill-Bates, D. A., Agostinelli, E., Przybytkowski, E., and Mondovi, B. (1994) Aldehyde dehydrogenase and cytotoxicity of purified bovine serum amine oxidase and spermine in Chinese hamster ovary cells. *Biochem. Cell. Biol.* **72,** 36–42.
17. Kims, B. W., and Morris, D. R. (1971) Inhibition of nucleic acid and protein synthesis in *Escherichia coli* by oxidized polyamines and acrolein. *Biochim. Biophys. Acta* **228,** 235–244.
18. Ayusawa, D., Iwata, K., and Seno, T. (1981) Alteration of ribonucleotide reductase in aphidicolin-resistant mutants of mouse FM3A cells with associated resistance to arabinosyladenine and arabinosylcytosine. *Somatic Cell Genet.* **7,** 27–42.
19. Igarashi, K., Kashiwagi, K., Hamasaki, H., Miura, A., Kakegawa, T., Hirose, S., and Matsuzaki, S. (1986) Formation of a compensatory polyamine by *Escherichia coli* polyamine-requiring mutants during growth in the absence of polyamines. *J. Bacteriol.* **166,** 128–134.
20. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275.
21. Alarcon, R. A. (1968) Fluorometric determination of acrolein and related compounds with *m*-aminophenol. *Anal. Chem.* **40,** 1704–1708.
22. Seyfried, C. E., and Morris, D. R. (1983) Methods for the study of the physiological effects of inhibitors of polyamine biosynthesis in mitogen-activated lymphocytes. *Methods Enzymol.* **94,** 373–389.
23. Gahl, W. A., and Pitot, H. C. (1982) Polyamine degradation in foetal and adult bovine serum. *Biochem. J.* **202,** 603–611.
24. Kehrer, J. P., and Biswal, S. S. (2000) The molecular effects of acrolein. *Toxicol. Sci.* **57,** 6–15.
25. Brunton, V. G., Grant, M. H., and Wallace, H. M. (1991) Mech-

anisms of spermine toxicity in baby-hamster kidney (BHK) cells. The role of amine oxidases and oxidative stress. *Biochem. J.* **280,** 193–198.

26. He, Y., Kashiwagi, K., Fukuchi, J., Terao, K., Shirahata, A., and Igarashi, K. (1993) Correlation between the inhibition of cell growth by accumulated polyamines and the decrease of magnesium and ATP. *Eur. J. Biochem.* **217,** 89–96.
27. Bitonti, A. J., Dumont, J. A., Bush, T. L., Stemerick, D. M., Edwards, M. L., and McCann, P. P. (1990) Bis(benzyl)polyamine analogs as novel substrates for polyamine oxidase. *J. Biol. Chem.* **265,** 382–388.
28. Igarashi, K., Sugawara, K., and Hirose, S. (1975) Effect on ribosomes of the substitution of spermidine or divalent cations for magnesium ions. *J. Biochem. (Tokyo)* **77,** 753–759.