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Mechanism of Abnormal Active Oxygen Generation in Tissue Cells by Puromycin Aminonucleoside

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Summary. **Puromycin aminonucleoside (PA) is known as a toxic substance which induces heavy proteinuria. However, the mechanism of inducing proteinuria has not been clarified. We have reported that PA increases active oxygen generation in isolated rat hepatocytes. In this paper, we investigated the mechanism of abnormally active oxygen generation in isolated rat hepatocytes. Active oxygen generation was measured by the conversion rate of creatinine to methylguanidine (MG), a radical adduct. MK-447, which is an active oxygen scavenger and affects arachidonic acid metabolism, markedly inhibited MG synthesis at 1 μM. CV6504, which completely inhibited proteinuria induced by PA, is an inhibitor of 5-lipoxygenase and thromboxane A_2 synthetase and also is an active oxygen scavenger. CV6504 at 50 μM almost completely inhibited MG synthesis increased by PA. AA861, a 5-lipoxgenase inhibitor, at 1 μM also inhibited MG synthesis increased by PA, but higher concentrations were not effective. Cyclooxygenase inhibitors, indomethacin and pyroxicam, inhibited MG synthesis increased by PA. These results suggested that the abnormally active oxygen generation by PA comes from arachidonic acid metabolism. However, thromboxane A_2 synthetase is not related to active oxygen generation in the arachidonic acid cascade. OKY-046, which is an inhibitor of thromboxane A_2 synthetase, inhibited abnormally active oxygen generation by PA. Stable thromboxane A_2, ONO-11114, had no effect on MG synthesis. An antagonist of platelet activating factor, YM-264, which decreases proteinuria induced by PA, also inhibited abnormally active oxygen generation by PA. These results suggest that abnormally active oxygen may come from the arachidonic acid cascade, but there might be other unknown mechanisms which regulate active oxygen generation in tissue cells.**

INTRODUCTION

Recently, it has been reported that the oxygen radical plays an important role in kidney diseases.[1-6] We have reported that methylguanidine (MG), which is a uremic toxin,[7,8] was formed by oxygen radical reaction with creatinine *in vitro*[9] and in isolated rat hepatocytes.[10-13] In addition, MG synthesis in isolated rat hepatocytes was inhibited by adenosine, adenosine potentiators (i.e. dipyridamole and dilazep), and adenosine analogues.[11-13] We have also reported that aminonucleoside of puromycin (PA), which induces severe proteinuria in rats,[14] stimulated MG synthesis in isolated rat hepatocytes, while adenosine, adenosine analogues and its potentiators inhibited the MG synthesis stimulated by PA.[11-13]

In this paper, we further investigated the details of these phenomena, as well as the mechanism of abnormal oxygen radical generation by PA in isolated hepatocytes, by measuring the formation of MG from creatinine by reaction with active oxygen.

METHODS

Preparation of isolated rat hepatocytes

Male Wistar rats weighing 300-500 g were used in all experiments. The rats were allowed free access to water and laboratory chow containing 25% protein. Isolated hepatocytes were prepared essentially according to the method of Berry and Friend[15] as described previously.[16] We calculated that 9.8×10^7 cells corresponded to 1 g of liver (wet weight).[17]

Incubation of cells

Cells were incubated in 6 ml of Krebs-Henseleit bicarbonate buffer containing 3% bovine serum albumin, 10 mM sodium lactate, 17.6 mM creatinine and indicated substances with shaking at 60 cycles/min in a 30 ml conical flask with a rubber cap under 95% oxygen and 5% carbon dioxide at 37℃ for 4 or 6 h

(except for the experiment on time dependence). In the experiments to check the effect of dipyridamole, cells were preincubated with dipyridamole for 5 min. Equilibration of the buffer was repeated every hour. To measure the rate of non-biological conversion of creatinine to MG,[10] incubations were carried out without cells. The amount of cells used for each experiment is indicated in the results section. The reaction was stopped by the addition of 0.6 ml of 100% (wt/v) trichloroacetic acid. After sonication, the supernatant was obtained by centrifugation at 1700 g for 15 min at 0℃ and 0.25 ml of the extract was used for MG measurement. MG was determined by high-performance liquid chromatographic analysis using 9,10-phenanthrenequinone for post-labeling, as described previously.[10] PA, adenosine, 2-chloroadenosine and dibutyryl cAMP were purchased from Sigma Chemical Co., St Louis, MO. N^6-monomethyl adenosine was purchased from Aldrich Biochemical Co., Milwaukee, WI. Dipyridamole was kindly donated by Böehringer Ingelheim Ltd. (E)-3-(p-(1H-Imidazol-1-ylmethyl)phenyl)-2-propenic acid (OXY-046) and (+)-9,11,epithia-11,12-methano-TXA_2 (ONO-1113) were gifts from Ono Pharmaceutical Co; 2,3,5-Trimethyl-6-(3-pyridylmethyl)-1,4-bezoquinone hydrochloride (CV6504) and 2-(12-Hydroxydodeca-5,10-dinyl)-3,5,6-trimethyl-1,4-benzoquinone (AA861) were gifts from Takeda Pharmaceutical Co; and 1-(3-phenylpropyl)-4-[2-(3-pyrodyl) thiazolidin-4-ylcarbonyl] piperadin (YM-461) was a gift from Yamanouchi Pharmacy Co.

RESULTS

Effect of adenosine and dipyridamole on MG synthesis in isolated rat hepatocytes

Incubation of isolated rat hepatocytes with adenosine at concentrations ranging from 0.1 μM to 100 μM resulted in the inhibition of MG synthesis. The inhibition was dose-dependent and was maximum at 100 μM adenosine (60±2%) (Fig. 1). The addition of 1 mM dipyridomole, which inhibits the uptake of adenosine into the cell, enhanced the effect of adenosine. The co-operative effect of dipyridamole and adenosine is shown in Fig. 2. In the absence of adenosine in the medium, dipyridamole inhibited MG synthesis. This result suggested the existence of endogenous adenosine in the medium. The effect of dipyridamole was dose dependent at the concentration range from 20 μM to 1 mM.

Specificity of adenosine analogues for the inhibition of MG synthesis

It was demonstrated that 2-chloroadenosine, which is not metabolized by adenosine deaminase, inhibited MG synthesis by 50% at a cencentration of 100 μM

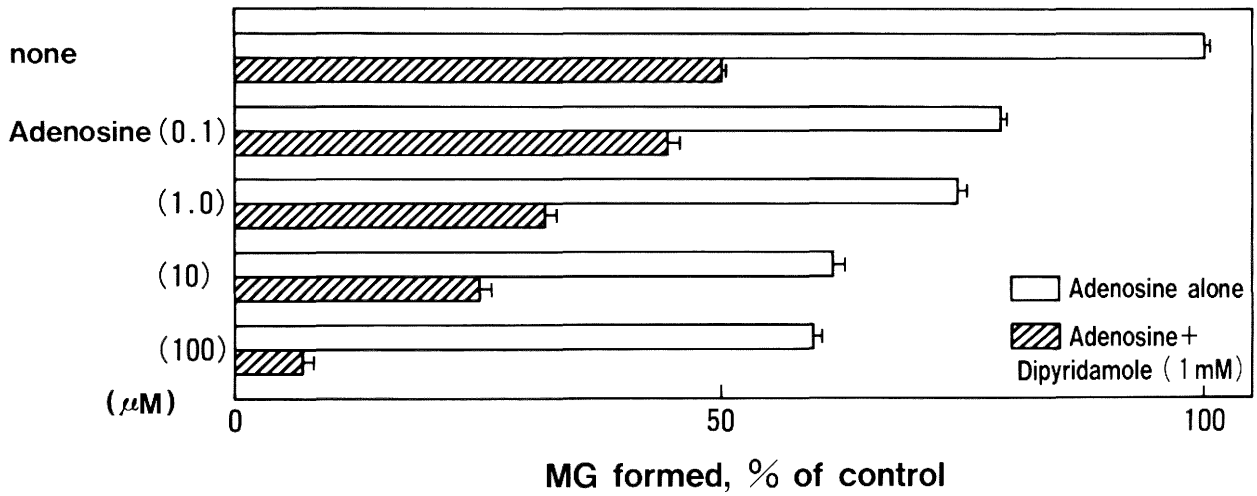

Fig. 1. Effect of adenosine and dipyridamole on MG synthesis in isolated rat hepatocytes. Hepatocytes (0.076 g of wet weight) were incubated for 4 h with adenosine in the absence (□) or in the presence of 1 mM dipyridamole (▨) as described in the Methods section. Various concentrations of adenosine were added to the incubation medium 5 min after the addition of dipyridamole. Each column represents the mean value of duplicate incubations. Bars represent the range of each determination.

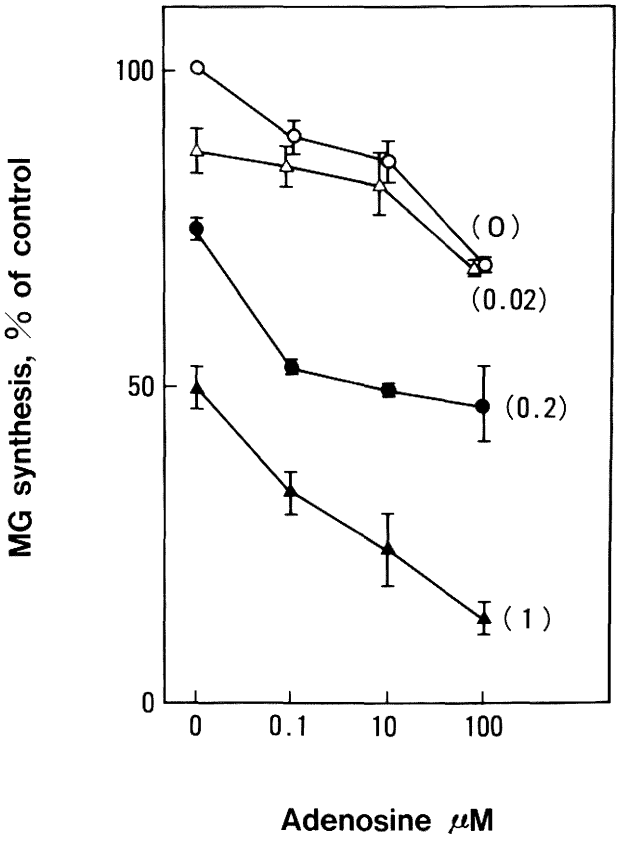

Fig. 2. Cooperative effect of adenosine and dipyridamole on MG synthesis in isolated rat hepatocytes. Hepatocytes (0.085 g of wet weight) were incubated for 4 h with various concentrations of adenosine and dipyridamole as described in the Methods section. Adenosine was added to the incubation medium 5 min after the addition of dipyridamole. The concentration of dipyridamole is given (as mM) in parenthesis. Each point represents the mean value of duplicate incubations. Bars represent the range of each determination.

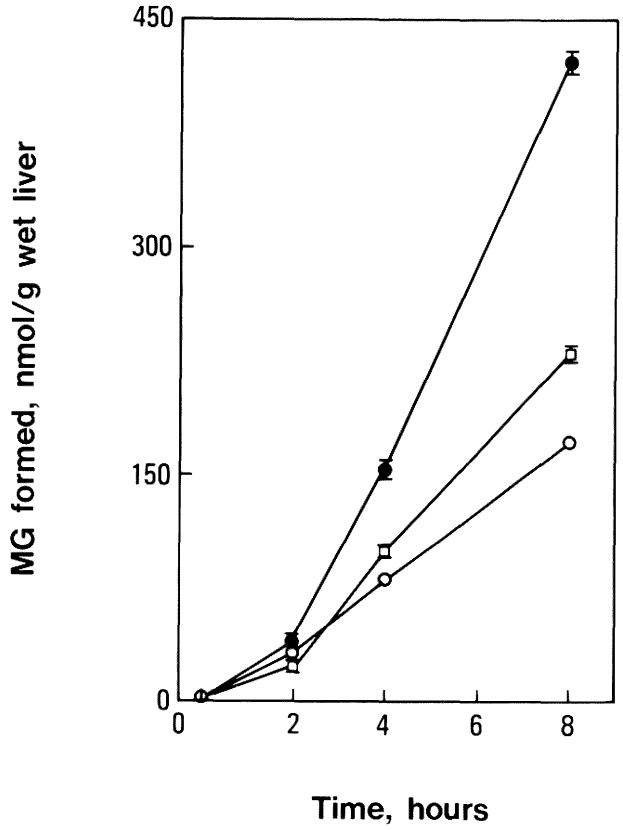

Fig. 3. Effect of PA on MG synthesis in isolated hepatocytes. Cells (0.18 g wet wedght) were incubated for 2, 4 or 8 h in the absence (○) or in the presence of 0.2 (□) or 1.9 mM (●) PA as described in the Methods section. Each point represents the mean value of duplicate incubations. Bars represent the range of each determination.

Table 1. Effect of adenosine analogues on MG synthesis

	MG formed	
reagent (mM)	μmole/g/4 h	(%)
control	218±2	(100±0.9)
adenosine (0.1)	130±4	(60±2)
2-chloroadenosine (0.1)	118±18	(54±8)
2'-deoxyadenosine (0.1)	179±8	(82±4)
inosine (0.1)	198±10	(90±5)
hypoxanthine (0.1)	241±16	(110±7)

Hepatocytes (0.14 g wet weight) were incubated with various reagents for 4 h. Each value represents the mean value of duplicate incubations ± the range of each determination.

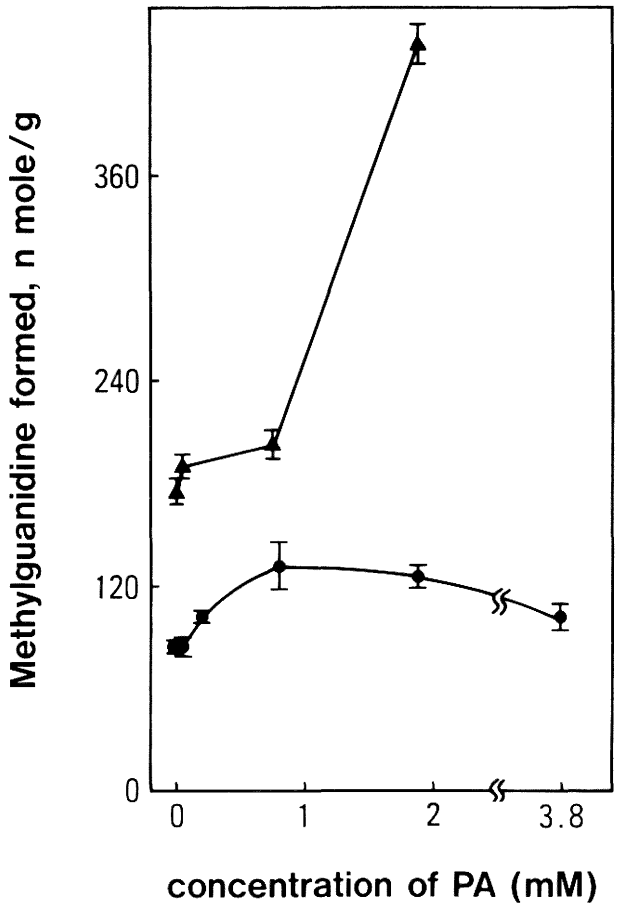

Fig. 4. Dose dependence in the effect of PA on MG synthesis. Cells (0.18 g wet weight) were incubated for 4 (○) or 8 (▲) h with various concentrations of PA as described in the Methods section. Each point represents the mean value of each determination.

(Table I). In contrast, 2-deoxyadenosine, a naturally occurring analog modified in the ribose moiety, did not inhibit MG synthesis. Adenosine is degraded to inosine and hypoxanthine.[18] Hypoxanthine and inosine at 100 μM had little effect on MG synthesis under the present conditions.

Stimulation of MG synthesis in isolated rat hepatocytes by PA

MG synthesis was increased in isolated rat hepatocytes incubated with PA, as shown in Fig. 3. The increase was apparent after incubation for 4 h, and markedly increased after 8 h. The maximum rate of MG synthesis was observed at 0.9 mM PA at 4 h, but MG synthesis was dose-dependent at 8 h (Fig. 4).

Inhibition of PA stimulated MG synthesis by adenosine and 2-chloroadenosine

MG synthesis stimulated by PA was inhibited 32±2% with 200 μM adenosine, and inhibited 54±5% with 100 μM 2-chloroadenosine, as shown in Table 2.

Effect of dibutyryl-cAMP, papaverine and allopurinol on MG synthesis

Many of the effects of adenosine have been attributed to its ability to stimulate or inhibit adenylate cyclase, and these effects are mediated by distinct binding sites for the nucleoside.[19] The existence of stimulatory (Ka) sites and of low-affinity inhibitory (P) sites for the nucleoside has been reported in liver[20] and isolated rat hepatocytes.[21]

Table 2 Effect of adenosine and 2-chloroadenosine on MG synthesis

	MG formed	
reagents (mM)	nmol/g/6 h	(%)
none	110.9±0	(100±0)
adenosine (0.2)	90.3±2.6	(81.4±2.3)
PA (1.9)	162.5±2.5	(14.6±2.2)
PA (1.9) + adenosine (0.2)	114.8±1.2	(103.5±1.1)
PA (1.9) + 2-chloroadenosine (0.1)	74.7±8.1	(67.4±10)

Cells (0.21 g wet weight) were incubated for 6 h as described in the Methods section. Values are expressed as the mean of duplicate incubations ± the range of each incubation.

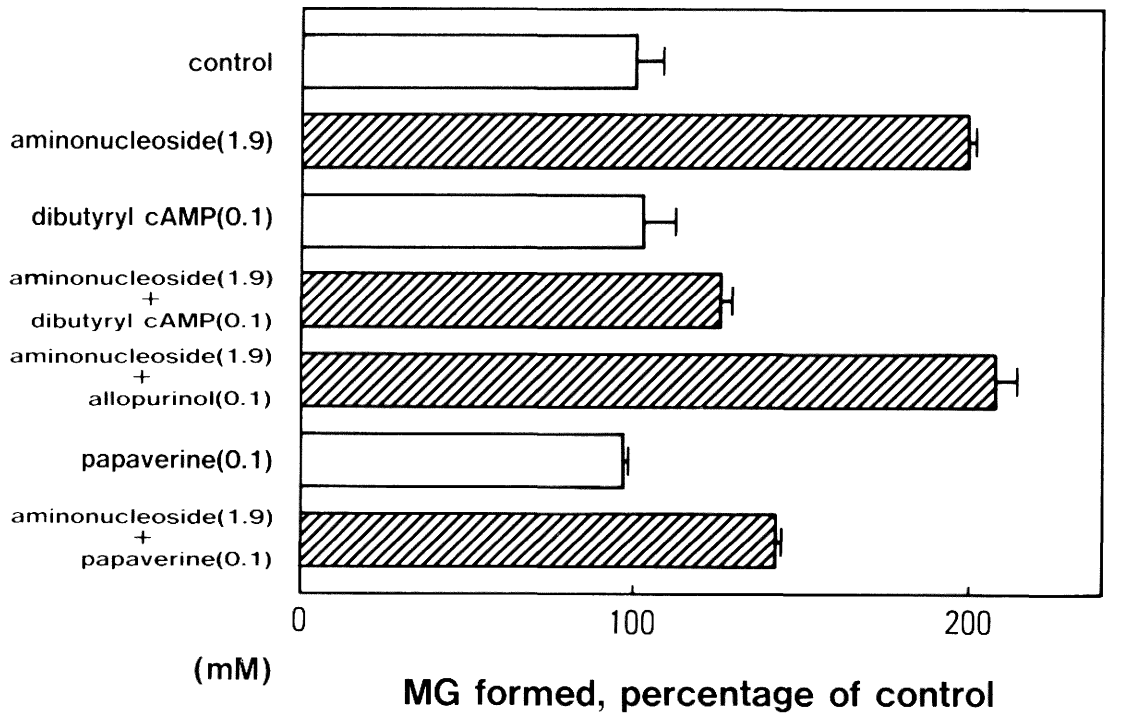

Fig. 5. Effect of dibutyryl cAMP, papaverine and allopurinol on MG synthesis. Cells (0.1 g wet weight) were incubated for 6 h as described in the Methods section. Each column represents the mean of duplicate incubations expressed as a percentage of the control value. Bars express the range of each incubation. The control value was 57.8±4 nmol/g/6 h.

Addition of 100 μM dibutyryl cAMP or 100 μM papaverine (which inhibits phosphodiesterase) inhibited MG synthesis stimulated by PA. However, MG synthesis in isolated hepatocytes incubated without PA was not inhibited by these reagents (Fig. 5).

In tissues, the hypoxanthine oxidase reaction is one of the metabolic pathways of oxygen radical generation.[22] To examine the role of this reaction in MG synthesis in isolated rat hepatocytes under our conditions, we tested the effect of allopurinol, which inhibits xanthine oxidase.[23] Allopurinol had no effect on MG synthesis stimulated by PA, as shown in Fig. 5, and also had no effect on MG synthesis in isolated hepatocytes incubated without PA.[13]

Effect of MK-447 on MG synthesis

MK-447 (2-aminomethyl-4-t-butyl-6-iodophenol HCl), a phenolic compound, scavenges oxygen-derived free radicals released during the conversion of prostagrandin (PG) G_2 to PG H_2.[24] MK-447 inhibited MG synthesis in isolated rat hepatocytes with or without PA at 1 μM as shown in Fig. 6. This inhibitory effect of MK-447 can not be explained as simple scavenging of active oxygen because of the high concentration of creatinine (17.6 mM) in the incubation medium. Rather, these results suggested the direct inhibition of cyclooxygenase by MK-447.

Effect of indomethacin and piroxicam on MG synthesis

Cyclooxygenase inhibitors, indomethacin and piroxicam, inhibited MG synthesis only when stimulated by PA at a concentration of 10 μM, and had no effect on MG synthesis without PA even at high concentrations of inhibitors as shown in Fig. 7. These results suggested excess of active oxygen comes from arachidonic acid metabolism by cyclooxygenase.

Effect of OKY-046 and ONO-11114 on MG synthesis

OKY-046 has been developed as a selective thromboxane A_2 synthetase inhibitor in platelets.[25] OKY-046 inhibited MG synthesis in isolated hepatocytes from a concentration of 1 μM as shown in Fig. 8. However, the extent of the inhibition of MG synthesis in the presence of PA is also higher than that without PA. Stable thromboxane A_2, ONO-11114, had

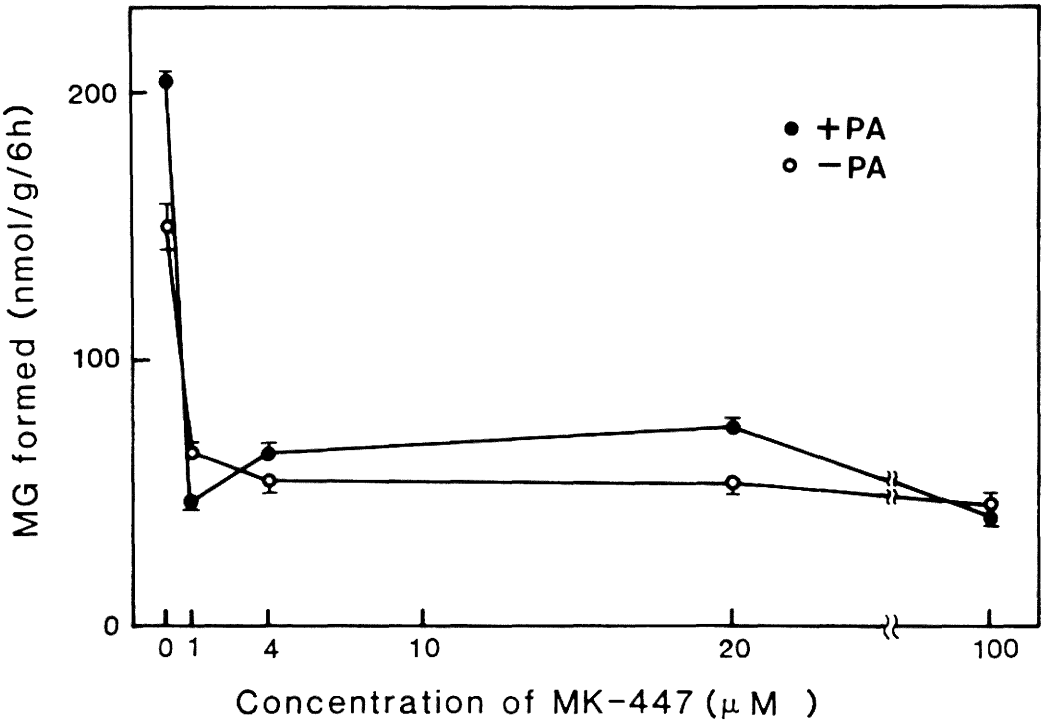

Fig. 6. Effect of MK-447 on MG synthesis. Cells vere incubated with various concentrations of MK-447 for 6 hours in the absence (○) or in the presence (●) of 1.9 mM PA.

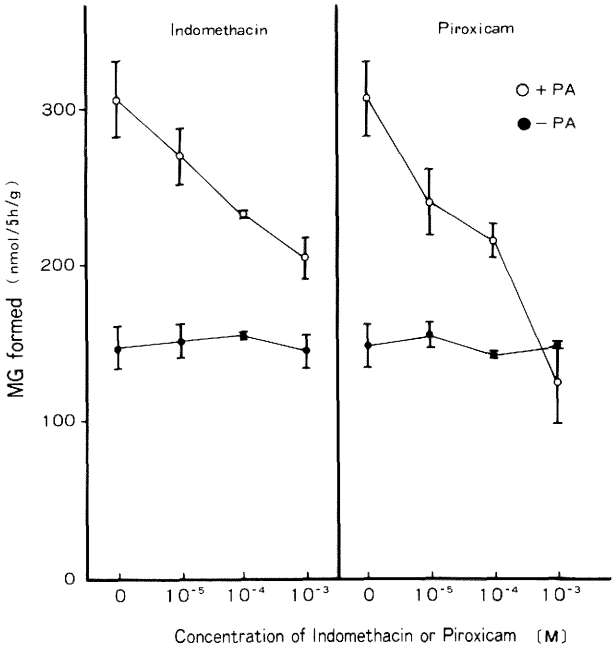

Fig. 7. Effect of indomethacin or piroxicam on MG synthesis. Cells were incubated with various concentrations of indomethacin (left) and piroxicam (right) for 6 hours without PA (●) or with 1.9 mM PA (○).

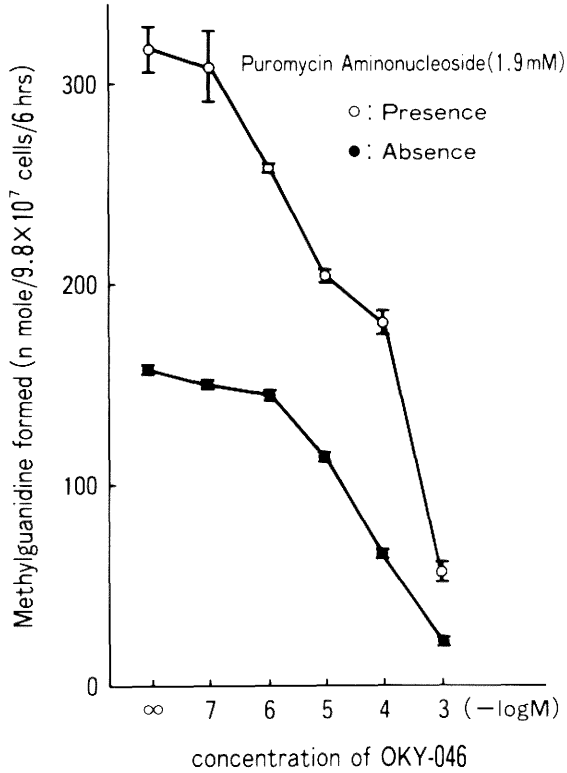

Fig. 8. Effect of OKY-046 on MG synthesis. Cells were incubated with various concentrations of OKY-046 without PA (●) or with 1.9 mM PA (○) for 6 hours.

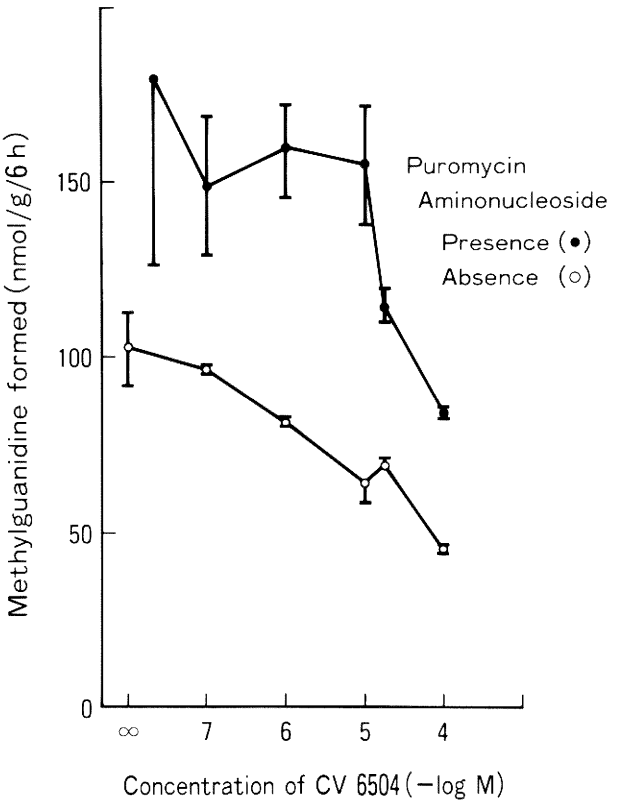

Fig. 9. Effect of CV6504 on MG synthesis. Cells were incubated with various concentrations of CV6504 without PA (○) or with 1.9 mM PA (●) for 6 hours.

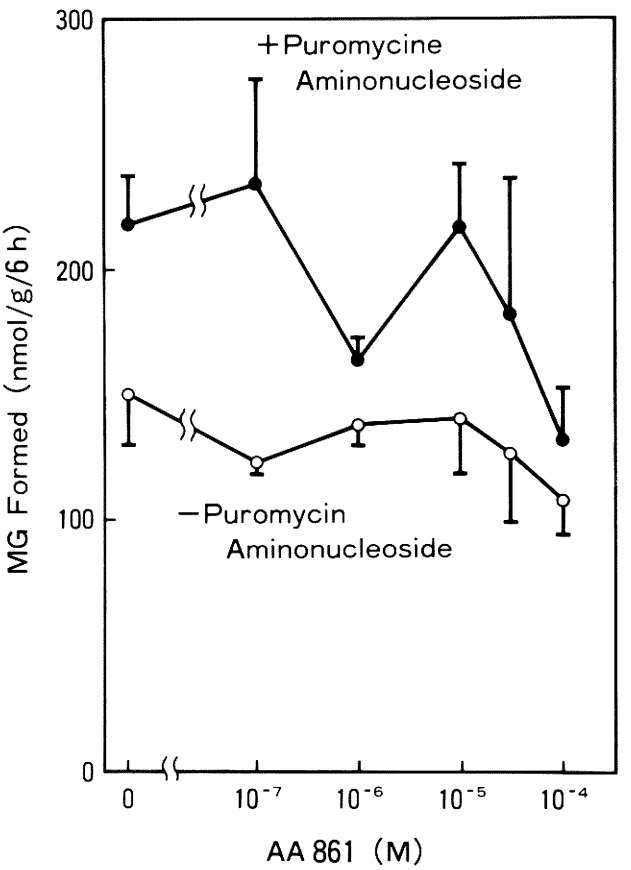

Fig. 10. Effect AA861. Cells were incubated with various concentrations of AA861 without PA (○) or with 1.9 mM PA (●) for 6 hours.

no effect on MG synthesis. These results suggested that thromboxane A_2 outside of the cell is not responsible for active oxygen generation or OKY-046 inhibits cyclooxygenase in the hepatocytes.

Effect of CV6504

CV6504 has been developed as an inhibitor which inhibits 5-lipoxygenase and thromboxane A_2 synthetase. This agent also scavenges active oxygen. CV6504 inhibited active oxygen generation as shown in Fig. 9. The extent of inhibition of MG synthesis is also higher than that without PA.

Effect of AA861

AA861 has been developed as a 5-lipoxygenase inhibitor. 5-lipoxigenase also generates hydroxyl radicals during leukotriene synthesis. AA861 inhibited active oxygen as shown in Fig. 10. The inhibition was not dose-dependent and the mechanism of inhibition of active oxygen generation is most likely complicated.

Effect of YM-461 on MG synthesis

We recognized that 1-(3-phenylpropyl)-4-[2-(3-pyridyl) thiazolidin-4-yl cabonnyl] piperidine, YM461, an antagonist of PAF[26] inhibited MG synthesis in isolated rat hepatocytes as shown in Fig. 11. The extent of inhibition of MG synthesis in the presence of PA was also higher than that without PA.

DISCUSSION

Activated oxygen radicals are thought to induce deleterious effects such as lipid peroxidation, inflammation, carcinogenesis, cataracts and atherosclerosis. Recently, it has been reported that active oxygen

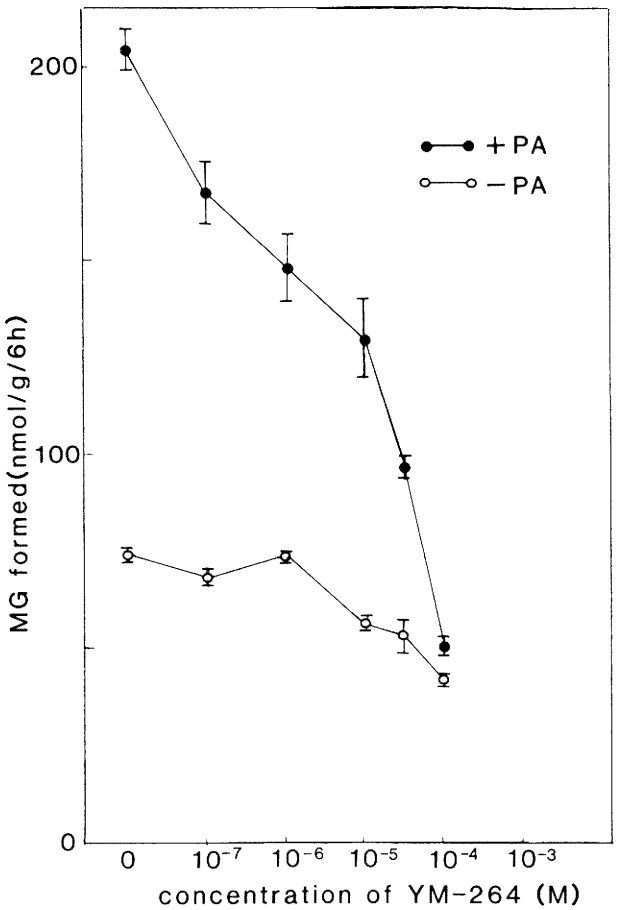

Fig. 11. Effect of YM461 on MG synthesis. Cells were incubated with various concentrations of YM461 in the absence of PA (○) or in the presence of 1.9 mM PA (●) for 6 hours.

plays an important role in the pathogenesis of acute renal failure[1,5] and glomerular injury.[2-5] We have reported that MG, a uremic toxin, was formed from creatinine by active oxygen *in vitro*[9] and in isolated rat hepatocytes.[11-13] Diamond et al. reported that PA-induced nephrosis was inhibited by superoxide dismutase and allopurinol.[27] They suggested that the increase of hypoxanthine (which is a substrate for xanthine oxidase) by the degradation of PA and/or the change of the enzyme form from NAD-reducing dehydrogenase (Type D) to superoxide-producing oxidase (Type O) led to an excess of oxygen radical generation. It was reported that kidney transplanted 15 min after PA injection developed severe proteinuria.[28] This result suggested irreversible binding (or quick reaction to PA by the cell) rather than the increase of hypoxanthine derived from PA degradation. We have found that adenosine inhibited MG synthesis in the presence and absence of PA. Further, it has been reported that adenosine inhibited superoxide anion generation in human neutrophils at physiological concentration,[29] and the injury to endothelial cells caused by neutrophils was inhibited by adenosine and its agonist.[30] In our system, MG synthesis was inhibited by low concentrations of adenosine and dipyridamole (which inhibits the uptake of adenosine into cells). Furthermore, MG synthesis stimulated by PA was inhibited by the addition of dibutyryl cAMP and papaverine, both of which increase the cAMP content in hepatocytes. Addition of 10 μM 2-chloroadenosine to hepatocytes increased the cAMP level in isolated rat hepatocytes.[21] These results suggested that the inhibition of oxygen radical generation in neutrophils and glomerular epithelial cells by adenosine potentiators may explain in part their proteinuria-reducing effect in human glomerulonephritis[31,32] and in PA nephrosis in rats.[33,34]

It has been reported that hydroxyl radicals are released by the arachidonic acid cascade. Cyclooxygenase inhibitors, indomethacin and piroxicam, inhibited active oxygen generation increased by PA but did not inhibit active oxygen generation without PA. Therefore, excess active oxygen generation by PA must be derived from PGG_2 to PG H_2. CV6504 which inhibits cyclooxygenase and 5-lipoxigenase and also scavenges active oxygen was reported to ameliorate PA induced nephrosis and immune complex mediated nephritis. Thromboxane A_2 synthesis has been reported to be related to the progression of kidney disease. OKY-046, which is a selective thromboxane A_2 synthesis inhibitor, ameliorates PA nephrosis and immune mediated nephritis.[35] In our hepatocyte system, 1 μM OKY-046 inhibited active oxygen generation by PA. Therefore, OKY-046 may have another effect such as cyclooxygenase inhibition in addition to its role as a thromboxane A_2 synthetase inhibitor. Anti-PAF drug, YM264, also was reported to ameliorate PA induced proteinuria and immune glomerulonephritis. YM461 also inhibited active oxygen generation. However, this effect may not depend on the anti-PAF effects at receptors of PAF. It has been reported that some anti-PAF drugs are effective when they are present intracellularly.[36]

We propose that PA increases active oxygen generation by arachidonate metabolism as shown in Fig. 12. This excess active oxygen generation may lead to the stimulation of active oxygen generation such as the conversion of hypoxanthine dehydrogenase to hypoxanthine oxidase and may generate a large amount of active oxygen. Many drugs which ameliorate kidney diseases may depend on the inhibition of

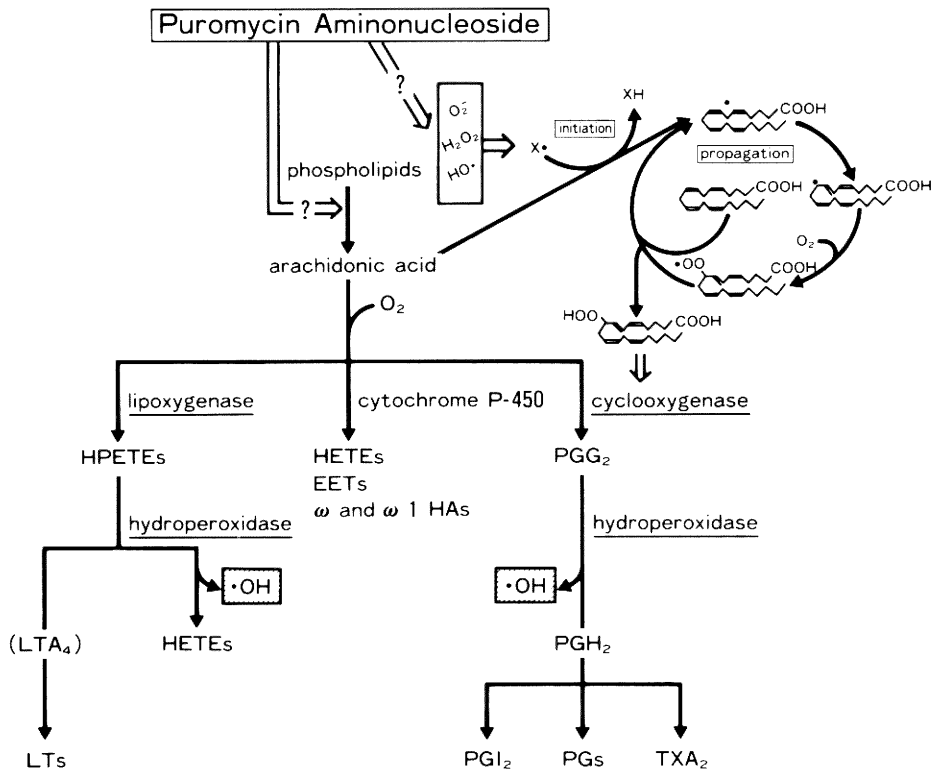

Fig. 12. Proposed mechanism of excess active oxygen generation by PA and inhibitory effect of adenosine on active oxygen generation.

excess active oxygen generation.

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REFERENCES

1) Paller MS, Hoidal JR, Ferris TF: Oxygen radical in ischemic acute renal failure in the rat. *J Clin Invest* **74**: 1156-1164, 1984.
2) Rehan A, Johnson KJ, Wiggs RC, Kunkel RG, Ward PA: Evidence for the role of oxygen radicals in acute nephrotoxic nephritis. *Lab Invest* **51**: 396-403, 1984.
3) Rehan A, Johnson KJ, Kunkel RG, Wiggs RC: Role of oxygen radicals in phorbol myristate acetate-induced glomerular injury. *Kidney Int* **27**: 503-511, 1985.
4) Adachi T, Fukuta M, Ito Y, Hirano K, Sugiura M, Sugiura M, Sugiura K: Effect of superoxide dismutase on glomerular nephritis. *Biochem Pharmacol* **35**: 34-345, 1986.
5) Dobyan DC, Bull JM, Strebel FR, Sunderland BA, Bulger RE: Protective effects of O-(β-hydroxyethyl)-rutoside on *cis*-platinum-induced acute renal failure in the rat. *Lab Invest* **55**: 557-563, 1986.
6) Holdsworth SR, Boyce NW: Hydroxyl radical mediation of immune renal injury by desferrioxamine. *Kidney Int* **30**: 813-817, 1986.
7) Giovannetti S, Biagini M, Balestri PL, Navasesi R, Giagnoni P, deMatteis A, Ferro-Milone P, Perfetti C: Uremia-like syndrome in dogs chronically intoxicated with methylguanidine and creatinine. *Clin Sci* **36**: 445-452, 1969.
8) Barsotti G, Bevilacqua G, Morelli E, Cappelli P, Balestri PL, Biovannetti S: Toxicity arising from quanidine compounds: Role of methylguanidine as a uremic toxin. *Kidney Int* **7**: s299-s301, 1975.
9) Nagase S, Aoyagi K, Narita M, Tojo S: Active oxygen in methlguanidine synthesis. *Nephron* **44**: 299-303, 1986.
10) Nagase S, Aoyagi K, Narita M, Tojo S: Biosynthesis of methylguanidine in isolated rat hepatocytes adn *in vivo*. *Nephron* **40**: 470-475, 1985.
11) Aoyagi K, Nagase S, Ohba S, Miyazaki M, Sa-

kamoto M, Narita M, Tojo S: Significance of active oxygen in methylguanidine synthesis in isolated hepatocytes. *Jap J Nephrol* **28**: 536, 1986 (abstract of Japanese Nephrology Congress in 1985).
12) Aoyagi K, Narita M, Tojo S: Uremic toxin—recent progress of our study. *Medical Practice* Vol. 3, No. 2, 256-259, 1986.
13) Aoyagi K, Nagase S, Narita M, Tojo S: Role of active oxygen on methylguanidine synthesis in isolated rat hepatocytes. *Kidney Int.* (in press)
14) Lamnigan R, Kark R, Pollak VE: The effect of a single intravenous injection of aminonucleoside of puromycin on the rat kidney. *J Pathol Bacteriol* **83**: 357-362, 1962.
15) Berry NM, Friend DS: High-yield preparation of isolated liver cells. *J Cell Biol* **43**: 506-520, 1969.
16) Aoyagi K, Ohba S, Narita M, Tojo S: Regulation of biosynthesis of gunidinosuccinic acid in isolated rat hepatocytes and *in vivo*. *Kidney Int* **24**: s224-s228, 1983.
17) Zahlten RN, Stratman FW, Lardy HA: Regulatioon of glucose synthesis in hormone-sensitive isolated rat hepatocytes. *Proc Nat Acad Sci USA* **70**: 3213-3218, 1973.
18) Granger DN, Rutil G, McCord J: Superoxide radicals in feline intestinal ischemia. *Gastroenterol* **81**: 22-29, 1981.
19) Sattin A, Rall TW: The effect of adenosine and adenosine nucleotides on the cyclic adenosine 3', 5'-phosphate content of guinea pig cerebral cortex slices. *Mol Pharmacol* **6**: 13-24, 1973.
20) Cooper DMF, Londos C: Evaluation of the effects of adenosine on hepatic and adipocyte adenylate cyclase under conditions where adenosine is not generated endogenously. *J Cyclic Nucleotide Res* **5**: 289-302, 1979.
21) Claus TH, Anand-Srivastava MB, Johnson RA: Regulation of hepatocytes cAMP pyruvate kinase by site-specific analogs of adenosine. *Mol cell endocrinol* **26**: 269-279, 1982.
22) Parks DA, Granger DN: Ischemia-induced vascular changes: Role of xanthine oxidase and hydroxyl radicals. *Amer J Physiol* **245**: G285-G289, 1983.
23) Bulkley GB: The role of oxygen free radicals in human disease processes. *Surgery* **94**: 407-411, 1983.
24) Harada Y, Tanaka K, Katori M: Enhanced prostacyclin generation by phenolic compounds acting as a tryptophan-like cofactor of PG hydroperoxidase. *Prostagranding* **28**: 367-381, 1984.
25) Iizuka K, Akahane K, Momose D, Nakazawa M: Highly selective inhibitors of thromboxane synthetase. *J Med Chem* **24**: 1139-1148, 1981.
26) Yamada T, Tomioka K, Saito M, Horie M, Mase T, Hara H, Nagaoka H, Suzuki T: Pharmacological properties of YM264, a potent and orally active antagosist of PAF. Proceedings of 7th International Conference on prostaglandins and related compounds. Florence 1990, p. 253. (abstract)
27) Diamond JR, Bonventre JV, Karnovsky MJ: A role for oxygen free radicals in aminonucleoside nephrosis. *Kidney Int* **29**: 478-483, 1986.
28) Hoyer JR, Ratte J, Potter AH, Michael AF: Transfer of aminonucleoside nephrosis by renal transplantation. *J Clin Invest* **51**: 2777-2780, 1972.
29) Bruce N, Cronstein BN, Karmer SB, Weissmann GD, Hirschorn R: Adenosine: a physiological modulator of superoxdde anion generation by human neutrophils. *J Exp Med* **158**: 1160-1177, 1983.
30) Cronstein BN, Levin RI, Belanoff J, Weissmann G, Hirschorn R: Adenosine: an endogenous inhibitor of neutrophil-mediated injury to endothelial cells. *J Clin Invest* **78**: 760-770, 1986.
31) Kan K, Wada T, Kitamoto K, Konishi K, Ozawa Y, Kato E, Matsuki S: Dipyridamole for proteinuria supression: use in a patient with proliferative glomerulonephritis. *J A M A* **229**: 557-558, 1974.
32) Tojo S, Narita M, Koyama A, Sano M, Suzuki H, Tsuchiya T, Tsuchida H, Yamamoto S, Shishido H: Dipyridamole therapy in the nephrotic syndrome. *Contrib Nephrol* **9**: 111-127, 1978.
33) Kimura K, Endo H, Sakai F: Suppressive effect of dipyridamole on the proteinuria of aminonucleoside nephrosis in rat. *J Toxicol Sci* **4**: 1-10, 1978.
34) Nagase M, Kumagi H, Honda N: Suppression of proteinuria by dipyridamole in rats with aminonucleoside nephropathy. *Renal Physiol* **7**: 218-226, 1984.
35) Nagamatsu T, Tsukushi Y, Ito M, Kondo N, Suzuki Y: Antinephritic effect of OKY-046, a thromboxane A synthetase inhibitor. *Jap J Pharmacology* **49**: 501-509, 1989.
36) Remy E, Vandesteene C, Remacle J: Central role of intracellular PAF in the activation of bovine neutrophils. Proceedings of 7th International conference on prostoglandines and related compounds, Florence 1990, p. 248. (abstract)