Bull. Environ. Contam. Toxicol. (1996) 56:630-634

Bulletin of Environmental Contamination and Toxicology

Bismuth Induces Metallothionein but Does Not Protect Against Cadmium Cytotoxicity in Cultured Vascular Endothelial Cells

T. Kaji,[1] A. Mishima,[1] C. Yamamoto,[1] Y. Fujiwara,[1] M. Sakamoto,[1] H. Kozuka,[2] F. Koizumi[3]

[1]Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3 Kanagawa-machi, Kanazawa 920-11, Japan
[2]Faculty of Pharmaceutical Sciences and [3]Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan

Received: 15 May 1995/Accepted: 13 September 1995

Cadmium has been shown to be an inducer of cardiovascular lesions such as atherosclerosis (Revis et al 1981) and hypertension (Schroeder and Vinton 1962). The relationship between cadmium exposure and vascular diseases was shown by epidemiological data (Carroll 1966). We found that cadmium destroys the monolayer of cultured vascular endothelial cells (Kaji et al 1992a). This suggested that damage of vascular endothelial cells may be an important event of cadmium-induced vascular disorders.

Metallothionein induction is postulated to be in general the most important mechanism for protection against cadmium toxicity (Probst et al 1977). However, zinc protects vascular endothelial cells from cadmium cytotoxicity without metallothionein induction (Kaji et al 1992b); zinc was not an effective inducer of the protein (Kaji et al 1993a). Recently, we found that bismuth strongly induces metallothionein selectively in vascular endothelial cells (Kaji et al 1994). Although zinc protection against cadmium cytotoxicity in vascular endothelial cells mainly resulted from a decrease in the accumulation of intracellular cadmium (Kaji et al 1992b), it was likely that bismuth reduces the cytotoxicity of cadmium by the metallothionein-dependent mechanism in the cells.

In the present study, we investigated the effect of bismuth on the cytotoxicity of cadmium in cultured vascular endothelial cells. We found that bismuth alone induces metallothionein but does not protect against cadmium cytotoxicity in the cells.

MATERIALS AND METHODS

Vascular endothelial cells derived from bovine aorta were a gift from Drs. Y utaka Nakashima and Katsuo Sueishi (First Department of Pathology, Faculty of Medicine, Kyushu University, Fukuoka, Japan). Dulbecco's modified Eagle's medium and ASF 301 medium were purchased from Nissui Pharmaceutical (Tokyo, Japan) and Ajinomoto (Tokyo, Japan), respectively. Fetal bovine serum was from Whittaker (Walkersville, MD, USA). Tissue culture plates and dishes were obtained from Corning (Coming, NY, USA). [2,8-^{3}H]Adenine (710.4 GBq/mmol) was from New England Nuclear (Boston, MA, USA). Bovine hemoglobin, cadmium chloride, bismuth nitrate and other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Correspondence to: T. Kaji

To evaluate the cytotoxicity of cadmium in the presence or absence of bismuth by the [^{3}H]adenine release assay, vascular endothelial cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum in 24-well plates in a humid atmosphere of 5% CO_2 until confluent. The medium was changed to fresh Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and the cell layer was then incubated at 37°C for 24 hr in the presence of [^{3}H]adenine at 37 kBq/mL. After labeling, the medium was discarded and the cell layer was washed twice with serum-free ASF 301 medium. The cell layer was then incubated at 37°C for 24 hr in 0.25 mL of serum-free ASF 301 medium in the presence of cadmium chloride at 2 or 5 μ*M* combined with or without bismuth nitrate at 2 or 10 μ*M*. After incubation, the medium was collected and the cell layer was washed with 0.25 mL of Ca, Mg-free phosphate-buffered saline (CMF-PBS); the wash was combined with the medium. The cell lysate was prepared by addition of 0.25 mL of 0.5% sodium dodecyl sulfate; after collecting the cell lysate, the well was washed with 0.25 mL of CMF-PBS and the wash was combined with the cell lysate. The radioactivity either in the medium or in the cell lysate was measured by scintillation counting. The [^{3}H]adenine release (%) was calculated by dividing the radioactivity (dpm) in the medium by that (dpm) found in both the medium and the corresponding cell lysate.

The accumulation of intracellular cadmium and bismuth was determined as follows: Confluent cultures of vascular endothelial cells in 6-well plates were incubated at 37°C for 24 hr in serum-free ASF 301 medium in the presence of cadmium chloride at 2 or 5 μ*M* combined with or without bismuth nitrate at 2 or 10 μ*M*. After incubation, the medium was discarded and the cell layer was washed twice with CMF-PBS. The cell layer was then extracted with 1 mL of 0.1 *M* acetate buffer solution (pH 5.5) containing 10 m*M* EDTA at 4°C for 72 hr. The extract was collected and the cell layer was gently washed with 1 mL of the buffer; the wash was combined with the extract. The extract was analyzed for the content of cadmium and bismuth by flameless atomic absorption spectrophotometry. The cell layer after extraction was scraped off with a rubber policeman in the presence of 0.75 mL of CMF-PBS. After collection of the cell suspension, the well was washed with 0.75 mL of CMF-PBS; the wash was combined with the cell suspension. The cell homogenate was prepared by sonication and used for the determination of DNA content by the fluorometric method (Kissane and Robins 1958).

The content of metallothionein was measured by the method of Onosaka et al (1978) with some modifications as follows: Confluent cultures of vascular endothelial cells in 6-well plates were treated for 24 hr with cadmium chloride at 2 or 5 μ*M* combined with or without bismuth nitrate at 2 or 10 μ*M* in serum-free ASF 301 medium. The medium was then discarded and the cell layer was washed twice with CMF-PBS. The cell layer was scraped off with a rubber policeman in the presence of 0.5 mL of 0.25 *M* sucrose. After collecting the cell suspension, the well was washed with 0.5 mL of 0.25 *M* sucrose and the wash was combined with the cell suspension. The cell homogenate was prepared by sonication. A portion (0.5 mL) of the homogenate was transferred into a microtube and 0.5 mL of 0.1 *M* Tris-HCl buffer solution (pH 8.0) and 0.05 mL of 10 μg/ml cadmium chloride solution were added. After incubation for 10 min at room temperature, 0.1 mL of 2% bovine hemoglobin was added and the mixture was boiled for 2 min; the tube was centrifuged at 10,000 x*g* for 5 min. The addition of hemoglobin; boiling and the centrifugation was repeated three times. The supernatant was analyzed for cadmium content by flameless atomic absorption spectrophotometry and the metallothionein content was calculated.

Table 1. The release of [^{3}H]adenine, the accumulation of cadmium and bismuth and the content of metallothionein in cultured vascular endothelial cells after exposure to cadmium or bismuth or both.

	[^{3}H]Adenine released (%)	Cadmium (pmol/μg DNA)	Bismuth (pmol/μg DNA)	Metallothionein (ng/μg DNA)
Absence of cadmium				
Control	21.41 ± 0.49	ND	ND	4.92 ± 1.17
2 μM Bismuth	24.22 ± 0.43	ND	45 ± 2	6.76 ± 0.92*
10 μM Bismuth	24.41 ± 1.46	ND	150 ± 13	17.96 ± 2.00**
Presence of 2 μM cadmium				
Control	28.70 ± 0.71	404 ± 62	ND	26.42 ± 2.86
2 μM Bismuth	28.29 ± 0.20	480 ± 44	54 ± 1	24.62 ± 1.29
10 μM Bismuth	28.55 ± 0.61	446 ± 44	121 ± 2	29.35 ± 1.94
Presence of 5 μM cadmium				
Control	37.31 ± 1.01	1181 ± 57	ND	32.03 ± 3.95
2 μM Bismuth	36.60 ± 0.58	1188 ± 31	60 ± 3	26.00 ± 2.07
10 μM Bismuth	39.27 ± 0.85	1266 ± 27	121 ± 15	38.49 ± 0.40

Confluent cultures of bovine aortic endothelial cells labeled with or without [^{3}H]adenine were incubated at 37°C for 24 hr in the presence or absence of cadmium chloride at 2 or 5 μM and/or bismuth nitrate at 2 or 10 μM. Values are means ± SE of four samples. Significantly different from the corresponding control, *$P<0.05$; **$P<0.01$ ND, not detected.

RESULTS AND DISCUSSION

Results are summarized in Table 1. The release of [^{3}H]adenine was increased by cadmium in a concentration-dependent manner, suggesting that the metal exhibited the cytotoxicity. Bismuth failed to affect the [^{3}H]adenine release in the presence or absence of cadmium. Microscopically, it was also observed that the damage of endothelial cell monolayer caused by cadmium was not prevented by bismuth (not shown). Bismuth accumulated in a concentration-dependent manner, while the accumulation of intracellular cadmium was unaffected by bismuth. Either bismuth or cadmium induced metallothionein, however, their combination failed to show an additive induction. The distribution of cadmium to the particulate fraction and the cytosol fraction was similar in endothelial cells treated with cadmium alone and in the cells treated with both cadmium and bismuth (for example, the distribution of intracellular cadmium to the particulate fraction was 31% and 26% when treated with 5 μ*M* cadmium alone and with both 5 μ*M* cadmium and 10 μ*M* bismuth, respectively), suggesting that bismuth did not affect the behavior of cadmium within the cells. In the cytosol fraction, cadmium was bound to both the high-molecular-weight proteins and metallothionein when analyzed by Superdex 75 gel chromatography; the relative distribution was unaffected by bismuth (for example, the distribution of intracellular cadmium to the high-molecular-weight fraction was 5.2% and 6.5% when treated with 5 μ*M* cadmium alone and with both 5 μ*M* cadmium and 10 μ*M* bismuth, respectively), suggesting that bismuth did not interfere the binding of cadmium to metallothionein as well as did not affect the induction of the protein by cadmium.

We previously reported that cadmium-induced destruction of a cultured endothelial monolayer is prevented by essential trace elements such as zinc (Kaji et al 1993b) and copper (Kaji et al 1992c) but enhanced by a toxic heavy metal such as lead (Kaji et al 1995). These interactions of cadmium with other heavy metals were independent of metallothionein induction in all cases. In the present study, bismuth was an inducer of metallothionein, but did not protect against cadmium cytotoxicity in vascular endothelial cells. Although the possibility that metallothionein induction is importantly involved in the protection against cadmium cytotoxicity in endothelial cells as well as other cell types (Hildebrand et al 1979) cannot be excluded, the interaction between cadmium and bismuth shown in the present study is a case where an inducer of metallothionein failed to reduce the cytotoxicity of cadmium.

In the previous study, it was shown that the intracellular accumulation of cadmium is markedly decreased by zinc (Kaji et al 1992b) and is moderately decreased by copper (Kaji et al 1992c) and lead (Kaji et al 1995) in vascular endothelial cells. These results suggest that cations can compete with cadmium for the entrance into endothelial cells and/or can mimic cadmium and dilute the actual concentration of the metal within the cells. However, bismuth accumulated in endothelial cells but did not decrease the cadmium accumulation. It is thus suggested that failure of bismuth to protect against cadmium cytotoxicity may result from inability of bismuth to compete with cadmium on the cell membane and within the cells. In vascular endothelial cells, it is possible that the competition with cadmium for the uptake and the critical target may be more important for protection against the cytotoxicity of the metal than the induction of metallothionein.

In conclusion, bismuth was not capable of decreasing the intracellular cadmium accumulation; in addition, combination of bismuth with cadmium failed to cause an additional induction of metallothionein. Bismuth could not reduce the cytotoxicity of cadmium in vascular endothelial cells in spite of its ability of

metallothionein induction. This was a case that metallothionein inducer does not necessarily reduce the cytotoxicity of cadmium. The defense mechanism for protection against cadmium cytotoxicity in vascular endothelial cells remains to be elucidated.

REFERENCES

Carroll RE (1966) The relationship of cadmium in the air to cardiovascular disease death rates. J Am Med Assoc 198:267-269

Hildebrand CE, Tobey RA, Campbell EW, Enger MD (1979) A cadmium-resistant variant of the Chinese hamster (CHO) cell with increased metallothionein induction capacity. Exptl Cell Res 124:237-246

Kaji T, Mishima A, Yamamoto C, Sakamoto M, Koizumi F (1992a) Effect of cadmium on the monolayer maintenance of vascular endothelial cells in culture. Toxicology 71:267-276

Kaji T, Mishima A, Koyanagi E, Yamamoto C, Sakamoto M, Kozuka H (1992b) Possible mechanism for zinc protection against cadmium cytotoxicity in cultured vascular endothelial cells. Toxicology 76:257-270

Kaji T, Fujiwara Y, Koyanagi E, Yamamoto C, Mishima A, Sakamoto M, Kozuka H (1992c) Protective effect of copper against cadmium cytotoxicity on cultured vascular endothelial cells. Toxicol Lett 63: 13-20

Kaji T, Yamamoto C, Tsubaki S, Ohkawara S, Sakamoto M, Sato M, Kozuka H (1993a) Metallothionein induction by cadmium, cytokines, thrombin and endothelin-1 in cultured vascular endothelial cells. Life Sci 53: 1185-1191

Kaji T, Mishima A, Yamamoto C, Sakamoto M, Kozuka H (1993b) Zinc protection against cadmium-induced destruction of the monolayer of cultured vascular endothelial cells. Toxicol Lett 66:247-255

Kaji T, Suzuki M, Yamamoto C, Imaki Y, Mishima A, Fujiwara Y, Sakamoto M, Kozuka H (1994) Induction of metallothionein synthesis by bismuth in cultured vascular endothelial cells. Res Commun Mol Pathol Pharmacol 86:25-35

Kaji T, Suzuki M, Yamamoto C, Mishima A, Sakamoto M, Kozuka H (1995) Severe damage of cultured vascular endothelial cell monolayer after simultaneous exposure to cadmium and lead. Arch Environ Contam Toxicol 28: 168-172

Kissane JM and Robins E (1958) The fluorometric measurement of deoxyribonucleic acid in animal tissue with special reference to the central nervous system. J Biol Chem 233: 184-188

Onosaka S, Tanaka K, Doi M, Okahara K (1978) A simplified procedure for determination of metallothionein in animal tissue. Eisei Kagaku 24: 128-131

Probst GS, Bousquet WF, Miya TS (1977) Correlation of hepatic metallothionein concentrations with acute cadmium toxicity in the mouse. Toxicol Appl Pharmacol 39:61-69

Revis NW, Zinsmeister AR, Bull R (1981) Atherosclerosis and hypertension induction by lead and cadmium ions: An effect prevented by calcium ion. Proc Natl Acad Sci USA 78:6494-6498

Schroeder HA, Vinton WH (1962) Hypertension induced in rats by small dose of cadmium. Am J Physiol 202:515-518