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EXOGENOUS GLUTATHIONE DECREASES CELLULAR CADMIUM UPTAKE AND TOXICITY

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ABSTRACT:

The effect of intracellular glutathione (GSH) on cadmium metabolism and toxicity has been extensively investigated. However, little is known regarding the effect of extracellular GSH on cellular cadmium responses. Therefore, this study was conducted to investigate the effect of exogenously added GSH on cadmium toxicity in normal rat kidney fibroblasts (NRK-49F).

Exponentially growing NRK-49F cells were arrested by serum deprivation and then stimulated with epidermal growth factor (EGF). $CdCl_2$, at concentrations that range from 0.25 to 2 μM, was found to inhibit, in a dose-dependent fashion, the EGF-induced DNA synthesis (as judged by [^{3}H]thymidine incorporation) in the cells. A long-term survival assay revealed that $CdCl_2$ above 1 μM was toxic to the cells. Exogenous GSH had a dose-dependent antagonistic effect on cadmium inhibition of EGF-induced DNA synthesis, and 1 mM GSH was found to block completely cadmium inhibition of both EGF-induced DNA synthesis and cell survival. Exogenously added GSH did not increase intracellular GSH levels but decreased cadmium accumulation by the cells. This decrease was primarily caused by a reduced cadmium uptake. Further studies indicated that exogenous GSH would form a complex with cadmium outside of the cells preventing cellular cadmium uptake. This may explain the mechanism of action of the exogenous GSH in cytoprotection against cadmium. The results also suggested a practical potential for GSH as a cadmium chelator. If GSH were coadministered with a cadmium mobilizer and a γ-glutamyl transpeptidase inhibitor, it could enhance cadmium excretion from the body.

GSH[1] is a tripeptide thiol present in mammalian cells in concentrations that range from about 0.1 to 10 mM. It participates in a number of important cellular processes, including protection of cells against the toxic effects of oxygen, radiation, and other compounds (1–3). That GSH is involved in cytoprotection against cadmium in cultured cells has been demonstrated with human lung carcinoma A549 cells (4). These cells are highly cadmium-resistant and concomitantly have high levels of intracellular GSH. Treatment of these cells with buthionine sulfoximine, a specific inhibitor of GSH synthesis (5), or with diethylmaleate, which conjugates with GSH, has been shown to deplete cellular GSH and to sensitize the cells to cadmium. Additional studies have indicated that GSH is involved in the early phase of the cadmium cytoprotective response (6). Results from other studies also suggested that not only cellular GSH content, but also some other aspects of GSH metabolic status may be related to cadmium cytoprotection (7).

Extracellular GSH released from the intracellular GSH pool is at low concentrations and functions in amino acid transport, ion permeation, and protection of the cell membrane against toxic effects (8, 9). Studies have shown that GSH forms complexes with zinc and cadmium *in vitro* (10). A GSH inward transport system has been demonstrated in intestinal epithelial cells (11), in intestinal brush-border membranes (12), in renal basal-lateral membrane (13), as well as in other systems (14–16). Such a transport system is absent in some other cultured cells (17, 18). Thus, it is hypothesized that exogenously added GSH would be maintained in the culture medium if the cells were lacking a GSH inward transport system. The extracellular GSH would also form a complex with cadmium outside of the cells preventing cadmium uptake by the cells. The decrease in cadmium uptake would in turn result in a decreased cadmium toxicity. Therefore, this study was undertaken to investigate the effects of exogenously added GSH on cadmium uptake by, and toxicity in, NRK-49F.

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[1] Abbreviations used are: GSH, glutathione; NRK-49F, normal rat kidney fibroblasts; DTNB, 5,5′-dithiobis (2-nitrobenzoate); EGF, epidermal growth factor; PBS, phosphate-buffered saline; TCA, trichloroacetic acid; CUBS, 10 mM HEPES:0.9% NaCl:10 mM EDTA; CFE, colony-forming efficiency.

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Materials and Methods

GSH, GSH disulfide reductase, NADPH, DTNB, and EGF were obtained from Sigma Chemical Co. (St. Louis, MO). Tritiated [^{3}H] thymidine and $^{109}CdCl_2$ were the products of Amersham Co. (Arlington Heights, IL) and New England Nuclear Co. (Boston, MA), respectively. McCoy's 5A medium was purchased from GIBCO (Grand Island, NY) and calf serum from HyClone (Logan, VT). ScintiVerse BD and Isoton II were from Fisher Scientific (Chicago, IL). All other chemicals were from either Sigma or Aldrich (Milwaukee, WI).

Cells and Cell Culture. NRK-49F, obtained from the American Type Culture Collection (ATCC CRL 1570), were routinely grown in McCoy's 5A medium supplemented with 10% calf serum and 2.2 g/liter of sodium bicarbonate at 37°C, and with a pH of 7.0–7.2 in a humidified atmosphere of 95% air and 5% CO_2. Stock cultures were passaged at 3-day intervals. Tests for mycoplasma, using a MycoTest kit (GIBCO), were negative. Cells were removed from monolayer stock cultures with trypsin-EDTA solution (0.05% trypsin:0.53 mM EDTA-4Na), counted with a Coulter counter, plated at about 21,000 cells/dish in 35 mm tissue culture dishes, incubated for 72 hr, and growth-arrested by incubation in McCoy's 5A medium containing 0.1% calf serum for 36 hr prior to experimental treatments.

Determinations of [^{3}H]Thymidine Incorporation into DNA. Growth-

arrested cells were stimulated with EGF at 10 ng/ml. Some cells were treated also with $CdCl_2$ or $CdCl_2$ and GSH simultaneously with EGF. Cultures were then pulse-labeled with 1 μCi/ml [^{3}H]thymidine between 10 and 14 hr posttreatment. At the end of the labeling period, the monolayer was washed 3 times with PBS, and the cells were detached with trypsin-EDTA solution. After suspension in PBS-bovine serum albumin, an aliquot was diluted with Isoton II for determination of cell number with a Coulter counter (model Z_{B1} Coulter Electronics). Because potential artifacts may be encountered in cell number counts with a Coulter counter (such as changes in cell size or volume caused by treatments or stage of cell growth and debris from lysed cells), the aperature current and amplification of the Coulter counter were adjusted to maintain average pulse height, an upper discriminator was not used, and values obtained were calibrated with hemocytometer measurements. The remainder of the cell suspension was mixed with 20% TCA to give a final TCA concentration of 10% and centrifuged after 10 min at 4°C. The precipitate was suspended with 10% TCA, centrifuged, and then suspended with 5% potassium acetate in ethanol. After centrifugation, the final pellet was dissolved in 0.1 M NaOH, aliquots of which were mixed with ScintiVerse BD and counted in a liquid scintillation spectrometer.

Measurement of Cadmium Accumulation. Serum-arrested cells were stimulated with EGF and exposed to 0.4 μCi $^{109}CdCl_2/10^{-6}$ mol $CdCl_2$ at different concentrations of $CdCl_2$ for 12 hr. Another protocol involves that the serum-arrested cells were stimulated with EGF for 10 hr under different conditions as described in the text and figure legend, and then the cells were exposed to $CdCl_2$ at a concentration of 0.5 μM for different time periods. The monolayer cells were then washed with CUBS (10 mM HEPES:0.9% NaCl:10 mM EDTA) 3 times, the cells were detached with trypsin-CUBS, and an aliquot of the cell suspension was counted with a Coulter counter. The remaining cells were collected by centrifugation, and ^{109}Cd taken up by the cells was determined by lysing the cells with 0.1 M NaOH and counting aliquots in a liquid scintillation spectrometer.

Determination of Total Intracellular Levels of GSH. The DTNB-GSH disulfide reductase recycling assay (19) was used to determine total cellular glutathione content (GSH + GSSG). The cells were trypsinized, rinsed with cold PBS, centrifuged at 4°C, resuspended in cold 5% sulfosalicylic acid, mixed vigorously, and recentrifuged. The supernatant was assayed for total GSH by measuring the color change of DTNB at 412 nm in the presence of GSH disulfide reductase and NADPH.

CFE. After the cells were simultaneously stimulated with EGF and treated with $CdCl_2$ for 12 hr, the monolayers were removed by trypsin-EDTA, and the cells were replated in medium containing 10% calf serum at about 600 cells/dish in 60 mm dishes. After 10 days incubation, the colonies formed were stained with crystal violet and counted with a Biotran III (New Brunswick) colony counter.

Statistical Analysis. Data were initially analyzed by ANOVA. Duncan's multiple-range test and the t test were used to analyze the data further. A factorial design (2 × 2) was used for the experiment presented in fig. 3. All of the experiments were repeated more than 2 times, and data were presented from one experiment, with triplicate samples per treatment.

Results

Exponentially growing NRK-49F were arrested (in G0) by serum deprivation. Cells were then stimulated by directly adding EGF into the monolayer cultures. EGF at a concentration of 10 ng/ml stimulated DNA synthesis in the serum-arrested NRK-49F cells, and the rate of DNA synthesis reached a maximum value about 12 hr post-EGF stimulation (as judged by [^{3}H] thymidine incorporation, data not shown). This result agrees with the previous observation (20). As shown in fig. 1, treatment of serum-arrested NRK-49F cells with $CdCl_2$ at the time that cells were stimulated with EGF resulted in a dose-dependent inhibition of DNA synthesis (estimated from [^{3}H]thymidine incorporation between 10 and 14 hr post-EGF stimulation). A

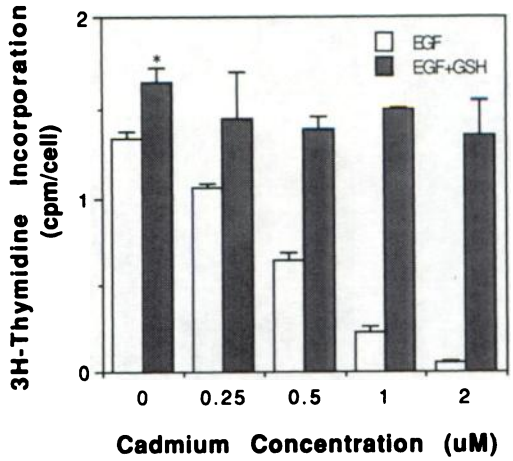

FIG. 1. *Inhibitory effect of $CdCl_2$ on EGF-induced [^{3}H]thymidine incorporation and modulation by exogenous GSH of this effect.*

Serum-arrested NRK-49F cells were stimulated with 10 ng/ml EGF, and $CdCl_2$ or $CdCl_2$ and 1 mM GSH was added to the cultures at the same time as EGF. Cells were harvested for [^{3}H]thymidine incorporation after 4 hr exposure to [^{3}H]thymidine between 10 and 14 hr post-EGF stimulation. Data represent mean ± SD from triplicate samples. Treatments with increasing cadmium concentration without GSH are very significantly different between each other ($p < 0.01$). Treatments with GSH are not significantly different ($p > 0.10$) between each other and from the control, but the one indicated by an *asterisk*, is significantly different from the control and other GSH treatment ($p < 0.05$).

$CdCl_2$ concentration of 0.5 μM represents the ED_{50} in these experiments. As also shown in fig. 1, exogenous GSH added into the monolayer cultures at a concentration of 1 mM completely blocked cadmium's inhibitory effect on EGF-induced DNA synthesis. Exogenously added GSH also enhanced EGF-stimulated DNA synthesis when added along with EGF (and without cadmium). A GSH dose-dependent effect on 0.5 μM $CdCl_2$ inhibition of EGF-induced DNA synthesis was shown in fig. 2. This is the basis for choosing 1 mM GSH in the rest of the study.

Below a concentration, usually in the submicromolar range, cadmium has a specific inhibitory effect on EGF-induced cellular responses (21, 22). Above this concentration, it may have an overt toxic effect on the cells. The cadmium cytotoxicity was thus determined with the NRK-49F cells. After the serum-arrested NRK-49F cells were stimulated with EGF and treated with varying concentrations of $CdCl_2$ for 12 hr, the medium was removed and the cells were replated in fresh medium containing 10% calf serum without cadmium to determine the effect of cadmium on CFE. Data shown in fig. 3 indicate that $CdCl_2$ at a concentration of 0.5 μM is not, as judged by the effect on CFE, toxic to the cells, and $CdCl_2$ at concentrations above 1 μM is toxic to the cells. It is also shown in fig. 3 that exogenous GSH of 1 mM, added with and maintained in the cultures for the same period of time as $CdCl_2$, completely protects cells against the toxic effects of cadmium for $CdCl_2$ concentrations used in this study.

GSH can be transported into some cells (11–16). It is not known whether GSH can be transported into the NRK-49F cells. Even if an intact GSH cannot be transported into the cells, exogenous GSH can be broken down and the products can be taken up by the cells. Therefore, cellular GSH may be increased through an enhanced synthesis. If intracellular GSH is increased by exogenously added GSH, the effect of GSH on cadmium

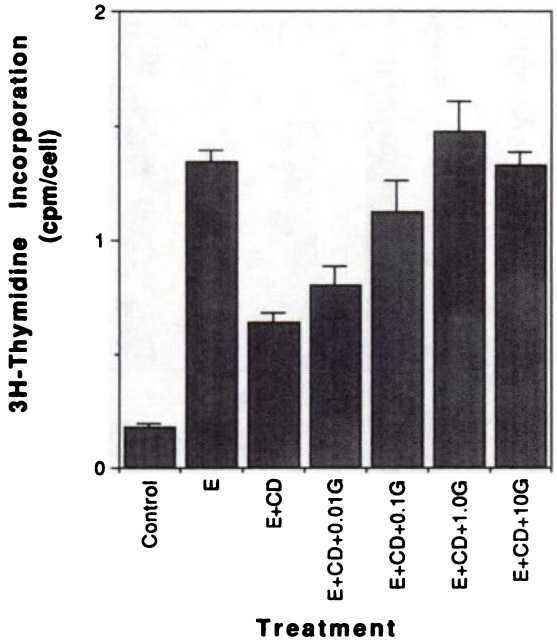

FIG. 2. *Dose-dependent effect of exogenous GSH on 0.5 μM $CdCl_2$ inhibition of EGF-induced [^{3}H]thymidine incorporation.*

Serum-arrested cells were stimulated with 10 ng/ml EGF (E). Some cells were treated with 0.5 μM $CdCl_2$ (CD) at the same time, or treated with 0.5 μM $CdCl_2$ and different concentrations of GSH (G, mM). Cells were then labeled with [^{3}H]thymidine for 4 hr between 10 and 14 hr post-EGF stimulation. Data represent mean ± SD from triplicate samples. All of the GSH and cadmium treatments are significantly different from the cadmium treatment alone and between each other ($p < 0.05$).

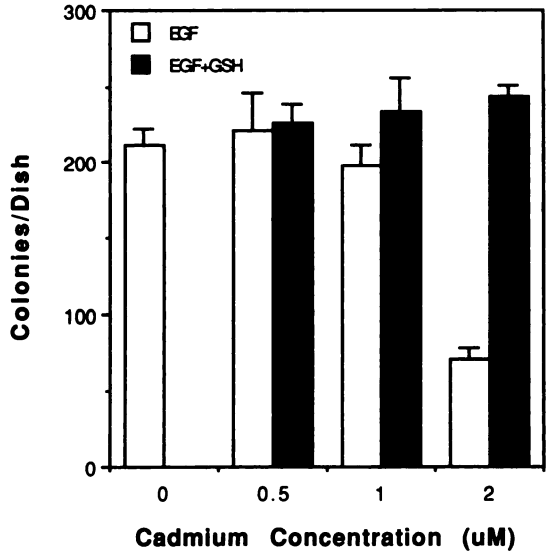

FIG. 3. *Effect of $CdCl_2$ or $CdCl_2$ and GSH on CFE in NRK-49F cells.*

Serum-arrested NRK-49F cells were stimulated with 10 ng/ml EGF and at the same time treated with $CdCl_2$ or $CdCl_2$ and 1 mM GSH for 12 hr. Cells were then replated in medium containing 10% calf serum but no $CdCl_2$ or GSH at about 600 cells/dish in 60 mm tissue culture dishes and cultured for 10 days for CFE determination. $CdCl_2$ at a concentration of 0.5 μM is not toxic ($p > 0.10$), and a toxic effect is evident at $CdCl_2$ concentrations above 1 μM ($p < 0.05$). There is no significant difference between the GSH-treated and EGF-stimulated controls ($p > 0.10$). Data presented are mean ± SD from triplicate samples.

toxicity would be related to its intracellular action rather than to its extracellular or cell membrane functions. However, as shown in fig. 4, neither GSH nor GSH plus EGF treatment increased intracellular GSH. In contrast, the combined treatment significantly decreased intracellular GSH levels ($p < 0.05$). The results suggest that GSH cannot be transported into the NRK-49F cells and that an enhanced synthesis of cellular GSH by exogenous GSH is absent in the NRK-49F cells. Thus, the added GSH probably acts at the extracellular level in cytoprotection against cadmium.

Determining the likely mechanism for the GSH antagonistic effect on cadmium cytotoxicity was approached by determining the effect of exogenously added GSH on cellular cadmium accumulation. The experiment involved exposing serum-arrested cells to $^{109}CdCl_2$ at different concentrations at the same time as the cells were stimulated with EGF. The ^{109}Cd accumulation by the cells was determined after 12 hr treatment. As shown in fig. 5, ^{109}Cd accumulation by the cells was $CdCl_2$ dose-dependent. Exogenous GSH, added at a concentration of 1 mM simultaneously with cadmium exposure, decreased cadmium accumulation by approximately 10-fold.

There may be two possible mechanisms by which exogenously added GSH affects cadmium accumulation by the cells. Exogenous GSH may directly act on membrane transport systems to decrease cadmium uptake by the cells. Alternatively, it may form a complex with cadmium in the culture medium to decrease the availability of free cadmium and therefore to prevent cadmium uptake by the cells. To investigate a possible mechanism responsible for the decreased cadmium accumulation, the serum-arrested cells were stimulated with EGF for 10 hr. $^{109}CdCl_2$ was

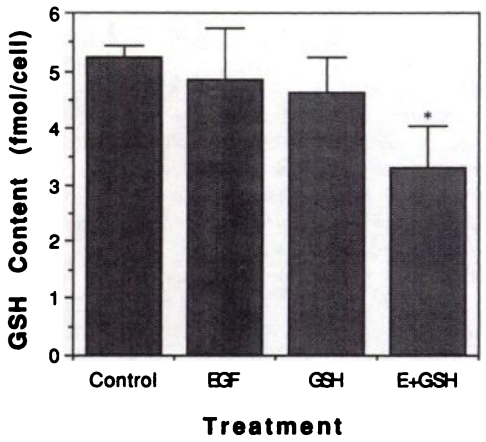

FIG. 4. *Effect of exogenous GSH on levels of intracellular GSH.*

Growth-arrested NRK-49F cells were stimulated with EGF and/or treated with GSH for 12 hr prior to harvesting for GSH determination. Data represent mean ± SD from triplicate samples. *Asterisk* indicates a significant difference from control ($p < 0.05$).

coincubated with exogenous GSH in the medium containing 0.1% calf serum, but no cells under the same condition as the cell cultures for 10 hr. Then, the conditioned medium was used to replace the monolayer culture medium to measure cadmium uptake. An additional protocol involved adding the exogenous GSH into cell cultures simultaneously with EGF for 10 hr. At the same time, $^{109}CdCl_2$ was incubated in medium containing 0.1% calf serum and EGF but no cells. The GSH-containing monolayer culture medium was then replaced by the $^{109}CdCl_2$-

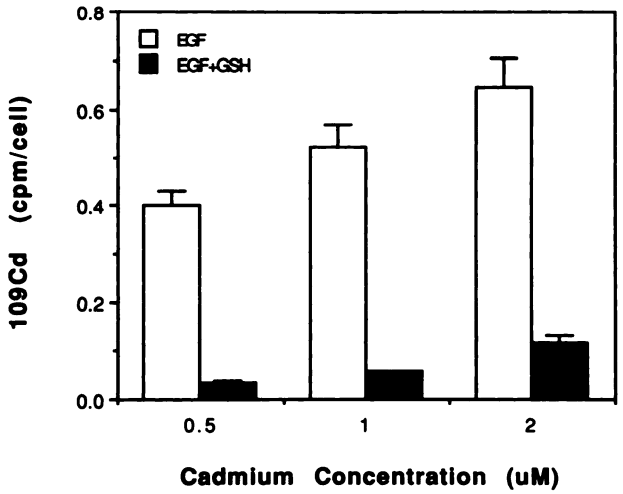

FIG. 5. *Effect of exogenous GSH on ^{109}Cd accumulation.*

At the time of EGF stimulation, NRK-49F cells were exposed to $^{109}CdCl_2$ at 0.4 μCi/10^{-6} mol $CdCl_2$. After 12 hr exposure, the monolayer was washed with CUBS for 3 times and detached with CUBS-trypsin. After centrifugation, one wash with CUBS, and recentrifugation, the pellet was dissolved with 0.1 M NaOH, aliquots of which were mixed with ScientiVerse for radioactivity determination with a liquid scintillation spectrometer. Data are mean ± SD from triplicate samples. A significant dose-dependent cadmium accumulation is observed ($p < 0.05$). Treatment with GSH very significantly decreased cadmium accumulation ($p < 0.01$).

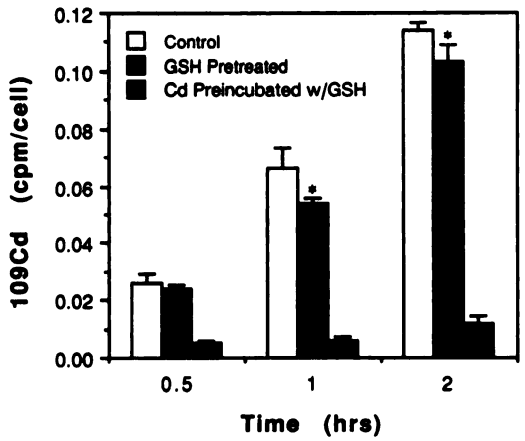

FIG. 6. *Effect of GSH on ^{109}Cd uptake.*

NRK-49F cells were exposed to 0.5 μM $CdCl_2$ at 0.4 μCi $^{109}Cd/10^{-6}$ mol $CdCl_2$ in three different ways: *1*) Cells were stimulated with EGF for 10 hr and then exposed to ^{109}Cd in fresh medium (control); *2*) cells were exposed to EGF in the presence of 1 mM GSH for 10 hr and then exposed to ^{109}Cd in fresh medium without GSH (GSH pretreated); and *3*) after 10 hr EGF stimulation, cells were exposed to ^{109}Cd, which is preincubated with 1 mM GSH for 10 hr (Cd preincubated with GSH). All data presented are mean ± SD from triplicate samples. *Asterisk* indicates a significant difference from other treatments ($p < 0.05$). Treatment of "Cd preincubated w/GSH" is very significantly different from other treatments ($p < 0.01$) and significantly different between each other ($p < 0.05$).

containing medium to measure cadmium uptake. The results presented in fig. 6 show that, although the exogenous GSH pretreatment alone had a significant effect ($p < 0.05$) on cadmium uptake, there was a dramatic decrease in cadmium uptake by the cells, under the condition of which the cells were exposed to the $^{109}CdCl_2$ in the GSH coincubated conditioned medium.

Discussion

Metabolism and functions of intracellular GSH related to protection against toxic agents, oxygen, radiation, and cadmium have been extensively studied. Conclusions drawn from these studies to date indicate the importance of intracellular levels of GSH in cytoprotection. However, little is known about metabolism and functions of extracellular GSH. Exogenous GSH has been added into the cell culture system and shown to protect cells from oxidative injury (11). This effect is primarily ascribed to the increased levels of intracellular GSH caused by a GSH inward transport. In this study, it was clearly shown that exogenously added GSH protected NRK-49F cells against cadmium. This protective process occurred at extracellular level rather than in the cytoplasm.

A GSH inward transport system has been demonstrated in other systems (11–16). However, results obtained from this study indicate that there may not be a GSH inward transport system existing in the NRK-49F cells. It was also shown that exogenously added GSH did not increase intracellular GSH levels through other mechanisms in the NRK-49F cells. This observation led to the study of the mechanism of action of exogenous GSH in protection against cadmium at the extracellular level.

GSH may have a direct effect on cell membrane (e.g., by reduction of cystine or some other disulfides to modulate cell membrane functions) (9). GSH also forms a complex with cadmium (10). The GSH-cadmium complex would prevent cadmium uptake by the cells. The results presented in this study strongly agree with this hypothesis. However, it cannot be excluded that GSH may also affect cell membrane transport system *per se* and thus decrease cadmium uptake by the cells. A significant effect of exogenous GSH pretreatment on ^{109}Cd uptake was observed, although this effect was not to the extent as that caused by the other treatment (fig. 6). The experiment (fig. 6) performed here was to preincubate the cells with exogenous GSH, and at the time of cadmium exposure, the GSH was removed from the culture medium. Some other reducing agents that can reduce cystine or some other disulfide compounds, but do not form a complex with cadmium, need to be screened to investigate a possible effect of reducing agents on cellular cadmium uptake.

This study also indicates that the GSH-cadmium complex is less or essentially nontoxic to the NRK-49F cells. This, together with the fact that the complex may not be transported into the cells, suggests a potential for GSH application as a cadmium chelator. There is, to date, no effective chelator available for cadmium intoxication. Recent studies (23) have suggested that synthesized esters of meso-2,3-dimercaptosuccinic acid, such as di(2′-methoxyethyl)meso-2,3-dimercaptosuccinate and di(2′-ethoxyethyl)meso-2,3-dimercaptosuccinate, mobilize intracellular cadmium and enhanced the fecal excretion of cadmium, but not the urinary excretion of this element. Exogenous GSH would form a complex with cadmium mobilized by the cadmium mobilizers if GSH were coadministrated. Because a GSH-cadmium complex would not be transported into the cells, the combined treatment would further enhance cadmium excretion. Kidney cells, however, have a high level of γ-glutamyl transpeptidase activity. Through the enzyme action, the GSH-mercury complex has been shown to be transported into the cells (24). This reaction may occur with the GSH-cadmium complex. This obstacle could be overcome by using an inhibitor of γ-glutamyl transpeptidase, such as acivicin. Therefore, an enhanced urinary, as well as fecal excretion of cadmium would be observed.

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References

1. B. A. Arrick, C. F. Nathan, O. W. Griffith, and Z. A. Cohn: Glutathione depletion sensitized tumor cells to oxidative cytolysis. *J. Biol. Chem.* **257**, 1231–1237 (1982).
2. J. E. Biaglow, M. E. Varnes, E. P. Clark, and E. R. Epp: The role of thiols in cellular response to radiation and drugs. *Radiat. Res.* **95**, 437–455 (1983).
3. T. R. Crook, R. L. Souhami, G. D. Whyman, and A. E. M. McLean: Glutathione depletion as a determinant of sensitivity of human leukemia cells to cyclophosphamide. *Cancer Res.* **46**, 5035–5038 (1986).
4. Y. J. Kang and M. D. Enger: Effect of cellular glutathione depletion on cadmium-induced cytotoxicity in human lung carcinoma cells. *Cell Biol. Toxicol.* **3**, 347–360 (1987).
5. O. W. Griffith and A. Meister: Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (*s-n*-butyl homocysteine sulfoximine). *J. Biol. Chem.* **254**, 7558–7560 (1979).
6. Y. J. Kang and M. D. Enger: Glutathione is involved in the early cadmium cytotoxic response in human lung carcinoma cells. *Toxicology* **48**, 93–101 (1988).
7. Y. J. Kang, S. T. Nuutero, J. A. Clapper, P. Jenkins, and M. D. Enger: Cellular cadmium responses in subpopulations T20 and T27 of human lung carcinoma A549 cells. *Toxicology* **61**, 195–203 (1990).
8. H. Sies, A. Wahllander, C. Waydhas, S. Soboll, and D. Haberle: Functions of intracellular glutathione in hepatic hydroperoxide and drug metabolism and the role of extracellular glutathione. *Adv. Enzyme Regul.* **18**, 303–320 (1980).
9. N. S. Kosower and E. M. Kosower: The glutathione status of cells. *Int. Rev. Cytol.* **54**, 109–160 (1978).
10. D. D. Perrin and A. E. Watt: Complex formation of zinc and cadmium with glutathione. *Biochim. Biophys. Acta* **230**, 96–104 (1971).
11. L. H. Lash, T. M. Hagen, and D. P. Jones: Exogenous glutathione protects intestinal epithelial cells from oxidative injury. *Proc. Natl. Acad. Sci. USA* **83**, 4641–4645 (1986).
12. M. T. Vincenzini, T. Iantomasi, and F. Favilli: Glutathione transport across intestinal brush-border membranes: effects of ions, pH. $\Delta\psi$, and inhibitors. *Biochim. Biophys. Acta* **987**, 29–37 (1989).
13. L. H. Lash and D. P. Jones: Renal glutathione transport characteristics of the sodium-dependent system in the basal-lateral membrane. *J. Biol. Chem.* **259**, 14508–14514 (1984).
14. R. G. Schnellmann: Renal mitochondrial glutathione transport. *Life Sci.* **49**, 393–398 (1991).
15. T. B. McKernan, E. B. Woods, and L. H. Lash: Uptake of glutathione by renal cortical mitochondria. *Arch. Biochem. Biophys.* **288**, 653–663 (1991).
16. T. M. Hagen and D. P. Jones: Transport of glutathione in intestine, kidney, and lung. *Adv. Biosci.* **65**, 107–114 (1987).
17. S. M. Deneke and B. L. Fanburg: Regulation of cellular glutathione. *Am. J. Physiol.* **257** (*Lung Cell. Mol. Physiol.* 1), L163–L173 (1989).
18. G. L. Jensen and A. Meister: Radioprotection of human lymphoid cells by exogenously supplied glutathione is mediated by γ-glutamyl transpeptidase. *Proc. Natl. Acad. Sci. USA* **80**, 4714–4717 (1983).
19. F. Tietze: Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: application to mammalian blood and other tissues. *Anal. Biochem.* **57**, 502–522 (1969).
20. Y. J. Kang and M. D. Enger: Increased glutathione levels in quiescent, serum-stimulated NRK-49F cells are associated not with a response to growth factors but with nutrient repletion. *J. Cell. Physiol.* **148**, 197–201 (1991).
21. M. D. Enger, F. A. Flomerfelt, P. L. Wall, and P. Jenkins: Cadmium produces a delayed mitogenic response and modulates the EGF response in quiescent NRK cells. *Cell Biol. Toxicol.* **3**, 407–416 (1987).
22. Y. J. Kang and M. D. Enger: Cadmium inhibits EGF-induced DNA synthesis but increases cellular glutathione levels in NRK-49F cells. *Toxicology* **66**, 325–333 (1991).
23. M. M. Jones, P. K. Singh, G. R. Gale, L. M. Atkins, and A. B. Smith: The mobilization of intracellular cadmium by alkoxyethyl esters of meso-2,3-dimercaptosuccinic acid, *Toxicol. Appl. Pharmacol.* **106**, 529–535 (1990).
24. T. Tanaka, A. Naganuma, and N. Imura: Role of gamma-glutamyltranspeptidase in renal uptake and toxicity of inorganic mercury in mice. *Toxicology* **60**, 1897–198 (1990).