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Mechanism of Cytotoxicity of Methylmercury

With Special Reference to Microtubule Disruption

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ABSTRACT

Mechanism of methylmercury cytotoxicity was investigated with special reference to its preferential action on microtubules and protein biosynthesis in cultured cells. The tubulin synthesis analyzed by autoradiography of two-dimensional electropherogram using ^{35}S-methionine was inhibited by 50–70% in mouse glioma cells exposed to 5×10^{-6} *M* methylmercury for 3 h, which almost completely depolymerized microtubules. Total protein synthesis monitored by incorporation of labeled methionine into acid insoluble fraction was decreased slightly but significantly and the protein bands other than tubulin on gradient urea-PAGE gel appeared to remain unchanged under the experimental condition used. These results suggest that the inhibition of protein synthesis observed on exposure to methylmercury can be ascribed, at least partly, to a possible autoregulatory depression in tubulin synthesis owing to the increase in the pool of tubulin subunits resulted from microtubule depolymerization by methylmercury.

Index Entries: Methylmercury cytotoxicity; microtubule disruption; tubulin biosynthesis; autoregulatory depression.

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INTRODUCTION

In the studies concerning the toxic action of methylmercury at the cellular and subcellular levels, a number of researchers have been interested in its adverse effects on macromolecule biosynthesis, cell mitosis, structural and functional components of cell membranes, and functions of neuronal cells (*1*). We have demonstrated that microtubules in cultured mouse glioma cells were specifically disrupted by methylmercury (*2–4*). Similar results have been obtained with some other cultured cells (*5–8*), suggesting that this cytoskeleton is a common and highly sensitive organelle on methylmercury exposure. In addition, it was confirmed by in vivo experiments that mitotic activity in the brains of mouse exposed to methylmercury *in utero* was selectively disturbed (*9,10*). On the other hand, protein biosynthesis has also been considered as one of the most sensitive targets for this metal alkyl.

In this paper, we demonstrate that the disruption of microtubules by methylmercury leads to an inhibition of new tubulin biosynthesis. This fact may, at least partly, account for the inhibition of protein biosynthesis in the cells exposed to methylmercury.

MATERIALS AND METHODS

Cell Culture and Labeling

Mouse glioma, SR-CDF$_1$DBT, was grown as monolayer cultures in Eagle's minimum essential medium supplemented with 10% fetal calf serum (GIBCO), as described previously (*2*). Subconfluent cultures were treated with various concentrations of methylmercuric chloride (CH_3HgCl) or colchicine (10^{-5} *M*) for 1,2,3, and 6 h.

The cells were pulse-labeled with 100 μCi/200 μL ^{35}S-methionine (Amersham) for 15 min in methionine-deficient medium containing 10% dialyzed fetal calf serum. After washing with phosphate buffered saline (pH 7.2), cells were lysed by the addition of a buffer (25 m*M* Tris-HCl pH 7.4) containing 0.4 *M* NaCl, 0.1% deoxycholate, 1% NP 40, and 0.5% SDS. Labeled cell extracts were prepared according to the methods described by Cleveland et al. (*11*).

Incorporation of ^{35}S-methionine into protein was monitored by measuring trichloroacetic acid (TCA)-precipitable counts according to the methods of Cleveland et al. (*11*).

Gel Electrophoresis

The pulse-labeled proteins from the monolayer cultures were analyzed by the two-dimensional gel electrophoresis technique described by O'Farrell (*12*). The pH gradient was 3.5–10. The samples were run on a

10% acrylamide slab gel in the second dimension. For detection of labeled proteins, the gels were allowed to expose Kodak X-ray film (XAR) at −80°C. Quantitation of radioactivity in individual spots of gels was performed with a scanning densitometry (SIMADZU, CS-9000). On the autoradiogram thus obtained the spot of α-tubulin was apparently overlapped by that of 58 K intermediate filament protein. Therefore, the spot of β-tubulin was used for quantitative determination of tubulin synthesis.

RESULTS AND DISCUSSION

Growth of mouse glioma cells were completely inhibited by 5×10^{-6} *M* methylmercury, as described previously (*2*). Indirect immunofluorescence study showed that microtubules disappeared completely within 1 h in the cells treated with 5×10^{-6} *M* methylmercury (*3,4*). The autoradiography subsequent to two-dimensional electrophoresis of the cytoplasmic proteins labeled with ^{35}S-methionine demonstrated that the labeling of β-tubulin was markedly reduced in the methylmercury treated cells compared with that in control cells. Extent of inhibition of β-tubulin synthesis measured by scanning densitometry was 50–70% as in the case of colchicine treatment (10^{-5} *M* for 3 h). Protein bands other than tubulin on gradient urea(1–4 *M*)-PAGE(4–16%) gel remained unchanged for 3 h in the cells treated with 5×10^{-6} *M* methylmercury, whereas marked decrease in labeling of various proteins was observed in the cells treated with 10^{-5} *M* methylmercury for 3 h. These results were well consistent with those obtained by the methods of immunoprecipitation of labeled β-tubulin. Total protein synthesis monitored by measuring TCA-prcipitable counts was slightly inhibited in the cells treated with 5×10^{-6} *M* methylmercury for 3 h. In neuroblastoma cells, a similar specific inhibition of tubulin synthesis by methylmercury was observed, as in glioma cells.

These results indicate that the disruption of microtubules by methylmercury leads to an inhibition of tubulin biosynthesis through the autoregulatory depression by increase in tubulin pool owing to microtubule depolymerization with methylmercury as reported by Ben-Ze'ev et al. using colchicine (*13*). This fact suggests that the inhibition of protein synthesis by methylmercury, which has been proposed as an important site in the methylmercury-induced cytotoxicity (*1*), can be ascribed to its specific depolymerizing effect on microtubules. And these results seem to be consistent with the fact obtained by the kinetic analysis of methylmercury-induced cell cycle disruption in CHO cells that the protein synthesis and mitosis are the most sensitive sites for methylmercury cytotoxicity (*14*).

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