Induction of Apoptosis by Mercuric Chloride in Rats

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Introduction
Toxicant-induced apoptosis has been demonstrated for toxic metals, such as cadmium, mercury, lead, and chromium in cultured cells. We have found that exposure of rats to mercuric chloride (HgCl₂) causes apoptosis in kidney. In the present study, inductions of apoptosis in various tissues of HgCl₂-treated rats were examined to clarify tissue difference in mercury-promoted apoptosis.

Methods
Animal Treatments — HgCl₂ was administered to male Wistar rats by s.c. injection at a dose of 4 mg/kg. The rats were killed by with drawing blood from heart under ether anesthesia at various times for collection of kidney, liver, thymus, spleen, and cerebellum samples.

Evaluation of Apoptosis — Fragmented DNA was quantitated as its derivatives with diphenylamine at 600 nm. DNA ladders were visualized on agarose gel electrophoresis.

Measurements of Mercury Level in Tissues — Samples (200 mg) were digested with nitric acid, potassium permanganate, and sulfuric acid at room temperature. Then, mercury concentrations were measured with stannous chloride as the reductant by flameless atomic absorption spectrometry.

Results and Discussion
Mercury levels in kidney reached a maximum at 7 h and then declined gradually, while mercury concentrations in liver, spleen, thymus, and cerebellum increased with time for 48 h from injection. Mercury levels in these tissues were extremely lower than those in kidney (cerebellum, 0.48%; thymus, 8.2%; spleen, 18%; liver, 36% of renal Hg concentration). A marked increase of DNA fragmentation was found in kidney at 16 h after administration (12 fold the control level), but not in cerebellum. In spleen, DNA fragmentation was significantly increased at 7 h after administration (1.5 fold the control level). The alteration in the apoptotic phenomenon was also detected in thymus and liver, but not significantly. According to the electrophoretic DNA analysis, increase in DNA ladders which are cleaved into oligonucleosome-size fragments were confirmed in liver, thymus, and spleen. While the ratio of increment for DNA fragmentation to mercury accumulation of kidney was close to that of liver and spleen in spite of its higher accumulation of mercury, the ratio of thymus was 10 fold-higher than that of the other tissues. Thus, the present results suggested that there are differential sensitivities to mercury in the induction of apoptosis among the tissues examined of HgCl₂-treated rats.

References