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Effect of mercuric chloride on cultured rat fibroblasts: survival, protein biosynthesis and binding of mercury to chromatin

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Exposure of animals to mercury leads to the accumulation of this metal in the cell nuclei [1]. The affinity of mercury for the nuclear material is high, its amount in the nuclei being considerable [2–4]. It has been established that mercury binds largely to the chromatin [3, 5–7], and the non-histone chromatin proteins are mainly responsible for this binding [4, 5]. Mercury studies on whole animals are difficult to interpret due to significantly different responses from tissue to tissue. Thus work on *in vitro* cell systems may reveal, more specifically, some of the cellular effects of this metal. However, little is known about the nature of mercury interaction with chromatin in cultured cells, except for a high incidence of chromosome aberrations induced by mercury in human lymphocytes [8], depression of DNA synthesis in mouse leukaemic [9] and HeLa cells [10], as well as reduction of rate of DNA replication in Chinese hamster ovary cells [11]. Experimental data on the binding of mercury by chromatin of cultured cells have been lacking until now.

This paper describes the effect of mercuric chloride on the survival of cultured fibroblasts and on the extent of mercury binding by the fibroblasts' chromatin.

Materials and methods

Experiments were performed on embryonic fibroblasts of Wistar rats. The fibroblasts were grown in a medium consisting of 75% MEM (Eagle's minimum essential medium) and 15% RTN (Hanks solution + 0.5% lactalbumin hydrolysate + 2% calf serum) supplemented with phenol red (for control of pH changes from the initial 7.2 to final 6.8), 10% fetal calf serum, 100 U penicillin/ml and 100 μg streptomycin/ml [12]. The fibroblasts were taken from skin tissue of 14–16-day old rat embryos subjected to digestion with 0.25% trypsin solution. Primary monolayer fibroblast culture was set up in rubber-stoppered 1-l. Legroux bottles at a concentration of 10^5 cells/ml and maintained for 6 days at 37°, changing the culture medium (100 ml) after every 3 days of incubation. The cultured cells were routinely passaged after 10–15 min trypsinization at 37°. Cell concentrations were determined using a haemocytometer, and cell viability was tested with 0.5% trypan blue. In the control cultures viability was found to be 92–96%. Cells after the third passage were used for experiments.

The effect of mercury on the survival of the cultured fibroblasts was studied by exposing them to a medium containing mercuric chloride in a concentration range from 5×10^{-7} to 1×10^{-4} M. Trypsinized cells passaged 2–3 times were used (amount: of 2×10^6 cells/bottle). Following 24-hr incubation, the culture medium was replaced by a fresh portion of medium containing mercuric chloride, and incubation was continued for another 24 hr. The cells were then suspended in Eagle's MEM medium and their concentration and viability were determined.

Incorporation of [^{14}C]leucine into proteins of the fibroblasts incubated in the presence of 10^{-6} and 10^{-5} M mercuric chloride was investigated in the monolayer cultures (2×10^6 cells). Following 23-hr incubation, [^{14}C]leucine (1.48 MBq) was added and incubation was continued for 1 hr (DL-[^{14}C]leucine; product of Institute of Nuclear Research, Warsaw, Poland; specific activity 389 MBq/mmole). After trypsinization and removal of non-incorporated labelled leucine by washing with culture medium, the cells were counted and monitored for viability, protein content and incorporation of radioactivity into acid-insoluble material after treatment of cell suspension with 10% trichloroacetic acid (1:1, v/v). The acid-insoluble precipitate was washed three times with trichloroacetic acid and dissolved in 1 ml NCS (Radiochemical Centre, Amersham, U.K.). The sample was neutralized with 0.1 ml of glacial

acetic acid, and 10 ml of scintillation liquid [13] containing 30% Triton X-100 was added. Radioactivity was measured in a scintillation counter (Wallac 81000, LKB).

The extent of mercury binding by cell nuclei and chromatin was determined after 24-hr incubation of the fibroblasts with $^{203}HgCl_2$(Rotop, G.D.R.; specific activity 13.9 GBq/g Hg) at concentrations of 10^{-6} and 10^{-5} M. The cell nuclei were isolated according to Mills and Means [14], using centrifugation through a solution of 2 M saccharose, 1 mM $CaCl_2$, 0.1 mM phenylmethane-sulfonyl fluoride (PMSF), 50 mM Tris–HCl, pH 7.5. The nuclei were examined for purity by methyl pyronine green staining. Chromatin was prepared by lysis of the nuclei with a solution of 0.25 M EDTA-Na_2, 0.08 M NaCl, pH 8.0, in the presence of 0.1 mM PMSF and washing with diluted 1:100 SSC (0.14 M NaCl, 0.014 M sodium citrate, pH 7.0). The analysis of ultraviolet absorption spectra in the range of 200–350 nm revealed a high purity of chromatin preparations.

The mercury level in the cells, cell nuclei and chromatin was determined radiometrically ($^{203}HgCl_2$) in a gamma spectrometer (Standard-70, Polon, Poland).

Protein was determined by the method of Lowry *et al.* [15].

Results and discussion

The survival curve of fibroblasts cultured in media containing increasing concentrations of mercury is biphasic (Fig. 1). Concentrations of mercuric chloride corresponding to the 'steep' portion of the curve (above 2.5×10^{-6} M) are critical for vital functions of the cells. Above 7.5×10^{-6} M $HgCl_2$, a considerable increase in the amount of fibroblasts detached from the vessel bottom is already observed, which seems to indicate cell damage. A similar pattern of the survival curve was obtained by Nakazawa *et al.* [9] for mouse lymphoma cells incubated with methyl mercuric chloride, and by Kasschau *et al.* [11] for Chinese hamster ovary cells treated with mercuric chloride. These authors found concentrations of 2.2×10^{-6} M in the case of methyl mercuric chloride and 4.5×10^{-5} M in the case of mercuric chloride to be critical for cell survival. The difference in the value of the critical mercuric chloride concentrations found by us for fibroblasts and that reported for Chinese hamster ovary cells may be due to the varying sensitivity of the investigated cells to mercury [16], and/or a protective effect of the plasma proteins present at higher concentration in the medium used by Kasschau *et al.* [11].

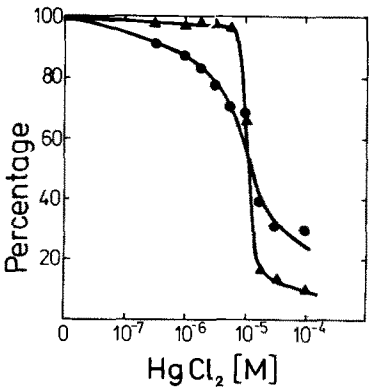

Fig. 1. Survival of the cultured fibroblasts after 24-hr incubation with mercuric chloride. Viable cells (●——●) and cells attached to the vessel bottom (▲——▲) as a percentage of cell number.

Table 1 shows the content of mercury in fibroblasts incubated with $HgCl_2$ at concentrations of 10^{-6} M (at which the cells were viable) and 10^{-5} M (at which about one third of the cells were dead). Culturing fibroblasts in a medium containing $HgCl_2$ at both chosen concentrations did not induce changes in the cellular protein content. It is noteworthy that cells cultured in the medium containing 10^{-5} M $HgCl_2$ bound only five times more metal than those from the culture containing ten times lower $HgCl_2$ concentration (10^{-6} M).

The intracellular distribution profile of mercury was different for both concentrations of mercuric chloride employed in the medium (Table 1). At 10^{-6} M $HgCl_2$, most of the metal was contained in the nuclear fraction (*ca* 70%) and in the chromatin (*ca* 50%). The metal-binding coefficient (μg Hg/mg protein) was five to six times higher for the cell nuclei and the chromatin than for the total cell homogenates. A similar intracellular distribution profile of mercury was found previously in *in vivo* studies for the liver and kidneys of rats exposed to mercuric chloride [4]. In spite of employment of the ten times higher $HgCl_2$ concentration, the mercury-binding coefficient for the chromatin was only slightly elevated whereas the metal content in the extranuclear fractions increased abruptly. Consequently, the relative contribution of the nuclear fraction and the chromatin to the binding of mercury by whole cells decreased for fibroblasts cultured in the presence of 10^{-5} M $HgCl_2$ compared with those cultured with 10^{-6} M $HgCl_2$.

Mercuric chloride at a concentration of 10^{-6} M induced a small (18%) decrease in the rate of protein biosynthesis in the fibroblasts, as estimated from [^{14}C]leucine incorporation (Table 2). However, at the higher concentration of mercury (10^{-5} M) and about two-thirds survival only, no changes were found in the amount of the incorporated label in comparison with control cells. Considering the viable cells in the culture only, it can be concluded that at this $HgCl_2$ concentration the rate of protein biosynthesis was augmented. The limited scope of the present study precludes the elucidation of this phenomenon. Recent data suggest metabolic effects of mercury compounds leading to a suppression of cellular protein biosynthesis [10, 16, 17]. However, stimulation of protein biosynthesis for some pro-

Table 1. Binding of mercury by the cultured fibroblasts, cell nuclei and chromatin after 24-hr incubation with $^{203}HgCl_2$ at concentration of 10^{-6} M (A) and 10^{-5} M (B)

	A		B	
	μg Hg / mg protein	% Hg	μg Hg / mg protein	% Hg
Fibroblast homogenate	0.09 ± 0.01	100	0.54 ± 0.06	100
Cell nuclei	0.55 ± 0.08	72	0.56 ± 0.07	22
Chromatin	0.61 ± 0.09	51	0.74 ± 0.09	16

Average values of eight independent experiments ± S.D. Intact fibroblasts in culture contained, on average, 0.07 and 0.38 μg Hg/10^6 cells for cultures incubated with 10^{-6} and 10^{-5} M $HgCl_2$, respectively. Cellular protein content was the same in both cases —0.76 mg per 10^6 cells.

Table 2. Effect of mercuric chloride on [^{14}C]leucine incorporation into proteins of cultured fibroblasts

Fibroblast culture	Viable cells (%)	Cpm/10^6 cells	% of control
Control	100	8088 ± 230	100
With 10^{-6} M $HgCl_2$	96	6636 ± 422	82
With 10^{-5} M $HgCl_2$	61	8042 ± 910	99

Means of five determinations ± S.D.

tein(s) and for certain range(s) of mercury concentrations cannot be excluded. For instance, Bonewitz and Howell [18] demonstrated that Zn and Cu at a concentration of 10 μg/ml medium lead to a stimulation of the synthesis of metallothionein-like proteins in cultured human skin fibroblasts. We observed [7] that the biosynthetic activity of metallothionein-like proteins in kidneys and liver of rats exposed to mercuric chloride at a dose of 5 mg Hg/kg body weight was higher than in the case of a dose of 1 mg $HgCl_2$.

In conclusion, the similarity of the intracellular distribution profile of mercury binding found in this study for cultured fibroblasts and that observed previously in the *in vivo* experiments suggests that cultured fibroblasts may be a useful model for further investigations on the interaction of mercury with chromatin.

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