Toxicity of Mercury to Hybridoma TA7 Cells

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Summary — Environmental mercury and mercury compound contamination has increased dramatically since the industrial revolution. This paper describes the toxic effects of mercury on a culture of hybridoma TA7 cells, which produce antibodies against the A-subunit of viskumin. Cells were cultivated on 96-well flat-bottomed plates with RPMI-1640 medium supplemented with 10% fetal calf serum at 37°C in 5% CO_2/95% air. The cells were exposed to 0.1nM/l–10μM/l $Hg_2(NO_3)_2.2H_2O$ (mercury nitrate) during the exponential growth phase. Toxicity was assessed by using the colorimetric MTT (tetrazolium) assay after exposure for 48 hours. Cell growth and cell survival were evaluated by using percentage indices of cellular content in exposed cells when compared to non-exposed control cells. The concentrations of the no-effect level, the lowest observed effect level and the the highest toxic effect level were registered. The toxic effects of the mercury compound on the hybridoma cells occurred between 0.1μM/l and 10μM/l.

Key words: mercury, hybridoma TA7 cells, toxicity, dose-effect.

Introduction

The existence of mercury has been known for a very long time. It is now widely used in many ways in industry and in medicine. Mercury exists in three forms: elemental mercury (Hg^0), inorganic mercury (Hg^+, Hg^{2+}), and organic mercury (CH_3Hg^+). The sources, pharmacokinetics and biological effects of these forms can vary. Elemental and inorganic mercury toxicity can result from industrial exposure, i.e. gold extraction processes (1), commercial application in scientific instruments and equipment (2), and production of caustic soda and button (disc) batteries (3). Organic mercury toxicity poisoning can result from methyl mercury pesticides (4), the burning of coal and other fossil fuels (5), or mining, smelting and refining processes. Mercury and mercury compounds can be washed from solid waste into lakes and streams, where micro-organisms transform them into their inorganic form, methyl mercury. This is then consumed and concentrated up the food chain, ultimately poisoning birds and mammals — including humans — that eat, for example, poisoned fish (6). Mercury pollution has now reached crisis proportions, especially in developed countries (7).

The main pathway of chronic mercury poisoning in Latvia is via the mouth and respiratory system. Toxic mercury vapours are released by its alloys (amalgams). Amalgams of tin and silver are widely used in dentistry (8). According to data provided by the Statistical Board of Latvia, 1794 medical workers were employed in dentistry in 1998 (9). Of these, 55% used unprotected composite material from amalgams, and were therefore exposed to the effects of mercury vapours. Physicians also administer mercury, for example, as anti-syphilitic remedies and mercurial diuretics, to treat medical problems (10, 11).

Mercury is absorbed in varying amounts when it is taken into the human body via air, water or food. It accumulates in the liver, kidneys, brain and blood, and can cause both acute and chronic health prob-

lems, depending on the form of mercury involved (11).

The Multicenter Evaluation of *In Vitro* Cytotoxicity (MEIC) programme was set up to evaluate the relevance of *in vitro* cytotoxicity tests in predicting acute human systemic toxicity. By the end of the programme in 1996, 29 laboratories had tested 50 chemicals, including mercury chloride, in 61 cytotoxicity assays (12). The toxicity of mercury chloride was evaluated *in vitro* by using a variety of cell cultures, including cultures of keratinocytes, hepatocytes and neurons (13).

The aim of this study was to assess the *in vitro* toxicity of mercury by using a hybridoma cell culture, which had never previously been used for the evaluation of mercury compound toxicity, even though hybridoma technology has existed since 1975 (14). This model could solve many of the problems encountered in immunology and medicine in assessing the many factors which can harm the human body.

Materials and Methods

An established hybridoma cell line, TA7, was used. TA7 is derived from the hybridisation of myeloma Sp2/0 cells with immune spleen lymphocytes from Balb/c mice (Stolbovaja arboretum, Russia), and produces anitbodies against the A-subunit of viskulin (15). Hybridomas were clonally transformed by using the limit dissolution method. Hybridoma TA7 cells have been used for experimental oncology research at the Moscow State University. Our colleagues there kindly donated the hybridomas for use in our experiments.

For the maintenance of stock cultures, hybridoma TA7 cells were seeded at a density of 10,000 cells/25cm^2 flask, on a feeder layer of fresh spleen cells from Balb/c mice, and in 30ml RPMI-1640 medium (Sigma, St Louis, MO, USA), supplemented with 10% fetal calf serum.

For the experiments, 50,000 hybridoma TA7 cells were seeded in each well of a 96-well flat-bottomed plate, in RPMI-1640 medium with 10% fetal calf serum. The cells were exposed to 0.1nM/l–10μM/l $Hg(NO_3)_2.2H_2O$ (mercury nitrate) for 48 hours at 37°C in 5% CO_2/95% air.

The MTT assay (16) was used to evaluate the viability of the TA7 cells. Briefly, a stock solution of 5mg/ml MTT (Sigma) was made. The culture medium was removed from the wells of the 96-well plates and replaced with 100μl RPMI-1640 containing 0.5mg/ml MTT. After incubation for 3 hours at 37°C and then for 30 minutes at 4°C, the plates were centrifuged at 3300*g* for 20 minutes at 4°C. The supernatant was removed from each well, then 200μl 100% dimethyl sulphoxide was added to each well, followed by a further incubation for 10 minutes at 37°C. Colorimetric measurements were then made in a Multiwell spectrophotometer (ELISA reader) by using a Labsystems Multiskan® Plus (Finland) at a wavelength of 540nm, and the results for test wells were expressed as a percentage of the untreated control well values.

The TA7 hybridoma cells in our case were not used as a model of immunotoxicology, but as a novel approach for mercury toxicity monitoring. This work was performed after previous experiments with mercury compounds, by using methods we have developed to assess the toxicities of ecological pollutants (17). These experiments included the culture of chick embryo macrophages tested with the same concentrations of mercury nitrate as those used in the present study.

Statistical analyses were performed by using electronic tables in Excel 5.0 for Windows and commonly used methods for parametric variation and correlation statistics.

Results

The toxic dose-effect of mercury on hybridoma TA7 cells was studied in two series of tests. Table I shows the results of the two series of experiments, and the supposed standard control exponential values between the maximal and minimal means (0.1nM/l–10μM/l) for the data. The first series data concern an earlier experiment and was worked out from only three tests. Therefore, they have comparatively large standard deviations (SDs). The second series data are based on 12 tests and are closer to the control exponential values. No reliable distinctions between indices of cell survival in the range of concentrations of mercury 0.1nM/l–0.1μM/l were found in the first series, but the means of cell survival with concentrations 0.1–10μM/l were statistically significant ($p < 0.05$) in the second series. The correlation indices of the dose-effect data of all the experimental data were $r^2 = 0.95$ in the first series and $r^2 = 0.93$ in the second series. In the range 0.1nM/l–0.1μM/l of the experimental data, they were $r^2 = 0.07$ in the first series and $r^2 = 0.91$ in the second series. In the range 0.1–10μM/l of the experimental data, they were $r^2 = 0.98$ and $r^2 = 0.99$ accordingly. Therefore, concentrations between 0.1nM/l and 0.1μM/l can be considered as the no-effect level, and 0.1–10μM/l as the observed effect level (consisting of 0.1–1μM/l as the lowest observed effect level, 1–10μM/l as the highest effect level, and > 10μM/l as the 100% lethal concentration) in hybridoma TA7 cells.

The percentage indices (mean ± SD) of cell survival in chick embryo macrophage cultures exposed to mercury nitrate were 96 ± 11% (0.1nM/l), 94 ± 10% (1nM/l), 89 ± 9% (10nM/l), 84 ± 7% (0.1μM/l), 61 ± 8% (1μM/l) and 0% (10μM/l; 17). Statistically different data ($p < 0.05$) from the control data were found for concentrations between 1μM/l and 10μM/l mercury nitrate. The correlation between indices of mercury nitrate toxicity in hybridoma TA7 cells and chick embryo macrophage models was $r^2 = 0.98$.

Discussion

One of the main topics in modern toxicology is the search for new predictive tests (18), and particularly *in vitro* tests, taking into account the desire to replace animal experimentation (12, 13). At a workshop held in 1998, which was devoted to discussing the scientific and regulatory challenges for the reduction, refinement and replacement of animals in medical testing, a variety of cell culture systems, including those involving hybridomas, were highlighted (19)

Haitov *et al.* (14) discussed the value of hybridoma systems prepared by many different technologies for studies of proliferative, cytostatic and degenerative processes under the influence of different agents, including toxic agents. Moreover, hybridomas based on myelomas represent processes predominantly found in antibody-synthesising B-cells, while using myelomas with T-cell clones could permit hybridoma systems to be developed, which would display immunological processes such as T-cell cytokine activity. Ig-synthesis by B53 hybridoma cells was demonstrated in the work of Setum & Mathur (20). A human T-cell hybridoma was developed from T-cells of an individual allergic to honey bee venom, which produced an antigen-binding glycosylation inhibition factor having affinity for bee venom (21, 22). Also, a human T-T cell hybridoma produces a T-cell-derived supressor factor (23). It is important to stress that in the "New Methods and Models" section of an issue of one of the leading toxicological periodicals, *Toxicology in Vitro*, the use of the MCF-7-E3 cell proliferation assay for environmental pollutants and industrial chemicals (24), and also for the effects of potential oestrogens and non-oestrogenic substances (25, 26), was positively evaluated. The CFD-PBPK hybrid model for simulating gas and vapour uptake in the rat nose is also very useful (27).

Original cytotoxicity experiments on mercury with the TA7 hybridoma model can be substantiated by considering the results obtained in the light of the usefulness of hybridoma models for the cytotoxicity evaluation of various environmental, industrial or other agents. Another novel aspect of the toxicological experiments with mercury was the use of the MTT reduction assay for the quantification of the cytotoxic effects of mercury and their concentration limits. With this convenient test, we have measured the 100% lethal concentration, and the highest and the lowest cytotoxic levels of mercury nitrate for the TA7 hybridoma cells. Keisari (28) and Chiba *et al.* (29) stress the validity of the MTT assay as a useful and convenient

Table I: The cytotoxic dose-effect of mercury ($Hg_2[NO_3]_2.2H_2O$) on hybridoma TA7 cells in percentage survival of cells

	Percentage survival (mean ± SD)					
Experiment	**0.1nM/l mercury**	**1nM/l mercury**	**10nM/l mercury**	**0.1μM/l mercury**	**1μM/l mercury**	**10μM/l mercury**
First series	103 ± 15	92 ± 9	84 ± 7	90 ± 10	69 ± 6	0 ± 0.1
Second series	100 ± 9	96 ± 6	90 ± 5	88 ± 6	65 ± 5	0*
Control	100	97.4	94.8	86.9	63.4*	0*

**p < 0.05 compared to data produced after exposure to 0.1nM/ml–0.1μM/ml*

colorimetric test for cellular growth and viability, which is valuable for the testing of various types of agents.

The relevance of the TA7 cell model is connected with its high rate of replication, and its high sensitivity to such cytotoxic agents as mercury nitrate.

Conclusions

The original data about mercury compound $Hg_2(NO_3)_2.2H_2O$ toxicity *in vitro* on hybridoma TA7 cell cultures supports the usefulness, sensitivity and reproducibility of this testing approach. The colorimetric MTT assay is a convenient and useful assay for quantification of the cytotoxic effects of mercury nitrate on hybridoma TA7 cells. By using this methodological approach, the lowest and highest effect levels and the 100% lethal concentration to hybridoma TA7 cells for the mercury compound was measured. This data suggest that hybridoma TA7 cells might be useful in assessing the dose-effect in *in vitro* toxicological experiments.

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