Growth Response of Mouse Lymphoma Cells to Low Concentrations of Mercuric Chloride

Stephan Paul Jost*, Jane Cole, and Brian C. Goodwin**

M. R. C. Cell Mutation Unit, The University of Sussex, Falmer, Brighton, BN1 9QG, England

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Three growth rate experiments involving several sampling points were performed to investigate the previous finding that very low concentrations of $HgCl_2$ inhibit the growth of murine lymphoblasts *in vitro*. However, results presented here do not confirm this, there being no significant differences between the three independent growth rate experiments.

Introduction

The present study sought to investigate the finding that the growth of murine lymphoblasts *in vitro* was found to be inhibited by a continuous, 4-day exposure to 10^{-16} M and 10^{-17} M $HgCl_2$ solutions present in the culture medium [1, 2]. The expected toxicity of 10^{-5} M and 10^{-6} M $HgCl_2$ solutions was clearly documented during the same study [1, 2]. All other concentrations tested (*i.e.* 10^{-7} M to 10^{-15} M and 10^{-18} M to 10^{-25} M $HgCl_2$) did not significantly affect normal growth [1, 2].

Mercuric chloride had been chosen as a test agent for two reasons. Firstly, $HgCl_2$ is an easily soluble compound of this well known toxic metal and, secondly, no nutrient or catalytic functions are known [1, 2]. Murine lymphoblasts were chosen for this culture system as they seem sufficiently sensitive to growth-inhibiting agents. For example, Schöpf [3] found that the growth of lympho*cytes* used in his system was not affected by exposure to 10^{-5} M $HgCl_2$, contrary to the response of lympho*blasts*.

In the investigations here, the effect of a range of $HgCl_2$ concentrations on the growth rate behaviour of murine lymphoma cells (L5178Y cell line) was tested *in vitro*. Regular sampling of the growing cell populations was thought to be a more accurate way of detecting growth than the single sampling adopted by Amons and van Mansvelt [1, 2] after the termination of a 4-day incubation period.

* Reprint requests to S. P. Jost, Department of Anatomy, The University of Manchester, Stopford Building, Oxford Road, Manchester, M13 9PT, England (Telephone: 0044-61-273-8241 x 173).

** B. C. Goodwin, Professor of Biology, The Open University, Walton Hall, Milton Keynes, MK7 6AA, England.

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Materials and Methods

Weleda Laboratories supplied the $HgCl_2$ stock solutions, prepared according to their methods of dilution for homeopathic preparations, in concentrations of 10^{-3}, 10^{-8}, 10^{-10}, 10^{-14}, 10^{-15} and 10^{-16} g/ml. These were arbitrarily coded A to E and diluted 400-fold on pipetting 25 µl into 10 ml of cell suspension to give final concentrations of 0.9×10^{-n} M, where n = 5, 10, 12, 16, 17 and 18.

L5178Y mouse lymphoma cells were originally obtained from Professor G. A. Fischer. Stationary suspension cultures are routinely grown in our laboratory in 125 ml glass or plastic flasks in 20 ml Fischer's medium containing 10% horse serum, penicillin/streptomycin and 200 µg/ml sodium pyruvate. The flasks are incubated at 37 °C in a humidified 5% CO_2/air incubator. Under these conditions, the cells have a doubling time of approximately 12 hours, and are capable of growth from a single cell. Frozen stocks were kept in ampoules at about 2×10^6 cells/ml in medium and 10% dimethyl sulphoxide in liquid nitrogen. For the experiments described here, one ampoule was removed from liquid nitrogen and the cells cultured overnight at 10^5/µl. They were then counted and subcultured in standard fashion [4–6]. For each growth rate experiment, a culture of exponentially growing cells at a density of not more than 5×10^5/ml was resuspended carefully by pipetting to give a single cell suspension. Cells were counted using a haemocytometer and diluted to provide 250 ml at approximately 1×10^4/ml (experiment 1, Table I), 2×10^4/ml (experiment 2, Table I) and 2.5×10^4/ml (experiment 3, Table I). 10 ml of the cell suspension was added to each of 24 glass universal bottles, which were divided at random into 6 groups of 4 bottles each, A_{1-4}, B_{1-4}, C_{1-4}, D_{1-4}, E_{1-4} and $Control_{1-4}$. Bottles A–E were treated with 25 µl of the relevant stock $HgCl_2$ solution.

The final concentrations of $HgCl_2$ were: A: 0.9×10^{-17} M; B: 0.9×10^{-18} M; C: 0.9×10^{-12} M; D: 0.9×10^{-16} M and E: 0.9×10^{-10} M. The bottles were loosely capped and incubated at 37 °C in a humidified 5% CO_2/air incubator. At 4 intervals during the next 2–3 days (Table I) all the bottles were

removed from the incubator at the same time, the cells carefully resuspended by pipetting, and a 1 ml sample from each placed in a bijoux bottle. The universal bottles were then replaced in the incubator, and the samples counted using a haemocytometer. Although L5178Y cells are able to reach densities of about 1×10^6/ml, a lag in growth rate occurs at densities greater than $5-6 \times 10^5$/ml, and the cells die at densities greater than 10^6/ml. Therefore, counting continued until a cell density of $3-6 \times 10^5$/ml was obtained.

Results

There are no significant differences between any of the five treatments A–E and the control, or between the three growth rate experiments employing varying sampling regimes. A summary of the experimental regimes and results is presented in Table I. Each result represents the mean value (including standard deviation, ±) per experimental group. Each group consisted of four bottles, and each score per bottle was based on the average of eight haemocytometer counts.

The statistical analysis of the results is based on linear regression analysis, and is summarized in Table II. The analysis was based on fitting

$$\log n = a + bt,$$

so that

$$n = e^{a+bt} \text{ or } e^a e^{bt},$$

where

a = intercept,
b = slope,
t = time.

All treatments in all three experiments fit a linear regression very well.

Conclusions

The results of this study demonstrate that there are no inhibitory effects of mercuric chloride at the concentrations previously reported to inhibit the growth of murine lymphoblasts in batch culture [1, 2]. Our procedure of determining growth rate by a linear regression analysis of the logarithmically transformed exponential growth equation is a more sensitive measure of growth than that used by the previous investigators, which was based on a determination of increase of cell numbers during a 90 hour growth period. However, although we conclude that the effect of mercuric chloride on murine lymphoblasts is the expected one, there being no inhibition at very low concentrations, we cannot draw any conclusions about the effects of high dilutions of substances on the state of whole organisms, as reported in the homeopathic literature [7, 8].

Cells in culture, particularly transformed cells, are unlikely to be good models for such phenomena.

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Table I. Summary of regimes and results from three growth rate experiments.

Experiment	Initial N° of cells	Sampling time [hrs]	Control	A	B	C	D	E
1	1×10^4/ml	21	2.72 ± 0.54	3.00 ± 1.01	3.16 ± 1.03	3.25 ± 0.85	2.69 ± 0.26	2.94 ± 1.12
		42	10.72 ± 1.16	10.56 ± 1.33	10.75 ± 2.78	10.25 ± 2.08	10.09 ± 1.74	10.09 ± 2.49
		50	21.09 ± 2.71	21.25 ± 2.63	21.50 ± 2.95	23.09 ± 3.92	24.97 ± 1.84	23.19 ± 3.87
		64½	50.47 ± 10.67	51.00 ± 7.99	45.41 ± 11.86	60.28 ± 6.15	63.78 ± 5.79	57.72 ± 10.40
2	2×10^4/ml	17	5.56 ± 1.11	5.81 ± 0.55	6.38 ± 0.81	5.44 ± 1.11	5.97 ± 1.18	5.59 ± 0.81
		24	8.06 ± 0.48	7.81 ± 1.39	8.09 ± 1.09	7.44 ± 1.31	8.16 ± 1.40	8.03 ± 1.14
		39	21.97 ± 2.24	22.16 ± 1.57	20.78 ± 1.34	20.88 ± 3.05	22.81 ± 2.59	20.72 ± 2.65
		50	38.47 ± 1.87	44.16 ± 4.87	42.88 ± 2.62	39.56 ± 6.50	44.44 ± 1.78	42.31 ± 1.16
3	$2½ \times 10^4$/ml	10½	4.63 ± 0.31	4.25 ± 0.68	4.56 ± 0.72	4.28 ± 0.45	4.41 ± 0.69	4.31 ± 0.93
		19½	7.47 ± 0.53	7.09 ± 0.36	7.22 ± 1.78	7.47 ± 1.15	6.59 ± 1.19	6.75 ± 0.88
		32½	19.16 ± 1.87	17.25 ± 1.27	16.88 ± 1.75	18.63 ± 1.81	18.88 ± 2.74	18.03 ± 1.94
		42	31.94 ± 1.52	28.13 ± 2.72	30.03 ± 1.83	31.56 ± 3.70	31.56 ± 2.54	32.28 ± 1.65

Table II. Linear regression analysis of results.

Experiment and treatment	Intercept 'a' and 95% limits	Slope 'b'	Variance of 'b'	Does slope differ from control? (95% level)
1 Control	−0.4322 (−0.6973; −0.1672)	0.0678	6.92×10^{-6}	–
A	−0.3511 (−0.6536; −0.0485)	0.0664	9.02×10^{-6}	No
B	−0.2039 (−0.6175; +0.2096)	0.0627	16.85×10^{-6}	No
C	−0.3611 (−0.7158; −0.0065)	0.0683	12.39×10^{-6}	No
D	−0.6210 (−0.8929; −0.3491)	0.0740	7.28×10^{-6}	No
E	−0.5015 (−0.9286; −0.0744)	0.0704	17.97×10^{-6}	No
2 Control	+0.6709 (+0.4898; +0.8525)	0.0603	5.90×10^{-6}	–
A	+0.6172 (+0.4250; +0.8095)	0.0631	6.61×10^{-6}	No
B	+0.7562 (+0.5815; +0.9310)	0.0592	5.46×10^{-6}	No
C	+0.5808 (+0.3332; +0.8285)	0.0619	10.97×10^{-6}	No
D	+0.6543 (+0.4436; +0.8651)	0.0627	7.94×10^{-6}	No
E	+0.6244 (+0.4531; +0.7958)	0.0620	5.25×10^{-6}	No
3 Control	+0.8427 (+0.7347; +0.9508)	0.0630	3.08×10^{-6}	–
A	+0.7916 (+0.6602; +0.9229)	0.0613	4.55×10^{-6}	No
B	+0.8254 (+0.6300; +1.0208)	0.0611	10.08×10^{-6}	No
C	+0.7712 (+0.6200; +0.9224)	0.0644	6.03×10^{-6}	No
D	+0.7213 (+0.5112; +0.9314)	0.0655	11.65×10^{-6}	No
E	+0.7014 (+0.5234; +0.8794)	0.0661	8.36×10^{-6}	No

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