Mutation Research, 127 (1984) 61–64
Elsevier

MTR03862

Clastogenic effects of biosynthetic human growth hormone in Snell dwarf mice and CHO cells in vitro

Paul P.W. van Buul [1] and Sylvia van Buul-Offers [2]

[1] *Department of Radiation Genetics and Chemical Mutagenesis, State University of Leiden, Sylvius Laboratory, Wassenaarseweg 72, 2333 AL Leiden (The Netherlands) and J.A. Cohen Institute, Interuniversity Institute for Radiopathology and Radiation Protection, Leiden (The Netherlands) and* [2] *Department of Pediatrics, University Children's Hospital Het Wilhelmina Kinderziekenhuis, Nieuwe Gracht 137, 3512 LK Utrecht (The Netherlands)*

(Received 6 October 1983)
(Revision received 2 January 1984)
(Accepted 9 January 1984)

Summary

Treatment of Snell dwarf mice with high concentrations of human growth hormone from pituitaries as well as of bacterial origin, significantly increased the frequencies of chromosomal aberrations in bone-marrow cells, as measured by the micronucleus test. In vitro treatment of Chinese hamster ovary (CHO) cells with the two types of hormone likewise induced structural chromosomal aberrations.

The successful production of human growth hormone by bacterial systems (Goeddel et al., 1979; Stebbing et al., 1981) has largely increased the possibilities for its therapeutic application. Hormones influencing cell proliferation, such as somatotropic hormone and thyroxine, have shown positive chromosome-breaking effects (Duca-Marinescu and Simionescu, 1973; Duca-Marinescu and Negoescu, 1973; Judin and Antipenko, 1972). Recently we reported on the effects of growth hormone, insulin, testosterone and thyroxine on the induction of micronuclei (MN) in bone-marrow cells of dwarf mice (van Buul and van Buul-Offers, 1982). The present communication deals with a comparison of the clastogenic effects of human growth hormone of pituitary (hGH) or bacterial (bhGH) origin in dwarf mice in vivo, and in in vitro cell cultures of Chinese hamster ovary (CHO) cells.

Materials and methods

Human growth hormone (hGH) derived from cadaver pituitary glands (2 U/mg) lot Nos. DqP107 and 80511, and bacterially synthesized *N*-terminal methionyl hGH (bhGH) (2 U/mg) lot Nos. 81651 and 82412 were a kind gift from AB Kabi (Stockholm, Sweden).

Snell dwarf mice were bred and kept under standardized laboratory conditions as described elsewhere (van Buul-Offers and Van den Brande, 1978). Groups were composed of 5 males and 5 females, and data were pooled. Animals were treated with 150 mU hGH and bhGH by daily subcutaneous injections for 4 weeks (for details see van Buul-Offers and Van den Brande, 1984). At the end of the experiment, all animals were killed 2 h after the last injection by decapitation under ether anaesthesia. The femurs were removed and bone-marrow smears were made, according to the technique of Lederbur and Schmidt (1973). For each femur, 1000 polychromatic erythrocytes (PCE) were analysed for the presence of micronuclei (MN), indicative of chromosomal breakage or non-disjunction.

BrdU prelabeled (for 12 h) or unlabeled cells from an established Chinese hamster ovary cell line (CHO) were produced as described by

TABLE 1

FREQUENCIES OF MN IN BONE MARROW CELLS OF HORMONE TREATED DWARF MICE.

Treatment	Dose per day	Number of MN per 1000 polychromatic erythrocytes (Mean ± SEM)
0.9% NaCl	-	2.2 ± 0.5
hGH	150 mU	4.3 ± 0.7 [a]
bhGH	150 mU	5.3 ± 0.7 [a]

[a] Significantly different from controls (Student's t-test $p < 0.05$).

Natarajan and van Kesteren-van Leeuwen (1977) and treated for 18 h with different concentrations of hGH and bhGH. Chromosomal preparations were made using an air-dry technique following 1 h colcemid (0.00014%) treatment.

Staining and differentiation of sister chromatids were performed according to Natarajan and van Kesteren-van Leeuwen (1977).

Results

The results of the bone-marrow analysis are represented in Table 1; they indicate an increase in the frequency of micronucleated PCEs for both types of human growth hormone. Tables 2 and 3 summarize the results of the in vitro hormone treatments. From these it can be seen that BrdU incorporation sensitizes the system, and that in hormone-treated as well as in untreated cells, higher frequencies of chromosomal aberrations are observed, compared to unlabeled cells. In all experiments both hGH and bhGH cause a significant increase in aberrations, but no clear dose–effect relationship seems to be present. However, our protocol of continuous treatment for 18 h coupled with a low and variable frequency of first-division cells and the use of a single sampling time is not particularly suitable for dose–response analysis.

The frequencies of sister-chromatid exchanges (SCEs) were not affected by hormone treatment (Table 2). As can be judged from the frequencies of cells in their first mitosis (M_1 cells) after BrdU and hormone treatment, both hormones induce delay of the normal progression of cells through the cell cycle (Table 2), indicating some toxic effects.

Discussion

Analysis of bone-marrow cells from hGH and bhGH treated Snell dwarf mice showed a significant increase in the frequencies of PCEs with micronuclei, suggesting chromosome breaking effects of both treatments. The discrepancy between these results and those published previously, where no effect of hGH was observed in the micronucleus test (van Buul and van Buul-Offers, 1982), might be due to the higher dose levels employed here.

TABLE 2

INDUCTION OF CHROMOSOMAL CHANGES BY hGH AND bhGH IN BrdU PRELABELED CHO CELLS [a]

Treatment	Dose (mU/ml)	% abnormal cells	% aberrations	Aberrations per 100 cells				% M_1 cells	SCEs/cell [b]
				Gaps	Breaks	Exchanges	Double minutes		
0		21	25	14	6	4	1	4	7.2
0.9% NaCl		20	26	17	6	3	0	11	8.7
hGH	3.6	21	25	14	6	3	2	7	8.6
	36	24	45	17	16	3	9	9	8.0
	360	33	60	25	18	7	11	19	9.6
bhGH	3.6	17	23	11	6	4	3	25	8.4
	36	26	35	21	9	2	5	22	10.0
	360	34	48	29	15	3	2	38	9.0

[a] 200 cells were analysed for each group from 2 independent Expts.
[b] 25 cells were analysed for each group from 1 Expt.

TABLE 3

INDUCTION OF CHROMOSOMAL ABERRATIONS BY hGH AND bhGH IN CHO CELLS [a]

Treatment	Dose (mU/ml)	% abnormal cells	% aberrations	Aberrations per 100 cells			
				Gaps	Breaks	Exchanges	Double minutes
0	–	7	7	4	2	1	0
0.9% NaCl	–	6	6	1	1	2	1
hGH	3.6	5	5	0	5	0	0
	36	19	27	7	9	0	11
	360	13	15	3	10	2	0
bhGH	3.6	10	11	8	1	1	1
	36	8	18	8	1	0	9
	360	15	17	9	5	3	0

[a] 100 cells were analysed from 1 Expt.

The observed increase in the frequencies of micronuclei has to be considered in the light of the known insensitivity of dwarf mice to the induction of chromosomal aberrations by clastogenic agents. We have demonstrated earlier that treatment of dwarf mice with X-rays or mitomycin C produced only half as many micronuclei as observed in normal mice. The observations of Bielschowsky and Bielschowsky (1959, 1960, 1961) of a lower or retarded tumor induction in dwarf mice by several chemical carcinogens, point in the same direction, and this could suggest that even higher numbers of chromosomal aberrations would have been obtained had normal mice been treated. On the other hand, it should be kept in mind that the dwarf mouse is a model for human panhypopituitarism, and in man, one does not regularly treat normal individuals with growth hormones.

Treatment of CHO cells in vitro with hGH and bhGH increases the frequencies of structural chromosomal abnormalities. Similar results have been obtained for other hormones such as diethylstilboestrol (DES) (Natarajan and van Kesteren-van Leeuwen, 1977). However, it should be noted that both positive and negative results are reported for DES in the mouse bone-marrow micronucleus test (Chrisman and Baumgarten, 1979; de Serres and Ashby, 1981). Furthermore, DES and some of its metabolites induced SCEs in human cultured fibroblasts and human lymphocytes in vitro (Rüdinger et al., 1979; Hill and Wolff, 1982). This contrasts with the results obtained with hGH and bhGH. The observed increase in frequency of double minutes may be of special interest (Tables 2 and 3). To date, this type of chromosomal aberration has frequently been observed in malignant cells, and it has been suggested that it involves amplification of genetic material significant in tumor development (Levan et al., 1977). With respect to the mechanism of chromosome aberration produced by growth hormones, really nothing is known. With our protocol, we have not been able to determine whether these agents are acting in a generalized or S-dependent manner.

Thus, we have demonstrated that human growth hormone from pituitaries as well as of bacterial origin exhibits clastogenic effects both in vivo and in vitro, there being no difference between the two types of hormone. The use of mutagenesis testing for assessment of carcinogenic risks is still a matter for debate, but there is growing evidence that chromosome aberration tests give the best correlation with carcinogenesis of chemicals (de Serres and Ashby, 1981; ICPEMC, 1982; Radman et al., 1982). Therefore, the results presented here suggest the need for extra caution if larger groups of patients are treated with bacterially synthesized human growth hormone.

Acknowledgements

We thank Johan Goudzwaard, Matty Feijlbrief and Joop Branger for their technical assistance and Marije Koopman for typing the manuscript.

We are grateful to Dr. B. Stringberg and Dr. L. Fryklund (Kabi, Stockholm) for their kind gift of hGH and bhGH.

This research was sponsored by the Association between Euratom and the University of Leiden, contract No. 052-64-I-BIAN, the International Atomic Energy Agency, contract No. 1745/R_2/RB, and the Dutch Foundation of Medical Research (FUNGO), grant No. 13-50-22.

References

Bielschowsky, F., and M. Bielschowsky (1959) Carcinogenesis in the pituitary dwarf mouse, The response to methylcholanthrene injected subcutaneously, Br. J. Cancer, 13, 302–305.

Bielschowsky, F., and M. Bielschowsky (1960) Carcinogenesis in the pituitary dwarf mouse, The response to 2-aminofluorene, Br. J. Cancer, 14, 195–199.

Bielschowsky, F., and M. Bielschowsky (1961) Carcinogenesis in the pituitary dwarf mouse, The response to dimethylbenzanthracene applied to the skin, Br. J. Cancer, 15, 257–262.

Buul, P.P.W. van, and S.C. van Buul-Offers (1982) Effect of hormone treatment on spontaneous and radiation-induced chromosomal breakage in normal and dwarf mice, Mutation Res., 106, 237–246.

Buul-Offers, S. van, and J.L. Van den Brande (1978) The Snell dwarf mouse, I. General growth pattern, before and during growth hormone and thyroxine therapy, Acta Endocrinol., 89, 632–645.

Buul-Offers, S. van, and J.L. Van den Brande (1984) Biosynthetic human growth hormone: effects on growth of Snell dwarf mice, Hormone Metab. Res., in press.

Chrisman, C.L., and A.P. Baumgarten (1979) Cytogenetic effects of diethylstilbestrol-diphosphate (DES-dp) on mouse bone marrow monitored by the micronucleus test, Mutation Res., 67, 157–160.

Duca-Marinescu D., and J. Negoescu, (1973) The mutagenic effect of thyroxin, Rev. Roum. Endocrinol., 10, 149–152.

Duca-Marinescu, D., and L. Simionescu (1973) The cytogenetic effects of the somatotropic hormone, Rev. Roum. Endocrinol., 10, 311–316.

Goeddel, D.V., H.L. Heyneker, T. Hozumi, R. Arentzen, K. Itakura, D.G. Yansura, M.J. Ross, G. Miozzari, R. Crea and P.H. Seeburg (1979) Direct expression in *Escherichia coli* of a DNA sequence coding for human growth hormone, Nature (London), 281, 544–548.

Hill, A., and S. Wolff (1982) Increased induction of sister chromatid exchange by diethylstilbestrol in lymphocytes from pregnant and premenopausal women, Cancer Res., 42, 893–896.

ICPEMC (1982) Mutagenesis testing as an approach to carcinogenesis, Committee 2 Report, Elsevier, Amsterdam.

Judin, G.L., and E.N. Antipenko (1972) Post-irradiation restoration of somatic cell chromosomes in mammals, The influence of thyroxine on the frequency of chromosome aberrations in liver cells of rat, Int. J. Radiat. Biol., 22, 501–506.

Lederbur, M., and W. Schmidt (1973) The micronucleus test, Methodological aspects, Mutation Res., 19, 109–117.

Levan, G., N. Mandahl, B.O. Bengtson and A. Levan (1977) Experimental elimination and recovery of double minute chromosomes in malignant cell populations, Hereditas, 86, 75–90.

Natarajan, A.T., and A.C. van Kesteren-van Leeuwen (1977) Mutagenic activity of 20 coded compounds in chromosome aberration sister chromatid exchanges assay using Chinese hamster ovary (CHO) cells, in: F.J. de Serres and J. Ashby (Eds.), Evaluation of Short-Term Tests for Carcinogens, Elsevier/North-Holland, New York, pp. 551–559.

Radman, M., P. Jeggo and R. Wagner (1982) Chromosomal rearrangement and carcinogenesis, Mutation Res., 98, 249–264.

Rüdiger, H.W., F. Haenisch, M. Metzler, F. Oesch and H.R. Glatt (1979) Metabolites of diethylstilboestrol induce sister chromatid exchange in human cultured fibroblasts, Nature (London), 281, 392–394.

Serres, F.J. de, and J. Ashby (Eds.) (1981) Evaluation of Short-Term Tests for Carcinogens, Elsevier/North-Holland, New York.

Stebbing, N., K. Olson, N., Lin, R.N. Harkins, C. Snider, M.J. Ross, F. Fields, L. May, J. Fenno, D. Fodge and G. Prender (1981) Biological comparison of natural and recombinant DNA-derived polypeptides, in: J.L. Gueriguian (Ed.), Insulins, Growth Hormone and Recombinant DNA Technology, Raven, New York, pp. 117–132.