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IMMUNOTOXICITY OF CHROMIUM COMPOUNDS: EFFECT OF SODIUM DICHROMATE ON THE T CELL ACTIVATION *IN VITRO*

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Human T cells, isolated from peripheral blood of healthy donors, were stimulated with phytohemagglutinin and cultured *in vitro* in the presence of sodium dichromate (10^{-3} — 10^{-9} M). A high ((10^{-3} M) concentration of sodium dichromate depressed T cell proliferation whereas low levels of dichromate (10^{-9} M) led to a significant decrease in interleukin 2 production. The results obtained suggest a direct toxic action of sodium dichromate on the functional activity of T cells.

The toxicity of chromium associated with occupation-related human disease and its potential health hazard as environmental pollutant have been cogently established and documented (1, 2). With increased attention focused on primary mechanisms by which metal elements affect biological systems, evidence now exists to indicate that certain metal compounds can affect the efficiency of immune mechanisms (3—12). Trace metals have been found to have a profound effect on cell-mediated immunity (8, 9), and some of them have been reported to have immunomodulatory properties (10—12). Cell-mediated immunity is based on a complex function of T cells as well as of several accessory cells. Many T cell functions are regulated by lymphokines, and interleukin 2 (IL—2) plays a key role in lymphocyte T activation and proliferation.

In the present paper, we report the effect of various concentrations of sodium dichromate on IL—2 production by phytohemagglutinin-stimulated human T cells and on T cell proliferation *in vitro*.

MATERIAL AND METHODS

Isolation of peripheral blood lymphocytes

Peripheral venous blood was obtained from healthy adult donors, and was drawn into heparinized syringes (10 IU/ml). Blood was diluted in an equal volume of phosphate-buffered saline pH 7.4 and centrifuged over Ficoll-Hypa-

que (Pharmacia Fine Chemicals, Piscataway, NJ). Mononuclear cells were recovered at the interface and washed twice in phosphate-buffered saline. Glass adherent cells were removed by incubation at 37 °C for 45 minutes on glass petri dishes in RPMI 1640 with 20% fetal calf serum (FCS). T cells were isolated from these nonadherent cells by rosetting with 2-aminoethylisothiouronium bromide hydrobromide-treated sheep red blood cells followed by centrifugation over Ficoll-Hypaque for 30 minutes at 300 g. The sheep red blood cells were lysed with Tris-NH_4Cl buffer. The cell population contained 95% T cells, as identified by the pan-T reagent Leu—5, and 1—2% peroxidase-positive cells.

The T cell population was washed with phosphate-buffered saline, and resuspended in RPMI 1640 supplemented with 10% FCS, L-glutamine, and penicillin-streptomycin. Phytohemagglutinin (PHA) (Burrough-Wellcome, Greenville, NC) was added to cultures, and cells were cultured in 95% air + 5% CO_2 at 37 °C during 48 h. Sodium dichromate was added to cultures in various final concentrations at the beginning of the culturing. Control media contained an equivalent concentration of sodium chloride. It was found that sodium chloride in used concentrations had no effect on cell proliferation or IL—2 production.

Measurement of interleukin 2 production

For IL—2 production 10^6 T cells were cultured at 37 °C in 1 ml RPMI 1640 supplemented with L-glutamine, 10% FCS and streptomycin-penicillin in 12 x 75 mm round bottom plastic culture tubes. PHA was added at the beginning of the culturing. Sodium dichromate was added as indicated.

At 48 h the culture supernates were collected and were dialyzed against 500 vol phosphate-buffered saline for 24 h at 4 °C with five changes of dialysis fluid. The IL—2 dependent cells used were an HT—2 cell line of BALB/C origin. The cell line has maintained in RPMI 1640 with 10% FCS and IL—2 at 60 U/ml. HT—2 were washed and adjusted to a concentration of 10^5 cells/ml with RPMI 1640, 10% FCS, 2mM L-glutamine and antibiotics. Cells were cultured in 100 μl aliquots in microtiter plates in the presence of 100 μl of IL—2 containing supernates, or those supernates diluted with complete media. After 22 h [^{3}H]-thymidine was added and at 26 h the cells were harvested and counted in a liquid scintillation counter. All cultures were performed in duplicate. Results were compared with a standard curve generated by adding known amounts of purified IL—2 (Electro-Nucleonics Inc., Silver Spring, MD) to HT—2 Cells.

Measurement of cell proliferation

T cells (10^6/ml) were cultured in the presence of 10 μg/ml of PHA in complete medium. They were incubated for 48 h at 37 °C in humidified 5% CO_2 and 95% air atmosphere; at 44 h of the culturing [^{3}H]-thymidine was added. Cells were harvested using a multiple sample harvester onto wool-glass paper. Dry filter discs were placed into scintillation fluid, and radioactivity was measured in a scintillation counter.

Cell viability

Trypan blue staining was used to determine cell viability. The number of stained cells, among 100, was counted after 5 min of the dye exposure.

RESULTS

Table 1 gives the results of T cell proliferation and T cell viability. Sodium dichromate at concentration 10^{-3} M had a profound depressing influence on T cell proliferation. A decrease in proliferation was caused by diminished viability of cells. The lower concentrations had no effect on proliferation or on the cell viability.

Table 2 summarizes the results of IL—2 production by T cells stimulated with PHA in the presence of various concentrations of sodium dichromate. It was found that all investigated levels of sodium dichromate led to a significant depression in IL—2 production.

DISCUSSION

A variety of cytotoxic effects of chromium compounds have been reported. Chromium (VI) compounds experimentally induce tumors in animals and produce cytogenetic and mutagenetic effects in mammalian cells cultured *in vitro* (13, 14). The reduced cell survival in some cell cultures was observed after exposure to chromium (15). *Hanon and Booth* (16) described depression of viral induction of interferon in mammalian cell monolayers pretreated with chromium. Metabolic changes, including a decrease in oxygen consumption and ATP level in rat thymocytes were reported (17).

The results described indicate a direct influence of dichromate upon IL—2 production by cultured human T cells. It was found that IL—2 production was much more sensitive to sodium dichromate than T cell proliferation. A relatively low level of dichromate (10^{-9} M) produced a significant depression in IL—2 production. A decrease in proliferation was observed only at a dichromate concentrations of 10^{-3} M, and was probably caused by direct cytotoxic activity of dichromate as indicated by low cell viability. The mechanism of depressed IL—2 production in the presence of low levels of dichromate is unclear. It is possible that metabolic alterations are induced by dichromate, and changed metabolic processes produce the decreased ability of the cells to synthetize lymphokines. On the other hand, IL—2 production is a necessary part of T cell activation and proliferation. It is possible that uneffected proliferation was induced by low levels of IL—2 which were still sufficient for cell proliferation.

The role of the described phenomena in altered cell-mediated host defence *in vivo* are obscure. It is possible that a decrease in IL—2 production leads to insufficient activation of immune mechanisms. Further studies to elucidate this hypothesis are needed.

Table 1

Effect of sodium dichromate on proliferation and viability of human T cells in vitro

PHA	Sodium dichromate (M)	Proliferation (cpm; % in brackets				Mean relative change (%)	Cell viability (%)
		Donor 1	Donor 2	Donor 3	Donor 4		
—	—	813±50 (2.0)	1,489±76 (1.8)	644±43 (1.9)	638±43 (1.0)	—	98
+	—	40,218±5,107 (100.0)	82,292±3,274 (100.0)	33,124±2,156 (100.0)	61,842±4,400 (100.0)	100.0	96
+	10^{-3}	145±27 (0.4)	261±14 (0.3)	87±17 (0.3)	127±26 (0.2)	0.3	38
+	10^{-5}	38.754±2,575 (96.4)	76,591±3,711 (93.1)	33,965±3,095 (102.5)	62,711±4,715 (101.4)	98.4	93
+	10^{-7}	41,654±3,706 (103.6)	81,375±4,256 (98.9)	35,274±2,756 (106.5)	64,276±3,825 (103.9)	103.1	92
+	10^{-9}	42,500±2,654 (105.7)	85,412±3,988 (103.8)	32,560±3,201 (98.3)	62,754±4,715 (101.5)	102.4	95

Table 2

Effect of sodium dichromate on interleukin 2 production

PHA	Sodium dichromate (M)	Interleukin 2 production (U/10^6 cells)*				Mean relative change %
		Donor 1	Donor 2	Donor 3	Donor 4	
—	—	3.6 (29.8)	2.1 (14.5)	1.7 (16.8)	3.2 (20.4)	19.46
+	—	12.1 (100.0)	14.5 (100.0)	10.1 (100.0)	15.7 (100.0)	100.00
+	10^{-3}	2.4 (19.8)	1.8 (12.4)	0.9 (8.9)	1.1 (7.0)	13.07
+	10^{-5}	2.5 (20.7)	2.7 (18.6)	2.3 (22.8)	3.2 (20.4)	20.63
+	10^{-7}	3.7 (30.6)	5.8 (40.0)	4.8 (47.5)	5.5 (35.0)	38.28
+	10^{-9}	4.5 (37.2)	6.9 (47.6)	6.2 (61.4)	7.1 (45.2)	47.85

* Mean of three determinations, percentage in brackets.

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Sažetak

IMUNOTOKSIČNOST SPOJEVA KROMA: UČINAK NATRIJEVOG DIKROMATA NA AKTIVACIJU T STANICA *IN VITRO*

Ljudske T stanice izolirane iz periferne krvi zdravih davalaca stimulirane su hemaglutininom i održavane u *in vitro* kulturi u prisustvu natrijevog dikromata (10^{-3} — 10^{-9} M). Visoka koncentracija (10^{-3} M) natrijevog dikromata smanjila je proliferaciju T stanica dok su niske razine dikromata (10^{-9} M) izazvale značajno smanjenje proizvodnje interleukina 2. Dobiveni rezultati upućuju na direktno toksično djelovanje natrijevog dikromata na funkcionalnu aktivnost T stanica.

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