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GRANULOCYTE RESERVE IN CHRONIC EXPERIMENTAL BENZENE POISONING IN RATS

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The normal two-fold increase in granulocytes in the peripheral circulation induced by corticosteroids was almost abolished in chronic benzene poisoning in rats.

Key terms: benzene poisoning, corticosteroids, granulocyte reserve assessment, haematotoxicity, peripheral blood.

Benzene haematotoxicity has been known since the past century (1, 2). Progressive impairment of the bone marrow function followed by a dose-dependent decrease of circulating blood elements in the periphery, aplastic anaemia and leukaemia has been described in chronic benzene poisoning (3–8). The methods available for early detection of the benzene induced haematotoxicity are still unsatisfactory (9). Detection of increased red cell mean corpuscular volume (10), decreased leucocyte phagocytic capacity (11), increased leucocyte alkaline phosphatase (12), increased number of lymphocyte chromosome aberrations or sister chromatid exchange abnormalities (13), altered serum immunoglobulin levels (14) and prolonged red cell glycerol haemolysis time (15) have been used with varying success.

The aim of this study was to determine granulocyte response to glucocorticoid stimulation in chronic benzene poisoning. The administration of glucocorticosteroids in normal conditions is followed by an increase in the polymorphonuclear neutrophil count in the peripheral blood, which reflects the size of the reserve pool of granulocytes in the bone marrow. Hence the name »granulocyte reserve«, which is known to be reduced in haematological and neoplastic diseases (16–20).

MATERIALS AND METHODS

Forty-eight three-month-old male albino rats weighing 250–300 g were randomly placed into four experimental groups of twelve animals each. The animals were housed in plastic cages, covered with stainless steel mesh, with three animals per cage. They were fed standard laboratory rat diet (Biotehnoški fakultet, Ljubljana, Yugoslavia) and had free access to water. The first, Control group, received no treatment at all. Rats in the second group (Oil) were administered subcutaneously 1 ml of olive oil (Ljekarne Zagreb, Zagreb, Yugoslavia) five days a week over three weeks, 15 doses in all. The third group (B 440) and the fourth group (B 880) were administered subcutaneously 440 mg/kg (vol 0.5 ml/kg) and 880 mg/kg (vol 1.0 ml/kg) of benzene in olive oil (50% v/v) respectively. The benzene was chromatography grade (Kemika, Zagreb, Yugoslavia).

At the end of the treatment all animals received intravenously a single dose of 1 mg dexamethasone-Na (Krka, Novo Mesto, Yugoslavia). Blood samples were taken from the tail vein and white blood cell and differential counts were performed before administration of dexamethasone and at 2, 4 and 6 hours thereafter. The total white cell count was determined in Bürker-Türk chambers after dilution with Türk solution 1:20 (Türk solution: acidum acetici glaciale 3, gentiana violet (1%) 1, aqua destilata ad 100) (21).

Peripheral blood smears were prepared and stained using a May-Grünwald-Giemsa stain and two hundred cells were counted under oil immersion (22). The number of leucocytes, lymphocytes and granulocytes was expressed in S.I. units ($\times 10^9/L$). The individual granulocyte responses were determined by subtracting the number of granulocytes before the administration of dexamethasone (initial) from the maximum number of granulocytes observed thereafter (final). The difference was called maximal response. The results were expressed as arithmetic means with standard errors and the significance of the difference was assessed by Student's t-test at $P < 0.05$ level.

RESULTS

Chronic benzene poisoning in both experimental groups was followed by a decreased number of leucocytes in the peripheral circulation in relation to control animals ($P < 0.05$) (Table 1). The impairment was more prominent with a higher dose of benzene (B 880) than with a lower dose (B 440) ($P < 0.05$) indicating a dose-dependent effect. Two animals on the high benzene dose (B 880) died before the end of the experimental treatment indicating that the sublethal dose/rate had been reached.

Peripheral lymphocytes appeared to be more sensitive to benzene poisoning than granulocytes (3, 4). Olive oil vehicles had a stimulating effect upon both lymphocytes and granulocytes in the peripheral circulation, probably owing to the inflammatory response. As olive oil was also used as a vehicle in the benzene poisoned rats, the deleterious effect of benzene on white cells was even more significant.

The number of granulocytes in the Control group increased more than doubly, indicating a normal response of the bone marrow to dexamethasone. The response of

Table 1.
White blood cells and granulocyte response in chronic benzene poisoning ($\times 10^9/L$)*

Group	Animals (n)	Leucocytes**	Lymphocytes**	Initial	Granulocytes Final	Maximal response
Control	12	9.51 ± 0.66 ^a	4.94 ± 0.49 ^a	3.58 ± 0.40 ^{ab}	13.95 ± 1.41 ^a	10.97 ± 1.36 ^a
Oil	12	13.08 ± 0.98 ^b	9.00 ± 1.05 ^b	5.69 ± 0.96 ^a	15.13 ± 2.43 ^a	10.90 ± 1.90 ^a
B 440	12	6.59 ± 0.88 ^c	2.05 ± 0.44 ^c	3.86 ± 0.48 ^{ab}	6.99 ± 1.16 ^b	3.12 ± 1.03 ^b
B 880	10 ^{***}	3.49 ± 0.95 ^d	0.87 ± 0.27 ^d	2.26 ± 0.64 ^b	4.99 ± 1.49 ^b	2.73 ± 1.23 ^b

* Mean (\bar{X}_{12}) ± SE ** Initial values only *** Two animals died before the end of treatment

a-d Means with various superscripts in the same column differ significantly (P < 0.05)

granulocytes in the Oil group was about twice the initial value too. Apparently, no response assessed by an increase in granulocytes in the peripheral blood, was observed in either group of the benzene treated rats. The maximal granulocyte response in both B 440 and B 880 rats was three times lesser than in either Control or Oil group ($P < 0.05$ for Control or Oil v.s. B 440 or B 880) (Table 1). The highest increase in granulocytes in the peripheral circulation was observed between the second and the fourth hours after the administration of corticosteroids in the Control and Oil groups (Figure 1).

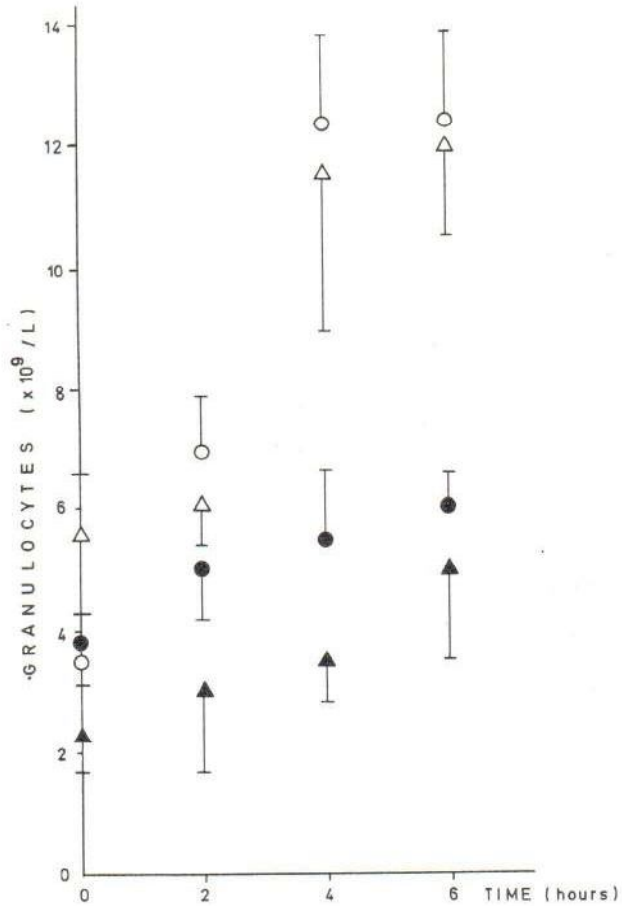


Figure 1. Kinetics of granulocyte response to single dexamethasone administration (1 mg i.v.) in chronic experimental benzene poisoning. (○ Control, △ Oil, ● B 440, ▲ B 880, Mean ± SE.)

DISCUSSION

Granulocyte reserve assessment is used in clinical practice to evaluate the myelotoxic effects of chemotherapy in patients with inflammatory and neoplastic diseases (16–20). After administration of corticosteroids mobilization of available granulocytes from the bone marrow takes place, reflecting the size of their reserve, indicating the therapeutic measures needed (16, 17). The results of this study indicate that evaluation of the granulocyte reserve may also be useful in assessing haematotoxic effects of chronic benzene exposure and poisoning. The granulocyte reserve was far below the normal response to corticosteroids in both groups of benzene poisoned animals. With the exception of the Oil group the initial granulocyte count was the same in the Control group and in both groups of benzene poisoned animals, indicating that standard granulocyte counting without data on granulocyte reserve may mask the already existing haematotoxicity. The results of this experiment confirm our previous clinical observation of impaired granulocyte reserve in people chronically exposed to benzene (23). Both human and animal data indicate that haematotoxic impairment in chronic benzene poisoning could be assessed by a careful study of granulocyte reserve.

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Sažetak

GRANULOCITNA REZERVA U KRONIČNOM EKSPERIMENTALNOM OTROVANJU BENZENOM U ŠTAKORA

Normalno očekivani dvostruki porast granulocita u perifernoj cirkulaciji nakon primjene kortikosteroida izostao je u štakora kronično otrovanih benzenom.

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Cljučne riječi: otrovanje benzenom, kortikosteroidi, procjena rezerve granulocita, hematotoksičnost, periferna cirkulacija.