THE RESPONSE OF BONE MARROW CELLS, THYMOCYTES AND OSTEOCLASTS TO HYDROCORTISONE

O. JOHNELL AND A. HULTH

From the Department of Orthopedic Surgery, Malmö General Hospital (University of Lund), S-214 01 Malmö, Sweden

Received for publication March 18, 1980

Summary. Hydrocortisone injections in young rats caused an increase in the proliferative response of bone marrow cells but a disintegration of thymus gland, the cells of which probably disappeared from thymus. Earlier investigations have shown that different kinds of trauma and antigen result in an increase in the mitotic rate of bone marrow and thymus cells and at the same time an increase in the number of osteoclasts. After steroids, however, no increase of osteoclasts occurred, probably because monocytes which develop into osteoclasts and macrophages are inhibited by hydrocortisone. The effect on serum calcium was minimal.

Recent studies in rats have shown that the mitotic rate of cells in bone marrow and thymus rises significantly 24–48 h after different kinds of trauma: bleeding (Perris et al., 1971), fractures (Hult and Johnell, 1976a), bone-marrow aspiration (Hult and Johnell, 1976b), and soft tissue incision (Johnell, 1977). The same phenomenon also occurs after antigenic challenge with sheep red blood corpuscles in rats (Hult and Johnell, 1978). The finding of a concomitant, constantly occurring increase in the number of osteoclasts in rib metaphyses (Johnell and Hult, 1977; Hult and Johnell, 1978) has made us suspect that there is some causal connection between stimulation of bone-marrow mitosis and increased osteoclasia.

Cortisone retards inflammatory reactions and immunological response in experimental animals. It is also known that cortisone to a certain extent impedes the healing of soft-tissue wounds (Green, 1965) and fracture healing (Hult and Olerud, 1964). Cortisone is used to stimulate bone marrow in bone marrow aplasia and represses the size of thymus in certain species, e.g. rats, mice and rabbits (Blomgren and Andersson, 1971). Thus, since cortisone acts on the defence system of the organism against traumatic and antigenic injuries, we considered it of interest to use the above-mentioned experimental model to study possible changes of cellular proliferation in the bone marrow and thymus after cortisone injections.

MATERIALS AND METHODS

Young rats weighing 100 g at the beginning of the experiment were used. Different experimental models were used. The number of animals was determined so that the statistical calculations could be made on identically treated groups of 10 animals. With the methods used, it is not possible to count colchicine metaphase mitoses and osteoclasts on the same animals, since colchicine inhibits the function and number of osteoclasts. That would necessitate doubling of the number of groups for the main experiment series (A).

A. Hydrocortisone acetate 1 mg/100 g body wt.—Except for 2 control groups, the animals were given hydrocortisone i.e. for 3 days, daily. The rats were killed in groups until 9 days after the last hydrocortisone injection (Days 1, 5, 7, 9).

Two additional groups of 10 animals each were given hydrocortisone once, and twice during 2 consecutive days respectively. These animals were killed the day after the first and the day after the second injection respectively. Both the last-mentioned groups were only used for mitosis counting.
B. Hydrocortisone acetate 0·1 mg/100 g body wt.—Except for one control group, the animals were given hydrocortisone daily for 3 days. The animals were killed in groups of 10. The first group was killed the day after having received 2 injections. The other animals were killed—as in Group A—on the 1st, the 5th, the 7th and 9th day after the third consecutive injection. The animals in Group B were only used for mitosis counting.

Counting of mitoses in bone marrow and thymus.—These animals were given 0·2 mg/100 g colchicine twice at 6 and 3 h before killing. The thymus gland and femoral bones were removed. Thymocyte and bone-marrow cell suspensions were prepared in a balanced glucose salts medium (5·5 mM glucose, 5·0 mM KCl, 0·63 mM CaCl₂, 1·0 mM MgSO₄, 5·0 mM Na₂HPO₄, 120 mM NaCl, 5·0 mM tris buffer, pH 7·2). The thymocyte suspension was prepared by mincing the gland in the medium with scissors; the resulting suspension being filtered through gauze. To prepare the suspension of bone-marrow cells, the ends of each femur were removed and the core of marrow was “washed out” with 1·5 ml of the medium and then dispersed by passing the tissue several times through a syringe with an 18-gauge needle. Both thymus and bone marrow suspensions were then gently centrifuged.

Samples of the cell suspensions were placed on slides and immediately fixed in alcohol and stained in haematoxylin and eosin. The slides were scored for the percentage of the total cell population in metaphase. Each preparation had 2 slides and at least 500 cells were counted on each (a total of at least 1000 cells were counted).

Counting of osteoclasts.—The other experimental animals and the other control groups were killed in groups without colchicine. In all rats, the right fourth and fifth rib were taken out. The bone was decalcified for approximately 20 h in 10% solution of EDTA, containing 0·1M tris buffer. The bone was then washed in cold saline, quickly frozen in liquid nitrogen and then cut in a cryostat, 5–6 sections 10 μm thick being made. The sections were cut at 30 μm intervals to minimize the appearance of the same osteoclast in different sections. The sections were stained for succinic dehydrogenase activity by the method of Pearse (1960) with nitroblue tetrazolium salt as the H-acceptor (Tatevossian, 1973).

The osteoclast count was carried out (a) in the trabecular bone of the metaphysis of the rib and (b) along a predetermined length of the cortex of the metaphysis, peri- and endosteally. The length was determined by a rule engraved on the eyepiece of the microscope. The very few osteoclasts occurring in the marrow cavity were also counted.

The serum albumin was determined by the bromeresol-green method; serum calcium being analysed by flame photometry. Haematocrit was also measured in the animals.

RESULTS

The body weight of the rats which had been given 3 doses of 1 mg/100 g hydrocortisone was significantly lower than that of the control rats and did not increase (Fig. 1). Even in rats given 0·1 mg growth was grossly retarded. The increase in weight of these animals followed that of the control animals during 9 days. The mitosis rate of the bone-marrow cells increased significantly in rats with multiple cortisone injections regardless of whether they had received high or low doses (Fig. 2). The high mitosis rate remained throughout the entire experiment period for the rats given 1 mg/100 g, but returned to normal at the 9th day in rats given the low doses of hydrocortisone. The curve shows the percentage increase in the mitosis rate of controls. The mitosis rate of thymus did not show any consistent change, neither in rats receiving high doses nor in those given low doses of hydrocortisone. It was, however, obvious that the thymus gland in the rats given 1 mg/100 g diminished in size and disintegrated. The mitosis
rate was therefore impossible to estimate owing to the low number of stained cells.

The amount of osteoclasts in the rib metaphyses in the rats given 1 mg/100 g hydrocortisone was perhaps slightly increased, but not significantly so, compared with the controls.

The total serum calcium was significantly higher in the rats given 3 injections of 1 mg/100 g than in the controls, 1 and 5 days after the end of the hydrocortisone administration, but when the concomitant increase in albumins is taken into account the hypercalcaemia becomes insignificant. However, the control and experimental animals given colchicine had a marked hypocalcaemia during the entire period and the hypocalcaemia was about the same in the two groups. The controls given colchicine had a serum calcium value 22% lower than the controls not given colchicine (2.07 ± 0.04 and 2.65 ± 0.03 mg/100 ml respectively). All rats given hydrocortisone both with and without colchicine had a significantly higher serum albumin level. The haematocrit increased significantly in rats given the high doses of hydrocortisone but not after the low doses (Fig. 3).

DISCUSSION

When the rat body has sustained a traumatic mesenchymal injury or an immunological challenge, the bone marrow and the thymus respond immediately by producing more cells for a day or two. Hypothetically, this new production of cells has something to do with the initiation of a reparative or immunological defence, e.g. production of leucocytes and macrophages.

Cortisone stimulates the bone marrow after marrow aplasia. In some species, such as rats and mice, cortisone causes an involution of the thymus gland (Blomgren and Andersson, 1971). In our experimental model, hydrocortisone injections in fact resulted in a significantly higher mitosis rate of bone marrow cells. The thymus gland sustained a continuous involution and in the rats given high hydrocortisone doses the thymocytes disappeared. There was no change in the rate of mitosis in rats given low doses.

It is impossible to say what types of cells proliferate in the bone marrow after
steroid injections and where the thymocytes go. Our experimental model gives information only on total proliferative activity. Both in thymus and bone marrow there are different populations of cells which probably react in different ways after trauma and steroids, respectively. Blomgren and Andersson (1971) showed, for example, that the thymus gland consists of at least two populations, a larger one in the cortex with cells sensitive to steroids and X-irradiation, and another with cells resistant to cortisone and irradiation.

As regards the difference in reaction after trauma and antigen, cortisone administration brings about an increased proliferative response of bone-marrow cells without being followed by an increased number of osteoclasts, which did not change at all. Steroids result in a rapid decrease in the number of monocytes in peripheral blood but do not diminish the number of already existing mature macrophages (Thompson and van Furth, 1970). Since osteoclasts originate from monocytes (Göthlin and Ericsson, 1973), it is probable that the lack of monocytes prevents an increase in osteoclasts.

The marked increase in serum calcium after 3 mg doses of hydrocortisone is certainly an effect of the concomitant increase in serum albumin. In clinical work, steroids are used in order to depress serum calcium in hypercalcaemia, e.g. in sarcoid (Winnacker, Becker and Kats, 1968) and in vitamin D intoxication (Myles and Daly, 1974). We did not see any depression at all, possibly owing to the high doses given. In animals receiving colchicine, however, the reverse occurred, namely a marked hypocalcaemia but still with hyperalbuminaemia. The hypocalcaemia is certainly due to the known osteoclast-impeding effect of colchicine, described by Heath, Palmer and Aurbach (1972).

Financial support was obtained from the Swedish Medical Research Council (Project No. B79-17X-0522-02).

REFERENCES


