Acta orthop. scand. 46, 25–30, 1975

Department of Experimental Pharmacology and Department of Orthopaedic Surgery, Athens University, Greece.

EFFECT OF CORTISONE AND AN ANABOLIC STEROID UPON PLASMA HYDROXYPROLINE DURING FRACTURE HEALING IN RABBITS

GEORGE LYRITIS, ZETA PAPADOPOULOU, PANAYOTIS NIKIFORIDIS, MENELAOS BATRINOS & DIONYSIOS VARONOS

Accepted 19.viii.74

Administration of hormones, affecting bone metabolism, is followed by alteration in hydroxyproline circulation and excretion (Kowalewski 1965).

In fracture experiments hydroxyproline circulation and excretion has been found to increase (Klein 1966, Kowalewski & Yong 1967, Fazakas et al. 1969, Struck et al. 1970). However, to our knowledge, the influence of cortisone and anabolic steroids on circulating hydroxyproline during fracture healing has not been investigated, although existing evidence, based on histological studies, indicates that cortisone treatment retards repair (Hulth & Olerud 1964, Koskinen 1965), whereas anabolics promote this procedure or antagonize the action of cortisone (Kowalewski & Gort 1959).

This has prompted us to investigate the effect of cortisone and anabolics on hydroxyproline excretion during fracture healing.

MATERIAL AND METHODS

Thirty-two male rabbits, body weight ranging from 1950–2200 g and 7–8 weeks old, housed individually and fed the same kind of food, were used. Under general anaesthesia with sodium pentobarbital, the animals sustained an operative fracture of the middle of the left radius. The rabbits were then divided into four groups and treated as follows; 1) controls, no treatment; 2) treated with hydrocortisone (hydrocortisone sodium succinate 5 mg per kg body weight every day); 3) treated with an anabolic steroid (norandrolone-19-phenyl-propionate, 5 mg per kg body weight intramuscularly) and 4) treated with hydrocortisone plus the anabolic steroid in the above dosages. Blood samples were taken by heart aspiration on day 3, 6, 9, 13, 16, 22, 25, 28, 33, 37, 45, 50 and 55 after the fracture. The amount of blood taken was

2 ml. The possibility of secondary anaemia was not taken into consideration. Total plasma hydroxyproline (HOP) was measured using the method of Prockop & Underfriend (1960).

Technique

Initially, blood samples were drawn into .01 vol heparin (heparin sodium, 1000 USP units per ml). The blood was centrifuged at 4° C for 30 minutes at 1000 × *g* and the plasma was separated. Routinely the plasma proteins were precipitated immediately.

Procedure

The procedure used for the assay of plasma HOP consists of precipitation of plasma proteins by cold ethanol, complete hydrolysis of the proteins by autoclaving overnight in alkali and measurement of HOP by the procedure of Prockop & Underfriend (1960).

The statistical analysis was carried out according to Student's t-test.

RESULTS

The results are shown in Figures 1–4. It is to be noted that in all groups of measurements the standard deviation was narrow.

In the group of animals serving as controls a statistically significant ($P < 0.01$) increase of plasma hydroxyproline was noted from day 3 to day 55 after the fracture. The hydroxyproline curve showed two peaks,

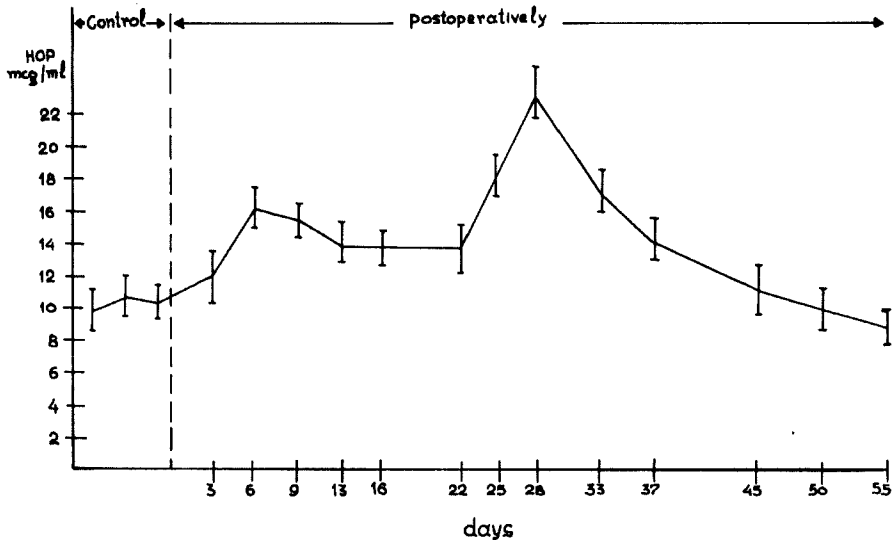

Figure 1.

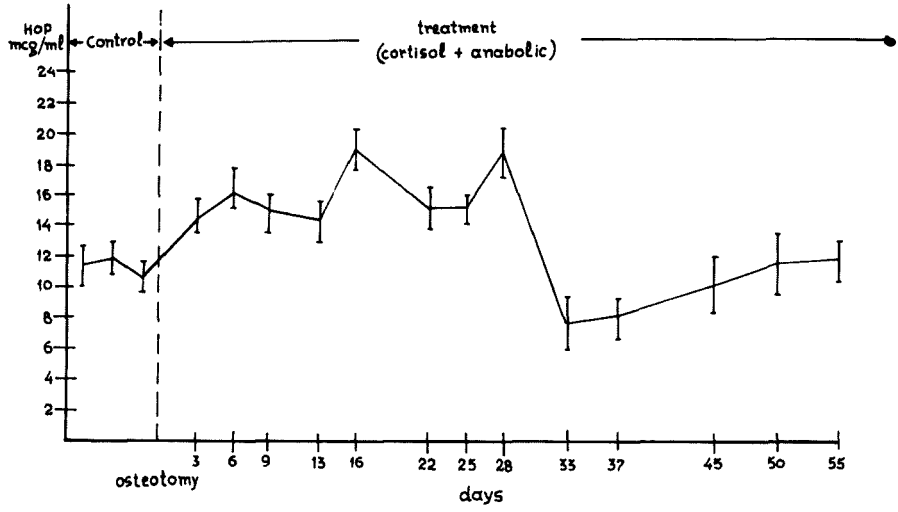

Figure 2.

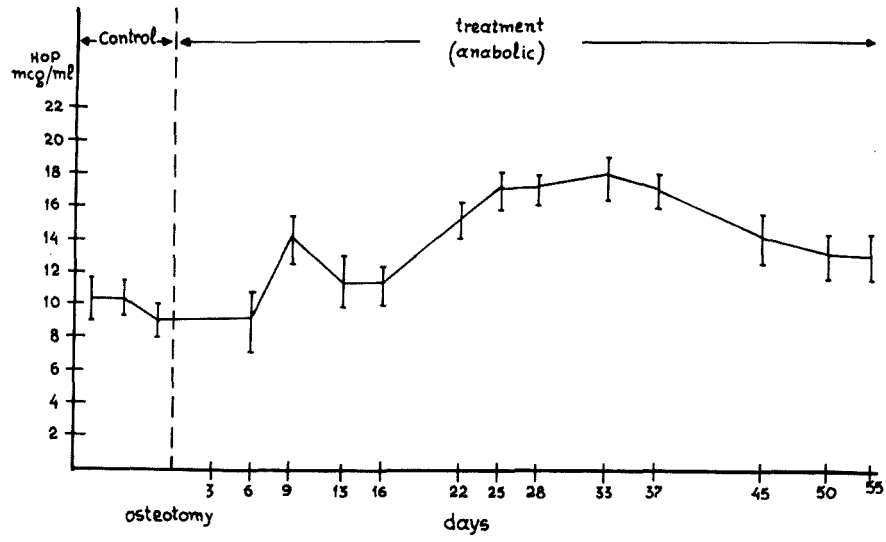

Figure 3.

one on the 3rd and one on the 28th day after the fracture, the second being higher than the first (Figure 1). In the group of hydrocortisone-treated animals a significant elevation of the HOP curve ($P < 0.01$) was noted from day 16, followed by a fall to low normal values, except for a small peak on about day 33 after the fracture (Figure 2). In the animals receiving the anabolic there was a sustained increase ($P < 0.01$)

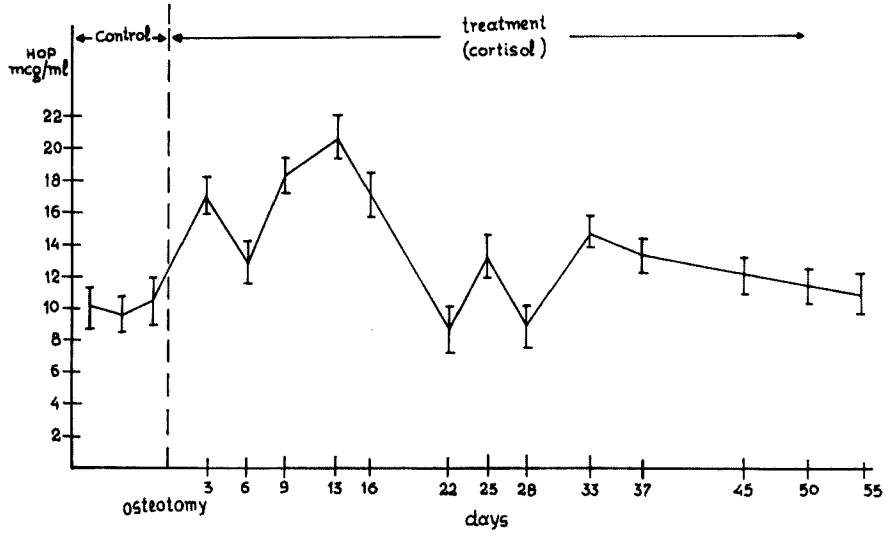

Figure 4.

of HOP starting from the 9th day after the administration of the drug and lasting throughout the experiment (Figure 3).

In the group of animals which received both hormones, a curve similar to that of the hydrocortisone-treated animals was obtained. HOP values were high during the first four weeks and decreased suddenly thereafter to lower than normal values (Figure 4). Both changes were statistically significant ($P < 0.01$).

DISCUSSION

An increase of hydroxyproline in plasma and urine is found when bone collagen is destroyed but can also be noticed in cases of intense collagen synthesis (Laitinen 1967, Prockop & Kivirikko 1967). Elevation of hydroxyproline values is also found in urine (Klein 1966) and serum (Struck et al. 1970) of normal animals with fractures.

Corticosteroids have been found to decrease free and total hydroxyproline while the protein-bound fraction remains unchanged (Kibrick & Milhorat 1969). Anabolic steroids, on the other hand, have an antagonizing effect on the action of corticosteroids, probably through the promotion of collagen synthesis (Kowalewski 1966).

The process of fracture healing involves both destruction and synthesis of bone in a sequence of events which may result in fluctuations of plasma hydroxyproline. In this respect, the early peak of plasma

hydroxyproline found in control animals may be attributed to the degradation of bone and to a lesser degree to synchronous matrix formation in the area of fracture, and the second rise, at about the 28th day, to callus remodelling expected to be occurring at this time. A similar phenomenon has been observed by Struck et al. (1970).

The significant increase of plasma hydroxyproline during the first three weeks in cortisone-treated animals is most probably due to a massive collagen destruction at the site of the fracture and in other bones as well. The retardation of callus maturation caused by cortisone (Sissons & Hadfield 1951) may be held responsible for the low hydroxyproline levels that follow the initial increase.

The anticatabolic effect of the anabolic drug on osteolysis has been shown to inhibit the initial catabolic phase normally observed in fractures (Koskinen 1965, Kowalewski 1966). The increase of hydroxyproline after the second week may be explained by the increase of collagen synthesis due to a prolonged remodelling of the primary callus.

It is interesting, however, that the protective influence of the anabolic steroid on bone catabolism manifested immediately after the fracture was almost abolished when cortisone was simultaneously administered at the dose used in the present study.

SUMMARY

The effect of cortisone and an anabolic steroid on plasma hydroxyproline (HOP) was investigated in young male rabbits, following operative fracture of the radius. The action of these hormones was studied in three groups of animals, a cortisone (hydrocortisone sodium succinate 5 mg/kg every day), an anabolic (norandronolone-19-phenylpropionate 5 mg/kg every other day) and a cortisone plus anabolic treated group. A fourth group of animals served as controls. Plasma HOP was found to increase during the fracture healing in control animals, particularly in the first week and during callus remodelling. Cortisone produced elevation of HOP level during the first two weeks followed by a decrease to low normal values. Animals treated with the anabolic did not present the initial rise but a sustained increase during callus remodelling. When both the anabolic and cortisone were administered, a curve similar to that of cortisone-treated animals was obtained. The initial increase of HOP is attributed to bone destruction and to a lesser degree to synchronous bone formation at the site of the fracture. This catabolic process seems to be enhanced by cortisone and inhibited by the anabolic. When, however, the two hormones are given together the protective anticatabolic effect of the anabolic is almost abolished.

ACKNOWLEDGEMENTS

This work was carried out with the technical assistance of Miss I. Gotsi. The study was supported by a grant from the National Institute of Research, Greece.

REFERENCES

Fazakas, I., Comes, V., Iorgurescu, E. & Gherman, E. (1969) Modificarile hidroxyprolinuriei in fracturi si unele osteoporoze. *Chirurgia (Pavia)* **18**, 625.

Hulth, A. & Olerud, S. (1964) Early fracture callus in normal and cortisone treated rats. *Acta orthop. scand.* **34**, 4.

Kibrick, A. & Milhorat, A. (1969) Prednisone effect on serum hydroxyproline fractions. *Proc. Soc. exp. Biol. (N.Y.)* **131**, 1424.

Klein, L. (1966) The relationship between urinary hydroxyproline, calcium and serum alkaline phosphatase during fracture healing and acute disuse osteoporosis. *Excerpta med.*, International Congress, Series No. 120.

Koskinen, E. V. S. (1965) The influence of hormonal treatment and orchiectomy, oophorectomy and thyroidectomy on experimental fractures. *Acta orthop. scand.* Suppl. 80.

Kowalewski, K. (1965) Effect of growth hormone and an anabolic steroid on excretion of hydroxyproline in the urine of rats. *Acta endocr. (Kbh.)* **50**, 321.

Kowalewski, K. (1966) Effect of an anabolic steroid upon various fractions of tissue hypothyroid rat with a long bone fracture. *Acta endocr. (Kbh.)* **56**, 547.

Kowalewski, K. & Gort, J. (1959) An anabolic androgen as a stimulant of bone healing in rats treated with cortisone. *Acta endocr. (Kbh.)* **30**, 273.

Kowalewski, K. & Yong, S. (1967) Bone and urinary hydroxyproline in normal and hypothyroid rat with a long bone fracture. *Acta endocr. (Kbh.)* **56**, 547.

Laitinen, O. (1967) The metabolism of collagen and its hormone control in the rat. *Acta endocr.* Suppl. 120.

Prockop, D. & Kivirikko, K. (1967) Relation of hydroxyproline excretion in urine to collagen metabolism. *Ann. intern. Med.* **66**, 6.

Prockop, D. & Underfriend, S. (1960) A specific method for the analysis of hydroxyproline in urine and tissues. *Analyt. Biochem.* **1**, 228.

Sissons, H. & Hadfield, G. J. (1951) The influence of cortisone on the repair of experimental fractures in the rabbit. *Brit. J. Surg.* **39**, 172.

Struck, H., Badew, D., Hernandez, R. & Befner, J. (1970) Klinisch Chemische befunde in Serum bei Experimenteller Fraktursetzung und verschiedener behandlung. *Z. klin. Chem. Biochem.* **8**, 453.

Key words: plasma hydroxyproline; fracture healing

Correspondence to:

George Lyritis, M.D.
Orthopaedic Registrar,
Orsett Hospital,
Orsett, Nr. Grays,
Essex, England.