Bone necrosis and urinary hydroxyproline excretion in rabbits

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Summary

1. Aseptic necrosis of bone is a serious chronic complication of deep-sea diving and compressed-air work.
2. The changes to the bone which occur in this condition take time to develop to the stage where they cause the radiographic signs of bone necrosis, and consequently there is a delay of some months between the causal incident and the first diagnosis by radiography.
3. As a possible method for the earlier detection of bone necrosis the 24 h urinary excretion of hydroxyproline was measured over a period before and after experimental production of bone necrosis in rabbits by the intra-arterial injection of glass microspheres.
4. Total hydroxyproline excretion rose significantly within a few days of the injection in those rabbits in which there was later shown to be histological evidence of bone necrosis. This rise occurred long before there was any radiographic change.
5. It is suggested that measurement of urinary hydroxyproline might be used to give an early indication of bone necrosis in man.

Key words: aseptic necrosis of bone, decompression sickness, hydroxyproline.

Introduction

Because exposure to a hyperbaric environment may result in aseptic necrosis of bone, this condition is a serious chronic complication of deep-sea diving and compressed-air work. Aseptic necrosis of bone has resulted in permanent disability of otherwise fit young men. In the British Royal Navy the incidence of bone necrosis is amongst the lowest in any professional group of divers, but even with such strictly controlled operations 5% of men are affected (Elliott & Harrison, 1970). In compressed-air workers the figure is at least 20% (MRC Decompression Sickness Panel, 1971). At the present time there may be more than 1000 divers at risk in the North Sea alone and with the world-wide expansion in sub-sea exploration the number of men affected will increase.

To date, the initial diagnosis is established by radiography; however, radiographic changes of aseptic bone necrosis usually take more than 3 months to appear (Walder, 1977). A safe method of detecting bone damage earlier than this would be a great advantage.

It has been suggested that since bone necrosis is associated with an increased turnover of collagen, the excretion of hydroxyproline (an imino acid found almost exclusively in collagen) is likely to be altered (Deiss, 1974).

Cox (1973) produced aseptic necrosis of bone in the femur of rabbits by the intra-arterial injection of glass microspheres, and recorded the earliest radiographic changes at 3 weeks. We used this animal model to determine whether aseptic necrosis of bone is associated with significant changes in the excretion of hydroxyproline and whether such changes occur earlier than those which are detected by radiography.

Methods

Eighteen mature female New Zealand White
rabbits were maintained in metabolic cages on a standard Oxoid diet no. 18 (Oxoid Ltd, London) with water ad lib. A single postero-anterior radiograph of the pelvis and femora of each animal was taken to confirm skeletal maturity and for comparison with radiographs taken at the conclusion of the experiment.

After an initial period of 3 days for the animals to acclimatize to their surroundings, their urine was collected, over 24 h into 0.1 ml of toluene, for 7 days. At the end of every 24 h the volume was measured and a 20 ml portion was frozen at −40°C for analysis.

Each rabbit was anaesthetized with intravenous Nembutal and the right external iliac artery exposed at laparotomy. To produce aseptic necrosis of one femur a suspension of 0.1 ml of glass microspheres (diameter 40–60 \( \mu \text{m} \)) in 0.7 ml of sodium chloride solution (150 mmol/l; saline) was injected into the right external iliac artery of 11 of the animals on the eighth day. During the injection the superficial femoral artery was temporarily clamped in order to direct the microspheres towards the femur and so minimize soft tissue damage. The remaining seven rabbits served as a control group to exclude the possibility that the operative procedure caused a change in the excretion of hydroxyproline: these animals underwent an identical procedure to the experimental animals but received an injection of saline without microspheres.

All 18 animals were given 50 mg of oxytetracycline intramuscularly for 5 days after the operation. Urine collections continued for 20 days after the operation. All animals were killed by an overdose of intravenous Nembutal on day 21. A second postero-anterior radiograph of the pelvis and femora of each rabbit was taken. The femora were then excised, bisected longitudinally and the cut surfaces inspected for gross abnormality. Longitudinal sections of each bone were prepared for histological examination.

After the final urine collection had been made the samples were thawed and duplicate estimations of total hydroxyproline performed by the method of Goverde & Veenkamp (1972). At the time of estimation the analyst did not know which animals formed the control group.

**Results**

Obvious macroscopic changes in the marrow of the right femur were seen in seven of the 11 animals injected with microspheres. These changes, together with changes in the cortex seen by microscopy, were typical of aseptic necrosis of bone. The right femora of the other four animals injected with microspheres and the seven animals in the control group appeared normal on bisection and histological examination. No radiographic changes of bone necrosis were detected in any of the rabbits.

From the results of the daily excretion of total hydroxyproline in the pre-operative period a mean daily excretion was established for each rabbit, which ranged from 16 to 85 \( \mu \text{mol}/24 \text{ h} \). The greatest variation for any one rabbit was 18–53 \( \mu \text{mol}/24 \text{ h} \) in a rabbit with a mean excretion of 32 \( \mu \text{mol}/24 \text{ h} \). No significant difference was found between the daily excretion of the animals in the control group and of the experimental animals.

As the purpose of the experiment was to see if a significant rise in hydroxyproline excretion occurs during the early stages of bone necrosis the daily postoperative excretion from those rabbits developing necrosis was compared with the daily postoperative excretion from the control group. The daily excretion from each rabbit in the postoperative period was expressed as a proportion of that rabbit’s pre-operative mean value. The mean and SEM of these daily postoperative proportions for the rabbits in the control group and those which developed bone necrosis are represented in Fig. 1.

The results show an increased postoperative excretion of total hydroxyproline by those rabbits which were subsequently found to have histological bone necrosis. This increase was significant on all the days evaluated except day 3. There was no significant change in the excretion of total hydroxyproline from the control group or from the four rabbits injected with microspheres and in which bone necrosis was not detected. The significance of the results was established by a paired \( t \)-test between the results for that day and the pre-operative mean values for the animals in that group.

**Discussion**

The apparent failure of the injection of microspheres to induce bone necrosis in every case was also noted by Cox (1973). As the site at which the injected microspheres eventually
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Fig. 1. Mean and SEM of the daily postoperative hydroxyproline excretion results for the rabbits in the control group (---) and the rabbits which developed bone necrosis (-----). The postoperative hydroxyproline excretion from each rabbit was expressed as a proportion of its mean pre-operative hydroxyproline excretion. Significance of mean values (P) is shown; NS = not significant. The horizontal line is the normalized mean.

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lodge cannot be precisely controlled it is likely that bone necrosis did not develop in four animals because the critical vessels were not occluded. The suggestion that this represents a failure to detect subliminal changes in the femur by histological examination seems unlikely because changes were so readily detected in the other seven animals.

Bone is the principal store of collagen in the body, and we believe that the significant rise in hydroxyproline excretion found in seven of the rabbits in this experiment originated from the bone and was associated with the development of the changes of aseptic necrosis of bone seen in the femur of these animals on histological examination. This view is supported by the absence of a significant rise in hydroxyproline excretion from the rabbits injected with microspheres but in which bone necrosis was not found. There was no macroscopic evidence of skin or muscle damage in any of the limbs injected with microspheres to support the view that these tissues, which contain a lower proportion of the collagen of the body, contributed significantly to the rise in hydroxyproline excretion.

From these initial animal experiments it is therefore suggested that the onset of bone necrosis in men who have been exposed to a hyperbaric environment might be detected by monitoring their urinary hydroxyproline excretion.

When this work was begun there was some doubt as to whether damage to such a small proportion of the skeleton as is seen in aseptic necrosis of bone would be sufficient to influence the hydroxyproline levels significantly, though the present results show that the technique appears to be very sensitive. The histology of autopsy material from divers and tunnel workers who had aseptic necrosis also reveals that the quantity of bone involved is much greater than is apparent by radiological examination (McCallum, Walder, Barnes, Catto, Davidson, Fryer, Golding & Paton, 1966; Weatherley, Gregg, Walder & Rannie, 1977), and this factor might be expected to favour the detection of significant changes in hydroxyproline excretion. Although they did not relate it to bone necrosis, Heyder & Tappan (1973) reported an increased excretion of hydroxyproline in men after hyperbaric exposure and suggested that changes in the metabolism of bone might be responsible. The increase reported fell within the limits of normal excretion. Results which we are now analysing do not suggest that significant changes always occur even on deep dives. However, before any change in the excretion of hydroxyproline from
divers can be related to the possible onset of bone necrosis it will be necessary to obtain a comprehensive record of hydroxyproline excretion after exposure to the different pressures and gas mixtures used in diving. In this way, increased or abnormal patterns of hydroxyproline excretion or other products of bone metabolism might serve as an early indicator of bone necrosis and greatly improve the management of this condition.

References

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