

A histopathological and morphometrical study of femur head cartilage in Wistar rats treated with prednisolone

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Summary. An analysis was made of histopathological developments in femur head cartilage in a group of Wistar rats receiving a daily intra-muscular injection of 2.5 mg prednisolone. This group was divided into four experimental batches, each consisting of 6 rats. Batches were sacrificed at 15, 30, 60 and 90 days after the start of the experiment.

Degeneration of the femur head cartilage was observed from the start of the experiment (15 days), and gave rise to chondrocyte necrosis by 90 days. Structural modifications are shown morphologically and morphometrically.

Key words: Cartilage, Femur head, Rat, Histology, Morphometry

Introduction

Cortisone, isolated by Kendall (1958) as Compound E, has been used for the last forty years in the treatment of the progressive chronic polyarthritis and various degenerative arthropathies. Administration has been both local and parenteral.

Later, certain unwanted effects, including Cushing's syndrome and suppression of the hypothalamus-hypophysis-adrenal axis, were observed in the prolonged systemic administration of these products. This led to the intra-articular administration of corticoids, which ensured a high concentration and maximum anti-inflammatory effect «in situ», while avoiding the general side-effects implied in systemic administration.

Hollander et al. (1951) reported that intra-articular administration of hydrocortisone acetate ensured a more prolonged and effective action.

Since hydrocortisone was only present in the articula-

tion for a short time, new corticoids were sought which would stay longer and have a more powerful anti-inflammatory effect. Hollander et al. (1955) and Chandler et al. (1958) used 3-butyl-hydrocortisone acetate, and Demartini et al. (1955) and Cohen et al., (1957) used prednisone and prednisolone, which had a more powerful anti-inflammatory effect.

Some years later, a derivative of the latter — methyl-prednisolone — was developed in the form of acetate salt, which coupled a strong anti-inflammatory effect with increased capacity for deposition in the articulation.

Chandler and Wright (1958) reported articular deterioration as a result of repeated articular injections of hydrocortisone in patients with rheumatoid arthritis. They referred to this phenomenon as «Cortisone arthropathy», and also observed that despite the clinical improvement recorded in all the cases studied, certain radiological changes took place ranging from slight deterioration to massive articular destruction. These lesions were not accompanied by painful clinical symptoms, which led them to consider it as a form of Charcot's neuropathic arthropathy.

Radiological changes may take two forms, depending on the method of corticoid administration used: a) the repeated infiltration of intra-articular injections may produce osteoporotic radiological changes in the articular extremities, with erosions similar to those characteristic of dissecting osteochondritis. These changes have become known as hydrocortisone-induced dissecting osteochondritis; b) systemic administration gives rise to bone erosion, particularly in the femur head which, although it does not initially affect the articular cartilage, later reflects the mechanical irregularity of the hip as articular deterioration becomes worse. This does not occur only in the case of exogenous corticoids; similar articular alterations have been reported in Cushing's syndrome (Murray, 1960; Louyot and Gaucher, 1963).

This paper presents a histopathological study of the behaviour of the articular cartilage following prolonged

systemic administration of prednisolone.

Materials and methods

The 32 Wistar rats used for this experiment weighed roughly 250 g and were free of infectious and parasitic disease.

8 rats were used as untreated controls, and the remaining 24 were subjected to a daily dosage of 0.5 mg/rat/day prednisolone (Solu-Darcotin). Experimental animals were divided into four batches and were sacrificed as shown in Table 1.

Samples of femur head cartilage for structural analysis were taken after vascular perfusion using 5% glutaraldehyde.

Histopathological analysis

Samples were fixed in 5% glutaraldehyde in phosphate buffer following the method recommended by Sabattini et al. (1963), and postfixed in 2% osmium tetroxide in phosphate buffer. Samples were then processed using propylene oxide and pre-embedded in a mixture of propylene oxide and araldite, prior to embedding in Durcupan A.C.M. (Araldite).

1 micron sections from the block thus obtained were stained with toluidine blue for light microscopic analysis (Nikon Optiphot).

Morphometrical analysis

Morphometrical analysis was carried out directly using histological sections of 3-5 µm thickness taken from the middle zone of the articular cartilage, embedded in araldite and stained with toluidine blue. These were observed through a Nikon Optiphot microscope equipped with a video camera. The image was reflected onto the screen of a semi-automatic image analyser (Olivetti M-24, with a VIDS-III programme).

Parameters studied in the morphometric analysis were as follows:

- Cells area (chondrocytes)
- Nucleus area (chondrocytes)
- Capsular area (chondroplasts)
- Territorial matrix area
- Chondrocyte nucleus/cytoplasm ratio (Nu/Ci)
- Cell area/capsular area ratio (Ce/Ca)

Statistical analysis

Statistical comparison on experimental batches with untreated controls and with other experimental batches was carried out in the case of area parameters using a Student t-test.

Results

Batch 1: Animals receiving 15 days' steroid treatment

Histological Analysis

After 15 days of prednisolone administration, chondrocytes in the cartilage parenchyma formed by «column» and «hypertrophic» zones showed vacuolised cytoplasm and an irregular nucleus displaced towards one of the cell poles. Chondrocytes had not lost their oval shape, and formed isogenic groups of three to four cells at most. Cell cytoplasm contained fatty droplets which gave it a foamy appearance.

Territorial substance was apparently normal, except for a slight focal thickening in some chondromae. The interterritorial matrix, however, showed no sign of alteration.

Morphometrical analysis

Cell area in the first experimental batch was slightly higher (46.79 square microns) than in controls, although the increase was not significant. Slight increases were also observed in nucleus area (7.18 square microns), territorial area (57.14 square microns) and capsular area (121.93 square microns). The nucleus/cytoplasm ratio and the cell area/capsular area ratio were also slightly higher, though the increase was not statistically significant (Tables 2-7) (Graphs 1-4).

Batch 2: Animals receiving 30 days' steroid treatment

Histopathological analysis

Structural changes in the parenchyma of experimental animals from the second batch were similar to those of batch 1. Chondrocyte cytoplasm had a foamy appearance and an irregularly-shaped, polarised pyknotic nucleus.

Anhistic areas were observed in the toluidine blue-stained territorial matrix, although no structural altera-

Table 1. Experimental Design. Inoculation of Prednisolone.

BATCH	EXPERIMENTAL ANIMALS	CONTROL	DOSE/DAYS	SACRIFICE
1	6	2	0.5 mg	15 Days
2	6	2	0.5 mg	30 Days
3	6	2	0.5 mg	60 Days
4	6	2	0.5 mg	90 Days

Table 2. Morphometrical and Statistical Studies of Cellular Area

VALUES (μm)	Control	BATCH 1	BATCH 2	BATCH 3	
Control	61.31 \pm 1.3	—			
BATCH 1	64.79 \pm 2.4	n.s.	—		
BATCH 2	76.40 \pm 2.4	n.s.	n.s.	—	
BATCH 3	107.56 \pm 4.2	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$	
BATCH 4	94.64 \pm 5.7	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$

Table 3. Morphometrical and Statistical Studies of Nuclear Area

VALUES (μm)	Control	BATCH 1	BATCH 2	BATCH 3	
Control	6.20 \pm 0.4	—			
BATCH 1	7.18 \pm 0.2	n.s.	—		
BATCH 2	7.20 \pm 0.2	n.s.	n.s.	—	
BATCH 3	6.90 \pm 0.3	n.s.	n.s.	n.s.	—
BATCH 4	4.15 \pm 0.5	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$

Table 4. Morphometrical and Statistical Studies of Capsular Area

VALUES (μm)	Control	BATCH 1	BATCH 2	BATCH 3	
Control	118.40 \pm 1.3	—			
BATCH 1	121.93 \pm 0.5	n.s.	—		
BATCH 2	122.50 \pm 0.2	n.s.	n.s.	—	
BATCH 3	122.78 \pm 0.3	n.s.	n.s.	n.s.	—
BATCH 4	102.55 \pm 0.2	n.s.	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$

Table 5. Morphometrical and Statistical Studies of Territorial Matrix Area

VALUES (μm)	Control	BATCH 1	BATCH 2	BATCH 3	
Control	57.09 \pm 0.5	—			
BATCH 1	57.14 \pm 0.3	n.s.	—		
BATCH 2	46.10 \pm 1.6	n.s.	n.s.	—	
BATCH 3	15.22 \pm 5.9	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$	—
BATCH 4	7.91 \pm 5.5	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$

Measurements = 300
 Values = Mean \pm Standard Deviation
 n.s. = No statistical differences has been observed
 $p \leq 0.01$ = Statistical differences for 99%
 $p \leq 0.05$ = Statistical differences for 95%

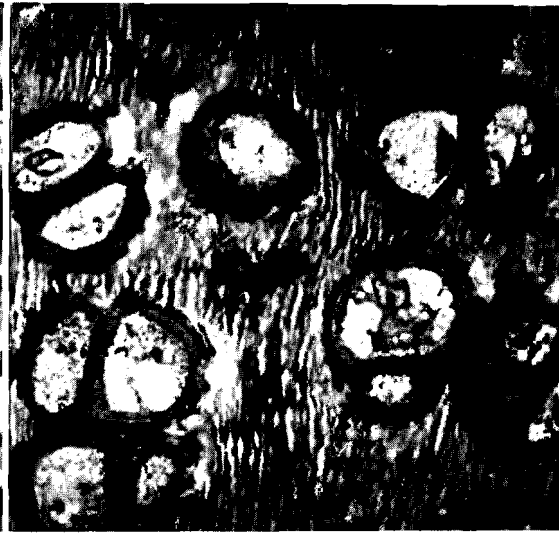
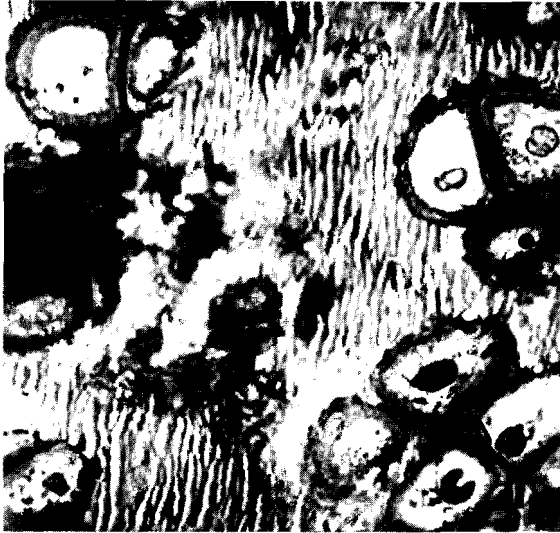


Fig. 1. Batch 2. Chondromae (detail) showing slight vacuolisation of chondrocyte cytoplasm (small arrow) and some pyknotic nuclei (large arrow). Toluidine blue, $\times 680$

Fig. 2. Batch 2. Detail showing intense vacuolisation of chondrocyte cytoplasm (arrow). Toluidine blue. $\times 680$

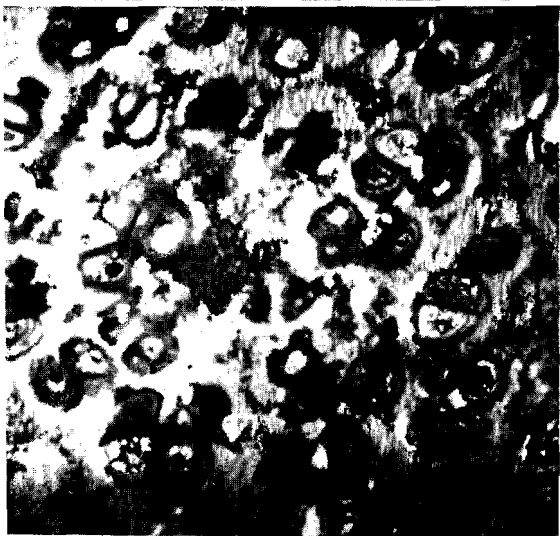


Fig. 3. Batch 3. Cartilage parenchyma (detail) showing chondrocytes with foamy cytoplasm and pyknotic nuclei (arrows). Toluidine blue. $\times 350$

Fig. 4. Batch 3. Chondromae (detail) showing cell components with foamy cytoplasm and peripheral displacement of nuclei. Toluidine blue. $\times 680$



Fig. 5. Batch 4. Chondrocytes showing intensely-vacuolised cytoplasm. Pyknotic nuclei with large polarised vacuoles (arrow). Toluidine blue. $\times 680$

Fig. 6. Batch 4. Necrotic chondrocytes (arrows). Toluidine blue, $\times 680$

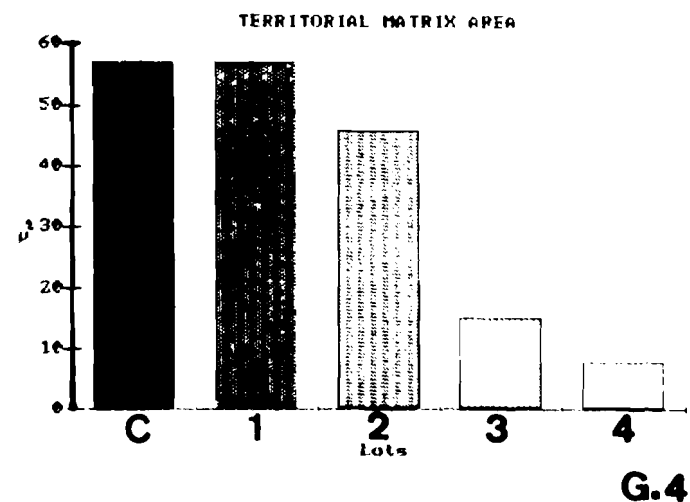
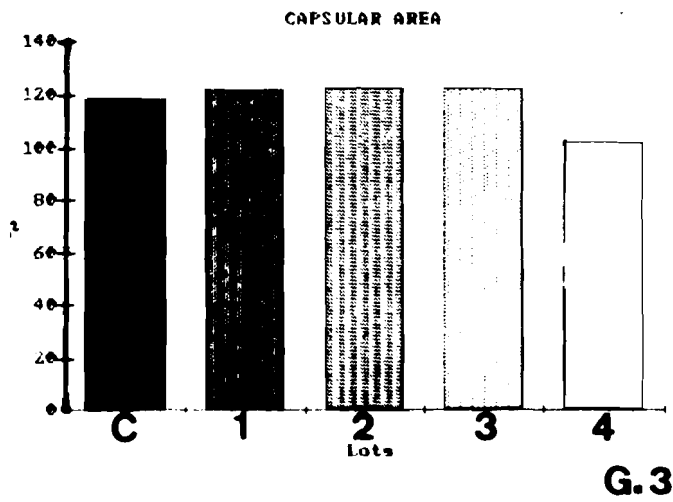
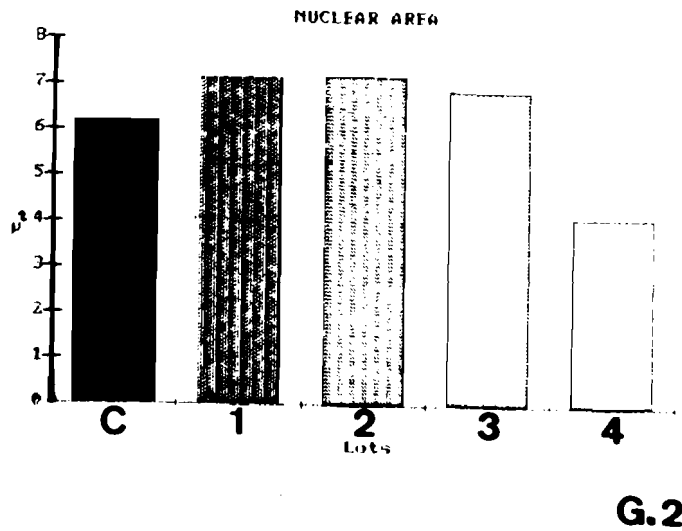
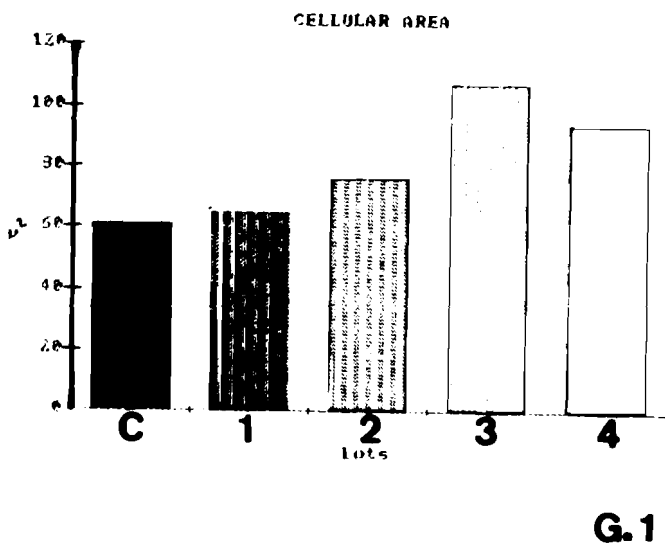
tions were found in interterritorial areas (Figs. 1, 2).

Morphometrical analysis

Morphometrical analysis of this batch revealed a

slight increase in the parameters measured, although it was not statistically significant.

Cell area was 76.40 square microns; nuclear area was 7.20 square microns; territorial area was 46.10 square



Graph 1. Graph of mean cell area (G. 1)

Graph 2. Graph of mean nucleus area (G. 2)

Graph 3. Graph of mean capsular area (G. 3)

Graph 4. Graph of mean territorial matrix area (G. 4)

microns and capsular area was 122.50 square microns. A slight, though not statistically significant, increase was also observed in the nucleus/cytoplasm ratio and the cell area/capsular area ratio (Tables 2-7) (Graphs 1-4).

Batch 3: Animals receiving 60 days' steroid treatment

Histopathological analysis

The middle zone of the parenchyma of animals from the third experimental batch was formed by isogenic Renault groups of three or four chondrocytes. Chondrocyte cytoplasm was anhistic, with central notches, and nuclei were displaced towards one of the poles. In some chondrocytes, the remaining cytoplasm was full of vacuoles or pseudogranular structures. Evidence of apoptosis was detected, principally in the more heavily-stained territorial areas.

The most evident changes in chondrocytes, territorial and interterritorial matrix were observed in the experimental batch. Progressive degeneration gave way to large areas of cell necrosis (Figs. 3, 4).

Morphometrical analysis

Considerable changes were detected in this batch, affecting both cell parameters and cartilage substance. Cell area was 107.56 square microns, territorial area was 15.22 square microns. These changes were significant not only in comparison with untreated controls, but also in comparison with other experimental batches. Changes in nuclear area (6.90 square microns) and capsular area (122.78 square microns) were not statistically significant.

Nucleus/cytoplasm ratio and cell area/territorial area ratio changes were significant in comparison both to controls and to other experimental batches (Tables 2-7) (Graphs 1-4).

Batch 4: Animals receiving 90 days' steroid treatment**Histopathological analysis**

By 90 days, middle zone cartilage parenchyma had undergone generalised necrosis as well as structural changes in the territorial and interterritorial matrix.

Chondrocytes showed evidence of generalised necrosis over the whole area studied, unlike in previous batches where necrosis was only focal. These cells presented foamy cytoplasm with irregularly-shaped nuclei and in some cases marked nuclear atrophy.

Both poles of the nucleus contained clusters of fatty droplets which occasionally compressed the nucleus. Territorial matrix was similar to that observed in previous experimental batches (Figs. 5, 6).

Morphometrical analysis

Morphopathological alterations were even more marked than in the previous batch, and affected cell parameters as well as territorial and interterritorial matrix parameters. Cell area (94.64 square microns) had increased with respect to controls and previous experimental batches, but the nucleus was significantly smaller (4.15 square microns).

Considerable increases were also observed in the nucleus/cytoplasm ratio and the cell area/capsular area ratio. These changes were statistically significant ($P \leq 0.01$) (Tables 2-7) (Graphs 1-4).

Discussion

Though previous studies have dealt with the effect of intra-articular administration of prednisolone on articular cartilage, this experiment was prompted by the widespread use of systemically-administered corticoids in the treatment of a range of ailments (Barrueco, 1987).

No structural alterations were observed in the middle zone of the articular cartilage in animals receiving 15 days' steroid treatment. We agree with Ishikawa et al. (1984) that the lack of change may be due partly to the short duration of treatment and partly to the fact that the changes may be adaptive in nature, and thus do not involve marked morphological changes. This is borne out by morphometrical analysis, which reveals measurements similar to those of untreated controls.

Similar findings concerning the territorial and interterritorial matrix may indicate that the functional architecture was not yet affected at this low dosage and over this brief period.

After 30 days' treatment, cell nuclei were displaced towards peripheral positions, due to the accumulation of metabolic products. These cell alterations are linked to processes of cell ageing and degeneration (Ohira and Ishikawa, 1986).

After 60 days' prednisolone administration, the overall state of the articular cartilage was similar to that observed in previous phases, although lesions were more acute. This suggests that cell changes have ceased to be

adaptive, and have become largely irreversible modifications. Higuchi et al. (1980), in studies using hydrocortisone, report that lesions begin at around 4 weeks and by 12 weeks have become irreversible.

The disparity between the cell parameters studied was most marked at this stage, since cell area and territorial area reach maximum divergence. This bears out the morphological findings observed.

The possible cell hypertrophy observed was similar to that reported by Higuchi et al. (1980) in studies using hydrocortisone.

The significant decrease in the size of territorial matrix was similar to that reported by Higuchi, who postulated a link between the marked decrease after the fourth week and the size of dose employed and the duration of treatment.

Unlike Higuchi et al. (1980) and Silberberg et al. (1966), we detected a decrease in the nucleus/cell ratio which is felt to reflect the progressive cell deterioration that — as analysis of the fourth batch showed — finally led to the death of cartilage cells.

In the final batch of experimental animals, which had received 3 months prednisolone treatment, cartilage destruction reached its maximum, with generalised chondrocyte necrosis and considerable nuclear alteration. Both the pyknotic appearance of the nucleus and the considerable degree of atrophy were evidence of reduced vitality, a morphological sign of irreversible lesion causing the destruction of the nucleus and the subsequent death of the chondrocyte (Ohira and Ishikawa, 1986).

Analysis of morphometrical parameters bore out the morphological findings, which were similar to those of previous batches, suggesting that alterations occurred at around 60 days, and stabilised or progressed more slowly thereafter. Thus cell area and capsular area, and the ratio between the two, were similar to those detected in the previous batch. The same holds true for the other morphometrical parameters (Barrueco, 1987).

In conclusion, it may be affirmed that intra-articular administration of prednisolone gives rise to degenerative processes in the femur head, leading to degenerative arthropathy. This indicates that parenteral administration of corticoids may, with time, give rise to what has been termed «cortisone arthropathy».

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