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Radiation Effects Produced by the Intra-articular Injection of ^{32}P

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The therapeutic use of intra-articular radioactive isotopes in colloidal solutions such as ^{198}Au, ^{90}Y or ^{32}P, has found acceptance in the past years.[1,15,18,20,24] It has been observed that ^{32}P, because of its properties,[2,3] is less dangerous and more effective than other isotopes. Injected into natural cavities in the form of true colloidal solution of chromic radiophosphate, it acts like an internal radiation source without or with little outward leakage, and whose effectiveness is long-lasting.[3] Other types of conventional radiation (X- and gamma-radiation from external sources) are known to cause important alterations of the joint tissues, especially of the growth cartilage.[5,6,10,12,13,19,22] The aim of this study was to analyze the radiation effects produced by the intra-articular injection of colloidal chromic phosphate (^{32}P) on the tissues of the articular cavity as well of the adjacent tissues, during brief and long periods of time.

MATERIALS AND METHODS

Twenty-five adult Wistar rats of 300 g each received an intra-articular injection in the knee of ^{32}P (B.A., Half life 14.5 days, 1.71 Mev) as true colloidal solution of chromic phosphate. The solution used was prepared according to Anjhileri and Marques[3] and the particles had a diameter of 10–30 mμ. The contralateral joint was used as control. The rats received 0.25 mCi and then were sacrificed in groups of 5, after 3, 7, 14, 28 and 56 days after the injection. The articular surfaces of the knee joint of man, dogs and rats, as well as of the human second interphalangeal joint of the forefinger, were also measured in order to establish an approximate relationship between the usual activity injected to human beings (4mCi) and that used by us in rats. The corresponding measurements were made by applying a piece of adhesive tape on to the articular surface, then lifting the tape off and superimposing it over a point network and counting the number of points enclosed on it.[7,23] According to the data obtained and assuming no leakage of the radioisotope out of the joint, the dosis in surface after 56 days was calculated in 70,000 rads total. The control of the injection was carried out by means of exposing the pieces of the first 3 groups directly in front of a conventional X-ray plate for 15–30 minutes and then developing and fixing them.

Both knee joints of all rats were fixed in neutral formaldehyde, decalcified in EDTA and em-

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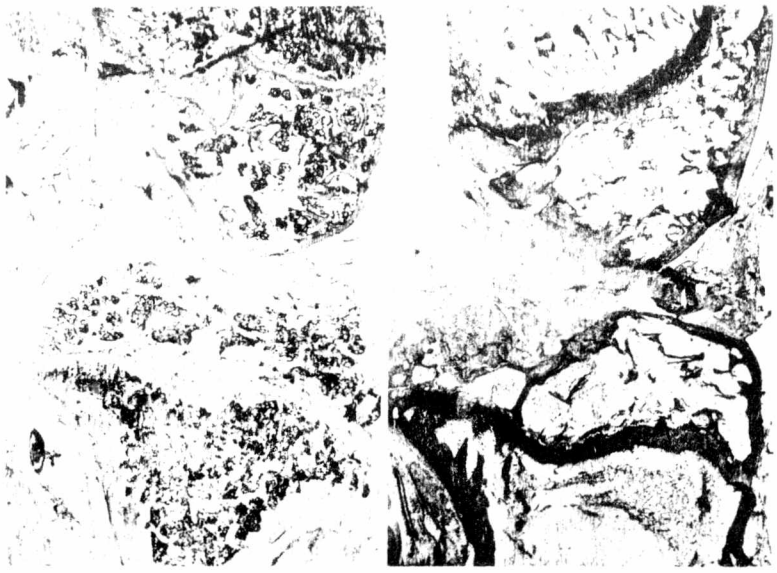

FIGS. 1A and B. (A, left) Knee joint of the control side (originally × 15). (B, right) Contralateral knee joint of the same animal which received an intra-articular injection of ^{32}P. See the substitution of the red bone marrow by yellow bone marrow, osteoporosis, increased width of the growth cartilage showing an evident alteration of staining affinity of its matrix (originally × 15).

bedded in paraffin. The sections made thereafter were stained with hematoxylin and eosin.

In order to check the localization of the isotope in tissues, sections of each case of the first 3 groups were used for microautoradiographs. They were prepared according to the dipping method, using NTB3 emulsion and developed after 14 days.

RESULTS

The macroautoradiographs showed that the isotopic solution was confined to the articular cavity. This was observed in the rats sacrificed on the first day after receiving the injection of ^{32}P solution, as well as in the rats sacrificed after 7 and 14 days.

The microscopic study did not reveal alterations in the rats sacrificed on the first day. The rats sacrificed after 14 and 28 days showed some histologic changes in the synovial membrane, such as chronic inflammatory infiltration, newly formed vessels and the presence of xantomatous cells. Furthermore, in some cases myxoid degen

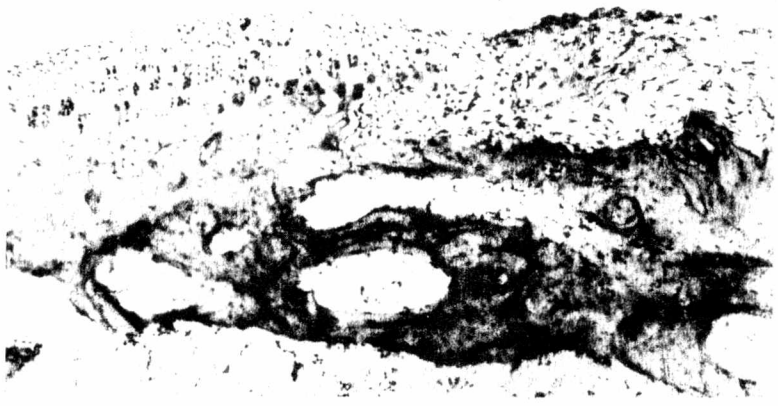

FIG. 2. Articular cartilage showing alteration in the disposition of chondrocyte nests (left). In the right hand area total cartilage atrophy can be observed. The zone previously occupied by the cartilage appeared replaced by a fibrous pannus (× 50).

eration could be observed. The animals sacrificed 56 days after the injection revealed important histological alterations (Figs. 1A and B). The articular cartilage showed areas of marked hypotrophy or even total atrophy. In the latter case the surface was in some cases covered by a fibrous pannus (Fig. 2). The remaining cartilage showed areas of total necrosis in association with synovial membrane necrosis. Other features were alterations of the usual disposition of the axial isogenous nests, formation of reactional osteophytes and an irregular staining affinity of the cartilage matrix. In the growth cartilage (Figs. 3 and 4) we observed an increase in width, disorganization of the axial isogenous nests, swollen chondrocytes, as well as pycnotic, hypertrophic and irregular grouping of cartilage cells. In addition, areas of altered staining affinity of the matrix as well as necrosis were observed. Histological alterations were also evident in the synovial membrane. Hypertrophy or complete loss of the surface cells, as well as a chronic inflammatory infiltration, vascular neoformation and areas of myxoid degeneration were noted. In addition, the disappearance of red bone marrow and its replacement by fatty bone marrow, as well as the decrease of bone trabeculae were noticeable.

The microautoradiographs showed a rather uniform and strong deposit of the radioisotope over the synovial membrane. We could also observe labelled macrophages near the synovial surface (Fig. 5), while over the cartilage the labelling was very little or nonexistent.

The measurements of the articular surface of the knee resulted as follows: 153 cm^2 for men, 13 cm^2 for dogs and 2 cm^2 for rats. The size relationship between knee surfaces of man and dogs was of 10:1 and of 70:1 between man and rat. The proximal articular surface of the proximal interphalangeal joint of the human forefinger measured 4 cm^2, giving a relationship with the articular surface of the rat of 2:1.

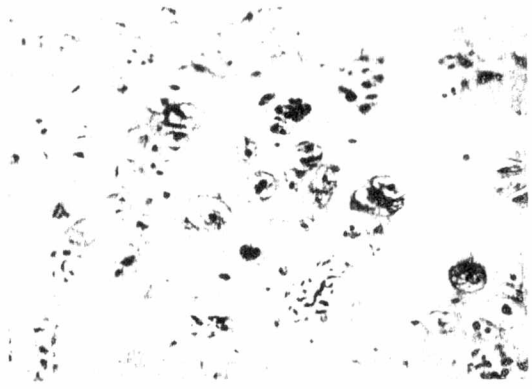

FIG. 3. Growth cartilage of the injected knee. Loss of the normal disposition of the isogenous chondrocyte nests and marked alteration of the individual chondrocytes (originally × 150).

DISCUSSION

The macroautoradiographs revealed the location of the isotope within the articular cavity. This observation proved that the injection was really intra-articular.

The histological study showed no changes in rats sacrificed one day after the injection. Few histological changes were noticed after 14 and 28 days, but they became clear and irrefutable 56 days after the injection. The lesions found by us at the level of the articular cartilage were not described hitherto as caused by the action of a

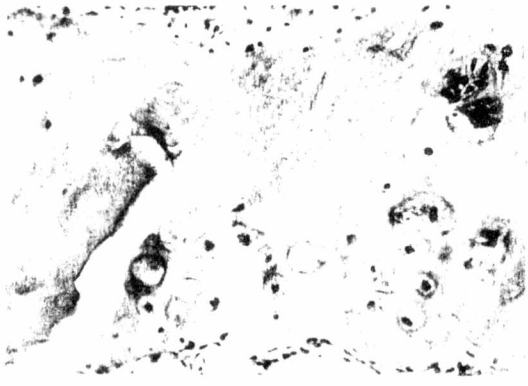

FIG. 4. Markedly irregular staining affinity of the cartilage matrix with areas of evident disorganization (originally × 150).

FIG. 5. Microautoradiography showing a deposit of ^{32}P over the synovial membrane and labeled macrophages. Rat killed 14 days after the intra-articular injection of ^{32}P, exposure time 14 days (originally × 100).

radioactive isotope. These finds of lesions, as lesions in general produced in the cartilaginous tissue, can be considered as irreparable, due to which the determination of the future evolution of a damaged joint would be very difficult. Our findings in the growth cartilage coincide with those described by other authors under different irradiation conditions. The disorganization of the growth cartilage and a simultaneous increase in width were described by Blackburn and Wells[5] after exposures of 1,000 rads, and by Sams[19] following 2,000 rads of X-irradiation, both in mice. Huibel[12] also in mice, and Klein-Szanto and Cabrini[13] in rats, reported similar results after 7,000 rads. The lesions found by Simmons, Hakim and Cummins[22] after β irradiation were also of the same nature.

The synovial tissues are known to be resistant to ionizing radiation, as described by Cottier[6]; however we have observed clear histological alterations similar to those described by Koptan and El-Zornaky[15] after injections of radioactive gold. On the other hand, Pavelka *et al.*[16] reported fibrosis, occlusion and vascular sclerosis following the intra-articular injection of ^{90}Y. It is through these phenomena that these authors explain the anti-inflammatory effects of intra-articular injected radioactive isotopes. Although the most striking changes are found in bone marrow tissues, it is a well known fact that these are the most radiosensitive tissues of all of those studied here. The proximity of the bone marrow to the articular cavity in rats and the penetration of ^{32}P in bone (4 mm) would explain the depletion discovered, which might not necessarily occur in human beings.

Microautoradiographical studies revealed that the true solution of colloidal chromic phosphate tends to be deposited on the surface of the synovial membrane. This deposit was also found in dog joints, as reported by Silberman *et al.*[21] and also confirmed by scintillographic countings. Nevertheless the histological changes caused by irradiation do not occur exclusively in the synovial membrane, but also, to a far greater degree, in the articular and growth cartilage and in the bone marrow.

On the basis of the relationship between the articular surfaces of the knee joint of man and rat, the injected activity in rats would be 16 times less than in man, if we consider that 4 mCi produce a beneficial clinical effect in man, and that we injected 0.25 mCi in our rats. Therefore in our experiments the injected activity in rats should be 4 times greater than the one injected in man, when receiving knee joint treatment. The articular surface of the second interphalangeal joint of the human forefinger, on the other hand, is only twice as large as the knee joint surface of the rat. The intra-articular injection in the interphalangeal joint, of the same activity given to the knee joint of man, would be only 2 times larger than the one we studied in rats. The data gathered in our experience should be taken as a warning in planning for treatment with radioactive isotopes, especially as regards the choice of the activity to be injected. It should also be considered that radiation causes cellular mutations related to the formation of malignant tumors. Tumor incidence is enhanced sometimes after X- or gamma-radiation[9,17] and even after isotopic incorporation.[4,8,14] In our experiments we found severe histological

changes in the chondrocytes; even the synovial cells showed alterations in the DNA content.[11]

Further studies, some of them already initiated by us, such as the effects after various postinjection periods of time, determination of DNA content, histochemistry of the cartilage matrix, histometric determinations and electron microscopy, should contribute to a clarification of the significance of the present findings.

SUMMARY

Colloidal forms of radioactive isotopes are being used for the treatment of some articular diseases. The radiation effects of intra-articular injection of 0.25 mCi of ^{32}P in the form of colloidal chromic phosphate were investigated in one of the knee joints of adult Wistar rats. The contralateral knee was used as a control. The animals were sacrificed one, 7, 14, 28 and 56 days later. Destructive changes were found after 56 days. The articular cartilage was atrophied and covered by a fibrous pannus. The growth cartilage was severely disorganized and there were deleterious effects on both cells and matrix.

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