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New Colloidal Chromic Radiophosphate (P^{32}) for Local Irradiation of the Central Nervous System

L. J. ANGHILERI* and R. MARQUÉS
Comisión Nacional de Energia Atómica, Buenos Aires, Argentina

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A method for the preparation of a true colloidal solution of chromic radiophosphate is given.

The measurement of the isotopic exchange with inactive ionic phosphate indicates good stability.

The study of the body distribution in rabbits after cisternal injection shows that approximately 80 per cent of the administrated colloid remains in the spinal cord and the rest diffuses into the organism, being incorporated mainly into liver and bone.

The physiological assay in rabbits indicates that this colloid induces only a slight neuroirritation, compared with the more critical effects observed with larger size particle phosphate colloids. This colloid provides a new potential tool for the irradiation *in situ* of the central nervous system which has advantages over the classic teletherapy.

UN NOUVEAU RADIOPHOSPHATE (P^{32}) CHROMIQUE COLLOIDAL POUR L'IRRADIATION LOCALE DU SYSTEME NERVEUX CENTRAL

Dans ce travail est décrite une méthode de préparation de solution colloidale véritable de radiophosphate chromique. Le test de l'échange isotopique avec phosphate ionique inactif indique une bonne stabilité.

L'étude de la répartition dans le corps de lapins après injection cisternale montre qu'environ 80 per cent du colloide administré demeure dans la chorde spinale tandis que le reste diffuse dans l'organisme, incorporé principalement dans le foie et le squelette.

Lés essais physiologiques chez le lapin indiquent que ce colloïde induit une légère neuroirritation, contrairement aux effects plus critiques observés avec des particules de phosphate de plus grandes dimension.

Ce colloïde fournit un nouveau matériel de valeur potentielle pour l'irradiation *in situ* du système nerveux central et présente des avantages sur la téléthérapie classique.

НОВЫЙ КОЛЛОИДАЛЬНЫЙ ХРОМОВЫЙ РАДИОФОСФАТ (P^{32}) ДЛЯ МЕСТНОГО ОБЛУЧЕНИЯ ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЫ

Дается метод изготовлепия истинно коллоидного раствора хромового радиофосфата.

Измерение изотопного обмена с неактивиым иоиным фосфатом указывает иа хорошую стабильность.

Изучение распределения в теле кроликов после вместилищиого введенин показывает, что приблизительио 80 проц. введенного коллопда остается в спинном мозге, а остаток распространяется в организме, усваиваясь главным образом в печени и кости.

Физиологическое определение у кроликов указывает на то, что этот коллоид вызывает только небольшое первное раздражение по сравнению с другими более серьезными эффектами, паблюдаемыми с фосфатными коллоидами с более крупными частицами. Этот коллоид дает новое орудие для облучения иа месте центральной нервной системы, которое имеет преимущества над обычными методами телетерапип.

NEUES KOLLOIDALES CHROMRADIOPHOSPHAT (P^{32}) FUR LOKALE BESTRAHLUNG DES ZENTRAL NERVENSYSTEMS

Es wird eine Methode für die Herstellung einer echten kolloidalen Lösung von Chromradiophosphat beschrieben.

* Present address: Deutsches Krebsforschungszentrum, Institut für Nuklearmedizin, Heidelberg, West Germany.

Die Messung des Isotopenaustausches mit inaktiven Ionenphosphat zeigt gute Stabilität an.

Die Untersuchung der Körperverteilung in Kaninchen nach zisternaler Einspritzung ergibt, dass etwa 80% des verabreichten Kolloids in der Wirbelsäule bleibt und dass der Rest im Körper diffundiert wird, hauptsächlich durch Einverleiben in Leber und Knochen.

Physiologische Proben in Kaninchen zeigen, dass dieses Kolloid nur eine leichte Nervenreizung induziert, im Vergleich zu den mehr kritischen Wirkungen, die mit Phosphatkolloiden von grösseren Korngrössen beobachtet werden. Dieses Kolloid ergibt somit ein Werkzeug, das verfügbar ist für eine *in situ* Bestrahlung des zentralen Nervensystems und einige Vorteile gegenüber der klassischen Therapie hat.

1. INTRODUCTION

UNTIL NOW the irradiation of the central nervous system for the treatment of malignant lesions such as neuroblastoma and its metastates has been done by external irradiation, mainly by teletherapy with Co^{60} or Cs^{137}. There are two principal factors which make successful treatment difficult with this technique:

1. The bone shield protecting the nervous tissues reduces considerably the percentage of the dose delivered at the soft tissue level, with respect to that applied on the body surface. For this reason the dose has to be much higher and can produce radiation damage or injuries in the other surrounding tissues.

2. When the malignant tissue is widely spread over the spinal cord it is difficult to treat the whole area with a homogeneous, localized, and prolonged irradiation.

Considering these difficulties, internal irradiation would be the treatment of choice, if it were possible to use a colloidal material without neuroirritation effects and with an even distribution, good stability and little diffusion to other organs.

The use of a γ-emitting radionuclide is undesirable because of irradiation of the surrounding tissues as in teletherapy.

A β-emitting radioisotope such as P^{32} with an energy of 1·7 MeV, a half-life of 14·3 days, and a maximum tissue penetration of 8 mm, seems to be the most convenient radionuclide.

Most of the colloidal radiophosphates (P^{32}) used in radiotherapy, particle sizes between 0·2 and 1·5 μ, sediment after a while giving an uneven distribution. This problem can be solved only by the use of a true colloidal solution and not a colloidal suspension.

This work describes the preparation and assay of a colloidal chromic radiophosphate (P^{32}) solution which can be used for internal irradiation of the spinal cord following intracisternal injection.

2. METHODS

2.1 *Preparation of colloidal chromic phosphate*

The colloidal chromic phosphate (P^{32}) solution was prepared as follows: 1·5 ml of H_3PO_4 solution (10 mg/ml), containing carrier-free P^{32}, was added to 1·8 ml of CrO_3 solution (10 mg/ml) in 5 ml of distilled water. The mixture was heated to 70–80°C and 100 mg of Na_2SO_3 dissolved in 3 ml of 2% gelatin solution were added. The mixture was heated for 10–20 min in a boiling water bath and then cooled to room temperature. To eliminate the remaining ions the clear greenish-blue true colloidal solution was dialyzed against distilled water until no more radioactivity was detectable in the water. The radioactive yield in several preparations was 30–40 per cent and the colloidal particle size was determined by electronmicrography being between 0·1–0·2 μ.

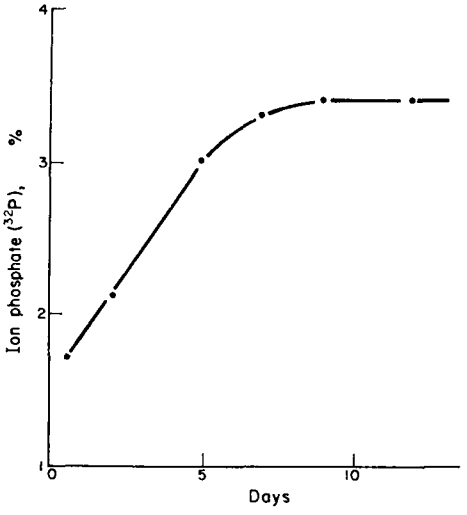

FIG. 1. Isotopic exchange of colloidal chromic radiophosphate with ionic phosphate.

After dialysis the colloid was sterilized by autoclaving and the absence of soluble phosphate was shown by electrophoresis using veronal buffer pH 8·6 and a voltage gradient of 16–18 V/cm. Under these experimental conditions the colloidal chromic phosphate does not migrate but the ionic phosphate does.

The stability of this colloid to isotope exchange with ionic phosphate was studied by incubation at 37°C, under sterile conditions, with isotonic phosphate solution at pH 7·2 (1 vol. 2·1 % KH_2PO_4 + 3 vol. 2·2 % $Na_2HPO_4 \cdot 2H_2O$). Samples were taken aseptically at different times and the activity present as ionic phosphate was determined by electrophoresis as described above. The results are shown in Fig. 1.

2.2 *Tests for neuroirritant action*

The assays for neuroirritation were done in sixty adult rabbits by injecting intrathecally 1 ml of solution containing between 250 and 2500 μc.

This colloid was well tolerated by the rabbits and only slight neuroirritation was observed, maximal between 10 and 15 min after the injection with complete recovery after $\frac{1}{2}$ hr. The use of anaesthetics such as pentothal diminished these effects.

An important finding has been the relationship between the irritant action and the particle size. Other colloidal suspensions with increasing particle sizes up to 2μ were tested. With these suspensions the effects were more violent, starting at 3–5 min after injection showing loss of stability, whimpers, increases in the cardiac and respiratory frequency, and running fits with a gait disturbance characterized by stiff legs. The animals, also presented tremors and symptoms of vestibular irritation. They can also develop cyanosis due to respiratory difficulty provoked by the body rigidity. Finally the animal may die from asphyxia. No important increase in the cerebrospinal fluid pressure has been observed. It starts at 100 mm, rises to a maximum of 150 mm at 45 min and returns to 100 mm after 1 hr.

The injection of gelatin alone, a Cr-gelatin complex, a suspension of insoluble anhydrous chromium chloride ($CrCl_3$) or a suspension of tricalcium phosphate $Ca_3(PO_4)_2$ caused no neuroirritation.

2.3 *Studies of tissue distribution*

For the study of the body distribution, rabbits were injected with 1500–2000 μc of the radiocolloid and sacrificed at different intervals. The organs were dissected out, ashed at 600–700°C and the ash was dissolved in concentrated HCl and diluted to 1 N. Finally aliquots of each ash solution were neutralized with 1 N NaOH, dried under an i.r. lamp and counted with a thin-window Geiger–Müller counter. The self-absorption corrections were made using a self-absorption curve, obtained by measuring samples containing different weights of mineralized material with the same added ^{32}P activity. The organs assayed were spleen, lung, intestine, kidney, heart and liver. The radioactivity was determined also in the spinal cord, muscle (leg), bone marrow (femur), and marrow-free bone (femur). Figure 2 shows the radioactivity, expressed as the percentage of the injected dose, found in the six organs after 1 day, 4 days, 9 days and 14 days. Table 1 gives similar data for the other tissues.

Between 32–48 hr after injection the radioactivity disappears from the spinal fluid and becomes localized on the meninges. The activity per unit surface area was found to be similar in different areas.

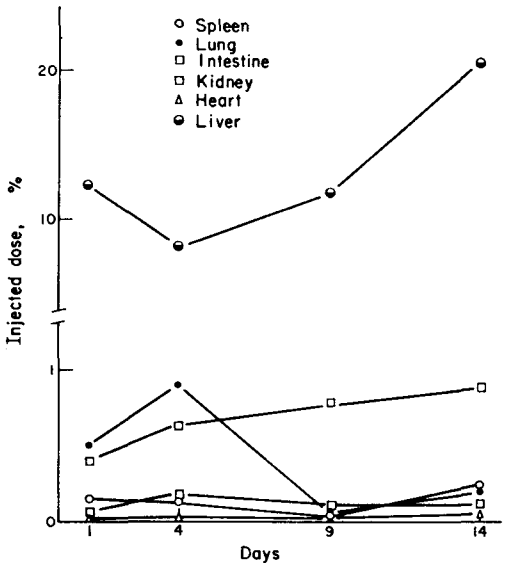

FIG. 2. Values of the radioactivity found at different times.

TABLE 1. Radioactivity found at different times in various tissues (as cpm/g/μc injected)*

	1 day	4 days	9 days	14 days
Muscle	0·6	1·3	1·0	1·1
Marrow-free bone	2·6	18·7	16·0	20·6
Bone marrow	43·2	54·8	14·8	27·2
Spinal cord	1326·3	2860·0	2234·0	1367·1

* These values are not corrected for decay.

The liver presents the highest activity of the organs tested, 12·5 per cent of the injected dose after 24 hr and 20·4 per cent after 14 days, these values are corrected for decay. In the other organs all the values are less than 1 per cent. These measurements (Fig. 2) indicate that approximately 80 per cent of the injected activity remains on the spinal cord.

Table 1 lists the activities found in the femoral bone marrow, marrow-free femur, spinal cord and leg muscle, expressed as cpm/g tissue/μc injected. In bone marrow there is a decrease from 43 cpm/g/μc at 1 day to 27 cpm/g/μc at 14 days, while in the marrow-free bone the opposite is seen, the radioactivity rising from 2·6 cpm/g/μc at 1 day to 20·6 cpm/g/μc at 14 days.

3. DISCUSSION

This technique for the preparation of chromic radiophosphate differs from the classical methods in the fact that the chromium is first bound, by coordinate covalency, to the gelatin, thus avoiding the process of molecular aggregation in a crystalline structure.

From the point of view of the isotopic exchange with ionic phosphate this preparation showed good stability. There was a release of only about 2 per cent of radioactivity after 24 hr and only about 3 per cent after 12 days (Fig. 1).

The study of the body distribution of this colloid shows a similar pattern to that observed previously[1] after intracavitary injection of a small particle size colloid, with relatively high amounts of radioactivity being incorporated into the liver and bone marrow. But in this case the diffusion of the colloid to the blood circulation, and later to the liver, is slower. The activity incorporated in the bone marrow (Table 1), which is important, is not as high as after intracavitary injection. As was observed previously[1] the radioactivity in bone marrow is higher than that in marrow-free bone. The decrease in the ratio of activity in bone marrow to activity in marrow-free bone indicates that the radioactivity which is first incorporated into the bone marrow, presumably as a colloid, is later mobilized and partially incorporated into the mineral constituents of bone.

This colloidal preparation has been well tolerated by the experimental animals and only a slight neuroirritation was observed. In general, the syndrome, which is more critical with larger particle colloids, is similar to that induced by intracerebral injections of other metal ions, such as copper, cadmium and zinc.[2] This neurosensitivity emphasizes the importance of the elimination of the residual chromic ion from the preparation.

Considering the suitability of this colloid for intrathecal injection, it has already been used with promising results in at least ten cases of human neuroblastoma. It is well tolerated by the patients and remarkable improvements of their conditions have been obtained.[3]

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