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Localization of Exogenous ^{14}C-Oxalate in Rats Determined by Whole-Body- and Microautoradiography

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Summary. A single dose of ^{14}C-oxalate was given to Wistar rats using a throat probe. The animals were sacrificed and the oxalate distribution was determined using whole-body-autoradiography. The results showed that a large portion of the absorbed oxalate had reached the bladder 30 min after administration. It is possible that this rapid renal excretion could lead to a short-term oxalate peak in the urine. The oxalate also showed a great affinity for bone. This suggests that there is a deep compartment for oxalate in bone. At the light microscopic level, the localization of ^{14}C-oxalate in bone and in the gastric wall was demonstrated using microautoradiography. This appears to show that there is gastric excretion of absorbed oxalate. Further pharmacokinetic investigations are necessary to confirm this conclusion.

Key words: Urolithiasis, Oxalate, Oxalic distribution, Autoradiography.

INTRODUCTION

It is generally agreed that oxalate plays an important role in the pathogenesis of calcium urolithiasis (12). Urinary oxalate is derived from both exogenous and endogenous sources and appears to be a non-essential end-product of metabolism (14). Past reports have indicated a wide variation of oxalate intake from food, ranging from 100 to 1000 mg/day (16). Also, the amount of oxalate absorbed from the intestines (16) has been reported to vary between 2 and 50% (3). Related studies have shown the plasma concentration of oxalate to vary between 10^{-6} to 10^{-4} mol/l, depending on the method used (7, 9). In addition, radioisotopic studies indicate that the miscible oxalate pool in the human ranges from 2 to 7 mg (8), while the biological half-life of oxalate is between 1 to 3 h. Finally, since the volume within which radioactive oxalate was distributed was found to correspond to about 80% of the body water (18), it seems that oxalate is not freely diffusible into all compartments of the body. However, the volume of oxalate distribution is only a calculated value which does not have an anatomical correlation. Furthermore, data about the time-dependent anatomical distribution of absorbed oxalate are not available at the present time. The aim of this study was to investigate the anatomical distribution of exogenous ^{14}C-oxalate by whole-body-autoradiography and microautoradiography.

MATERIAL AND METHODS

Male Wistar rats weighing 200 g received 0.5 ml of a ^{14}C-labelled 3% sodium oxalate solution (total activity 5 μC) using a throat probe (6). Two animals were then sacrificed 1/2, 2, 5, 24 and 72 h later by anaesthesia with an overdose of ether. The animals were then frozen by immersion in a dry ice/acetone mixture, followed by deep-freeze storage over liquid nitrogen. For the preparation of the longitudinal sections, the animals were first embedded in a wet cellulose powder and then re-frozen with liquid nitrogen. The animals were next sliced into sections of 2 mm thickness, using a special circular saw (10). The individual sections were then arranged over segments of X-ray film, and returned to a deep freeze at -25°C for an exposure period of 14 days. After development, the dark regions in the film image were correlated to specific tissue areas.

In a second experimental series, male Wistar rats weighing 200 g each received daily doses of 0.5 ml of 3% ^{14}C-labelled oxalate solution, con-

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taining an activity of 8.3 μC, through a throat probe. The animals were sacrificed from 1-7 days later.

For the microautoradiographical study, bone and soft-tissue specimens of 10 μm thickness were prepared from the deep-frozen sections. From these, slides were made which were covered with a photographic emulsion (Ilford G5) diluted with equal parts of distilled water. This preparation was carried out under a 15 W red lamp according to dipping technique instructions (1). The slides were then immediately returned to a temperature of -40°C in the deep freeze and exposed for 21 days in the dark. Following this period the slides were developed (Kodak D 13, 3 min), fixed, and stained (H & E) through the emulsion. The silver grains were then correlated to the tissue areas.

RESULTS

The results, using whole-body-autoradiography, show that 30 min after its application, the main ^{14}C-activity was localized in the stomach and upper portion of the small intestine. The oxalate already absorbed was rapidly eliminated by the kidneys and was found in the bladder which contained relatively high activity after 30 min. After 2 h the main activity was observed in the small intestine and partly still in the stomach. In addition, some activity could be demonstrated in bone. At the same time, a small degree of activity was also detected in the kidneys and to a lesser extent in the liver. The activity present in the blood, however, was so small that the large blood vessels were not visible. In other organs such as the spleen, the testes and in muscles no measurable activity could be found.

Five hours following oxalate application, no further changes in the activity pattern could be found, except for normal intestinal transport. After 24 h the wall of the stomach showed uptake, whereas at the same time the contents of the stomach were without activity. Finally, 72 h after oxalate administration only a small amount of activity was present in the intestines, bone and stomach wall, whereas the bulk of the activity had been almost completely eliminated.

In a second series of experiments in which the animals had been sacrificed after repeated doses of ^{14}C-oxalate, an accumulation of ^{14}C-oxalate was found in bone. It was then recorded that the activity in bone decreased with the time which had elapsed since the last application. Again the wall of the stomach showed activity at the same time that the contents of the stomach were without activity.

At the light microscopic level, the localisation of ^{14}C-occalate in bone and the gastric wall were studied by autoradiography: a high activity was observed in the subendosteal layer of bone trabeculae. The subendosteal zones and osteones and subperiosteal zones of compact bone were less heavily labelled. In the periosteal and endosteal regions several single cells were labelled, but it could not be stated with certainty whether they were osteoblasts or not, as the cells of the periosteum and the endosteum are difficult to differentiate. However, high activity was noted in matrix-like areas of osteocytes, localised at the periphery of bone tissue. Occasionally, the edge of the adjoining lacuna was found to contain high activity as well. Few silver grains were dispersed above the columnar cartilage and it appeared that the cell plasma as well as the calcified intercellular substance contained oxalate.

In the mucous membrane bearing the glands of the gastric wall, accumulations of silver grains were observed above adjoining cells of different levels between the gastric glands and the gastric lumen. In the middle of the accumulation, a crystal-like formation was observed which could be localised in the extracellular space of the gastric mucosa. Above the superficial mucosal cells the mucus was labelled as well.

DISCUSSION

As was expected, activity levels in the blood and other organs were found to be low (8, 9). Low activity was visible in the kidney 30 min after oxalate application. The activity was higher in the medulla than in the cortex. Studies concerning oxalate content of the cortex, medulla, and papilla in the rat kidney (15) demonstrated a concentration gradient between the renal cortex and the papilla which is comparable to the sodium and calcium gradients (13).

It was surprising to find that relatively high activity could be found in the bladder after 30 min. This indicates that exogenous oxalate was rapidly eliminated by the kidneys. This rapid excretion of exogenous oxalate might be of clinical significance since a large intestinal load may conceivably lead to a high urinary oxalate concentration peak of short duration. It was also noted that from 24 to 72 h after administration, only the wall of the stomach showed activity. The increasing activity of the gastric mucosa from the gastric lumen suggests a gradual incorporation of oxalate in and/or between the cells during their migration (11). In some areas this may lead to such a high concentration that crystallisation occurs in the region of the superficial mucosal cells. The occurrence of ^{14}C-oxalate in the gastric wall, when the contents of the stomach are without activity, and the activity in the mucus above the superficial mucosa cells indicate an oxalate excretion through the gastric glands. In studies using autoradiography, this path way of

excretion for bicarbonate has been demonstrated (5).

The high affinity of oxalate for bone is probably due to their high calcium content. In the literature (4), it is stated that even 3 weeks after a single dose of ^{14}C-oxalate, some activity could be detected in the bones.

After repeated applications of ^{14}C-oxalate by throat probe, the high affinity of oxalate for bone was confirmed; in addition, an accumulation of ^{14}C-oxalate was found. This indicates that oxalate penetrates into a deep compartment within the bone. Pharmacokinetic studies (7) do not confirm this conclusion because the actual observation times were probably not long enough to show any mobilization of oxalate from the bone. The complete kinetic behaviour of oxalate may be only correctly determined using a model which contains more than one compartment.

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