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Autoradiographic findings in experimental sinusitis in rabbits

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SUMMARY

In order to study the effect of a purulent infection in the sinus cavity on the uptake of radionuclide in the surrounding bone, two series of experiments were performed; one using ^{45}Ca-chloride ($T^1/_2 = 153$ d), that was given intravenously in a dose of 50 MBq to the previously infected animal, and in the other $^{99}Tc^m$-DP was used in a dose of 530-1600 MBq. The animals were killed 4-6 hours later and analyzed using an autoradiographic technique and histological examination. Chronic unilateral infection of the maxillary sinus was induced experimentally according to the technique described by Kumlien and Schiratzki (1985). An acute unilateral maxillary sinusitis was induced experimentally according to Johansson et al. (1988). An increased uptake of $^{99}Tc^m$-DP in the bone surrounding the sinus cavity with an infected mucosa could be seen. This uptake could be seen four days after induction of an acute, purulent, pneumococcal sinusitis, and five months after the induction of an purulent, chronic sinusitis. No increase in uptake could be seen in the mucosa of the sinus with purulent infection. The β-emitting nuclide gave better resolution in autoradiography than did the γ-emitting nuclide, and would give better possibilities in identifying structures; however, the specificity to bone of ^{45}Ca was not high enough to be suitable.

INTRODUCTION

Since the introduction of radionuclide methods for clinical examination, scintigraphy of the skeleton has gained wide application. It has been found to be a good supplement to other diagnostic procedures such as X-ray examinations. Within the head region, the technique has mainly been used for the diagnosis of infections and malignant tumours. Berg et al. (1981), used scintigraphic

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technique for the differentiation between a purulent and a non-purulent maxillary sinusitis. They used ^{99}Tc-methyldiphosphonate, ($^{99}Tc^{m}$-DP), and they found a statistically significant increased uptake in sinuses with a purulent sinusitis. However, the exact nature of the increased uptake and whether this uptake was localized to the bone or to the mucosa or both, was not analyzed. In a pilot experiment a rabbit with a unilateral, purulent maxillary sinusitis was examined with $^{99}Tc^{m}$-DP with the aid of a scintillation scanner camera. However, although a high dose of nuclide was used (≈200 MBq/kg), no significant difference in uptake between the healthy side and the infected side could be seen (unpublished data). A possible explanation might be that the molar region, having a high uptake, obscured a possible difference in uptake. The only projection possible, without interference from the molar region, is an anterior-posterior view, however, in this projection the rather small bony mass of the sinus walls are obscured by the incisors and the neurocranium. Our solution was to use autoradiography. As the clinically used nuclide, $^{99}Tc^{m}$-DP, is γ-emitting the resolution might be low in autoradiographic pictures due to the high energy of γ-rays. A β-emitting nuclide would from a theoretical standpoint give better resolution. The aims of this study were:

1. To investigate if a β-emitting nuclide (^{45}Ca-chloride) could be used.
2. To analyze whether an increase in uptake could be detected and if the uptake is localized to the sinus mucosa or the surrounding bone in the maxillary sinus.
3. To analyze if there is a difference in uptake between an acute purulent or a chronic purulent sinusitis.

MATERIAL AND METHOD

Eight New Zealand white rabbits with an average weight of 4 kg were used.

A. In this first series, the possibly better resolution of a β-emitting nuclide was to be studied. The indicator, ^{45}Ca-chloride ($T_{1/2}$ = 153 d), was given intravenously in a dose of 50 MBq to the previously infected animal (see below). 3–4 hours later the animal was anaesthesized with a mixture of fluanizonum 10 mg + fentanyl-citrate 0.315 mg/ml (Hypnorm®) given in a dose of 0.25 ml/kg body-weight intramuscularly and diazepam (Stesolid®) 0.5 mg/kg body-weight given intramuscularly. The animals were breathing spontaneously. The animals were then killed by an overdose of KCl given intravenously and were then decapitated. The skin was immediately removed and the head was then totally immersed in a mixture of water and carboxymethylcellulose. The head and the surrounding mixture was then frozen with the aid of a mixture of solid CO_2 and heptane. The frozen head was then cut into slices, 20 μm thick, using a freezer microtome (LKB®).

Sections were taken both for histological examination purposes and for the auto-

radiography. The sections for autoradiography were then put in contact with autoradiographic film (Structurix® by Gevaert®). The sections were in contact with the film for 3, 7 and 10 days in order to study different energy levels. The autoradiographic preparations were kept in a freezer during the exposure until development. The films were developed in D-19 by Kodak® for two minutes. The films were studied using a light microscope (Photomicroscope 111, by ZEISS). In order to discriminate possible differences in radioactivity in different areas within each section, the density of the film was analyzed using the microscope exposure measuring device as a "densitometer". The meter was set on "spotreading mode", for highest possible resolution.
The sections used for the autoradiography were, after the exposure, used for light microscopy, after histological preparation, in order to relate the findings on the autoradiographic film with the histological picture.

B. In a second series of experiments $^{99}Tc^{m}$-DP was used. The radioactivity of the nuclide was measured prior to injection. The indicator was given in a dose of 530–1600 MBq in about 2 ml intravenously. 3–6 hours later the animals were anaesthetized, killed, frozen and the head cut into slices as described above. The autoradiographic procedure was the same as described above. The exposure of the autoradiographic films was three days in this series. When sectioning the skull the sections in both series (A+B) were made horizontally to get right and left sinus cavity on the same slice/film in order to obtain identical levels from the sinuses and as identical exposure and development as possible. The freezing technique allows gross examination of the sections. If, however, examinations using high magnification are to be performed, another fixing technique should be chosen. For this purpose sections from other experiments (Kumlien and Schiratzki, 1985; Johansson et al., 1988) were used in order to see possible changes in the mucosa and bone that could be correlated to the increased uptake.

The rabbits were divided into two groups according to method used:
A. Three animals were used as follows: one animal served as a control, the second rabbit had an experimentally induced chronic unilateral infection of one maxillary sinus according to the technique described by Kumlien and Schiratzki (1985). The third rabbit had an acute unilateral maxillary sinusitis induced experimentally according to Johansson et al. (1988). The autoradiography was performed four days after the induction of acute sinusitis, and five months after the induction of chronic sinusitis.

B. In this series five animals were used. One served as a control. Two had unilateral, chronic maxillary sinusitis and two had unilateral, acute maxillary sinusitis, all induced according to the technique described above.

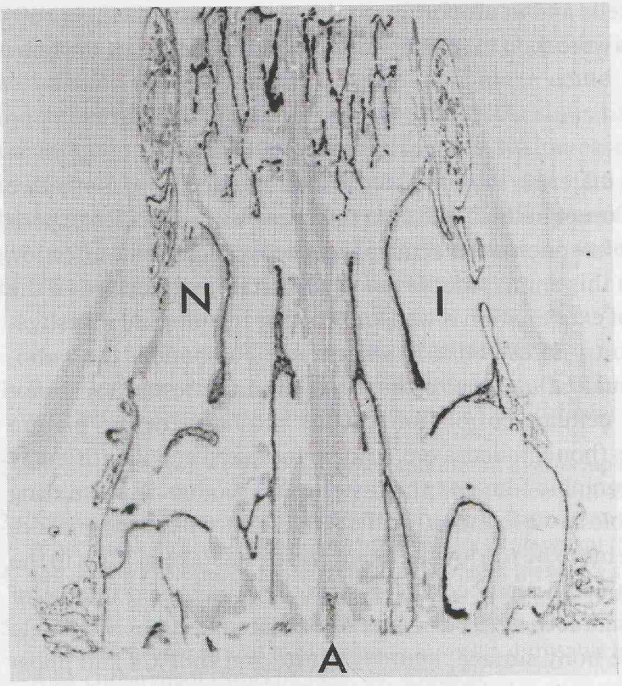
RESULTS

A. In this series all animals showed a marked uptake of ^{45}Ca in the studied bone of the sinus cavity as well as a slight uptake in the sinus and nasal mucosa. Also other soft tissues showed uptake to some extent. The resolution was very good, making localization of anatomical details and borders between mucosa and bone easy. No significant difference in uptake of ^{45}Ca in bone surrounding the normal and the infected sinus could, however, be detected. There was no difference between the rabbit with normal sinuses on both sides or the rabbits with a unilateral acute or chronic sinus infection. No difference in distribution of ^{45}Ca could be detected irrespective of exposure time used (3, 7 and 10 days).

B. All animals showed a dense uptake in the bone throughout the skull in the studied sections, and to some extent in the soft tissues including the sinus mucosa (Figure 1). There was much less uptake in the soft tissues with $^{99}Tc^{m}$-DP than with ^{45}Ca. The border between bone-soft tissue including mucosa was less distinct than when the β-emitting nuclide was used. There was a significant increase in radioactivity, as seen by the corresponding increased optical density on the film, in the bone surrounding the infected sinus compared to the non-infected in all animals. There was no difference between the animals with a chronic or the animals with an acute sinusitis. No difference in uptake of ^{99}Tc between the sinuses could be seen in the animal without infection. No increase in uptake could be found in the mucosa of the infected sinus (Table 1). The histological findings in acute sinusitis were: infiltration of granulocytes and lymphocytes in an oedematous mucosa. The mucosa sometimes displayed a discontinuity in the epithelial layer due to proliferating fibroblasts into the purulent mass in the sinus. Also osteoclasts could be seen on the bone, although regionally and in sparse numbers. In the sinus with chronic infection large number of granulocytes and lymphocytes could be seen infiltrating the sinus mucosa. The mucosa was oedematous. The epithelium had changed, there was a

Table 1. Main data of the different experiments.

rabbit /nuclide	induced infection /side	dose MBq	time between injection of nuclide and start of autoradiography (hours)	no. of sections studied
46/^{45}Ca	acute/left	50	3	9
47/^{45}Ca	control	50	4	8
29/^{45}Ca	chronic/left	50	4	5
75/^{99}Tc	chronic/right	530	6	5
126/^{99}Tc	acute/left	723	6	14
125/^{99}Tc	chronic/right	828	5	5
189/^{99}Tc	acute/right	1600	4	7
188/^{99}Tc	chronic/left	735	6.5	13



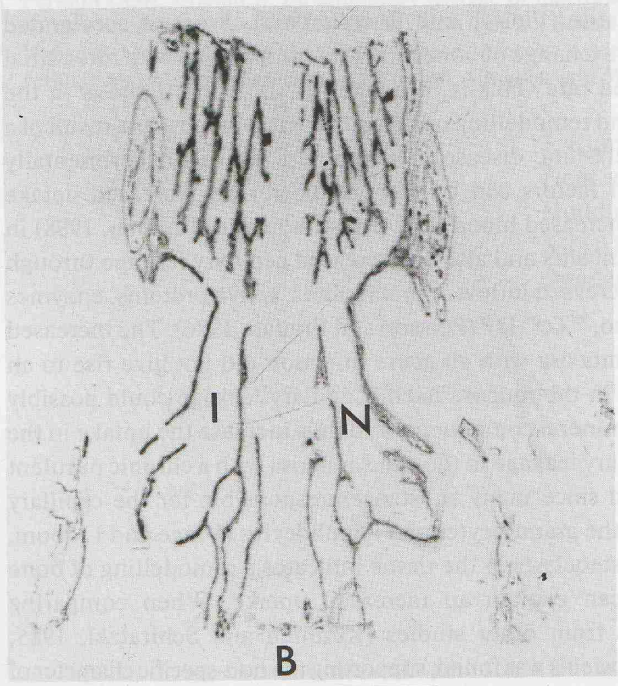


Figure 1.
Two autoradiographic films from an acute (A) and a chronic (B) sinusitis, rabbits nos. 126 and 75 respectively. The bone surrounding the infected sinus cavity (I) is darker, indicating an increased uptake of nuclide, compared to the non-infected (N) sinus.

large number of goblet cells and in proportion to this the number of ciliary cells was reduced. Osteoclasts were seen in sparse numbers on the surface of the bone surrounding the sinus.

DISCUSSION

When analyzing clinical material, the time factor is often uncertain, that is, we seldom know the time of onset of the disease. In this study we induced an experimental chronic, purulent infection or an acute pneumococcal purulent infection in the maxillary sinus. In this animal model the timespan from induction of the sinusitis to the moment of examination is well known. The histological investigations were carried out to try to establish in what tissue component the radionuclide had been accumulated in. Due to the γ-radiation the resolution is not high enough to permit cellular correlation to the autoradiographic picture although different tissues (bone, mucosa etc.) can be localized and identified by combining the autoradiographic film and the histological section. A β-emitting nuclide would be preferable as we found in the first series (A). On the other hand, the specificity to bone seems to be too low, as no increase in uptake of ^{45}Ca in the bone surrounding the infected sinus could be seen. The increased uptake of $^{99}Tc^{m}$-DP in bone found in this material can not be correlated to a specific, single factor. The crystals in the bony surface, hydroxyapatite and thus Ca and phosphorus are the major components suitable for tracer studies, and the diphosphonates interact with hydroxyapatite (Fleisch and Russel, 1969). Areas of accelerated bone turnover will allow exchange of bone minerals components with interstitial fluid ions at an increased rate. This is true whether the bone tissue is in the normal growth process and remodelling or if a pathological process as a result of a trauma, infection or metastatic disease is in progress. In this experimentally induced sinusitis several factors can be responsible for an increased uptake (turnover). There is an increased blood flow (Johansson and Kumlien, 1988) in the mucosa with acute sinusitis and also an increased capillary leakage through the tissue, allowing an increased inflow of metabolites, active proteins, enzymes and therefore possibly also, $^{99}Tc^{m}$-DP (Persson and Erjefält, 1986). The increased blood flow in the sinus mucosa with an acute infection did not give rise to an accumulation of nuclide in the mucosa itself. Capillary leakage could possibly increase the turnover of mineral components and thus increase the uptake in the bone. An increased capillary leakage in the sinus mucosa with a chronic purulent sinusitis can be expected since many substances responsible for the capillary leakage are produced by the granulocytes and lymphocytes (Vinge and Lidbom, 1981). The presence of osteoclasts in the tissue indicates a remodelling of bone tissue, which in turn, can explain an increased uptake. When comparing histological preparations from other studies (Kumlien and Schiratzki, 1985; Johansson et al., 1988) no osteitis was found, supporting the non-specific character of the increased uptake in the bone in the infected sinus.

CONCLUSION

An increased uptake of $^{99}Tc^{m}$-DP in the bone surrounding the sinus cavity with an infected mucosa could be seen. This uptake could be seen four days after induction of an acute, purulent, pneumococcal sinusitis, and five months after the induction of a purulent, chronic sinusitis. No increase in uptake could be seen in the mucosa of the sinus with purulent infection. The β-emitting nuclide gave better resolution in autoradiography than did the γ-emitting nuclide, and would give better possibilities in identifying structures, however the specificity to bone of ^{45}Ca was not high enough to be suitable.

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