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Double-labelling technique in the evaluation of nasal mucoadhesion of disodium cromoglycate microspheres

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Summary

^{99m}Tc-labelled disodium cromoglycate particles and ^{111}In-labelled microspheres of disodium cromoglycate and polyacrylic acid were administered simultaneously to the nasal cavities of four healthy volunteers. The initial deposition patterns of the inhaled powder mixture as well as the drug removal due to the mucociliary clearance were monitored by a gamma camera. The double-labelling technique enabled in vivo comparison of simultaneously administered plain drug particles and polyacrylic acid microspheres of disodium cromoglycate. After administration, both the plain drug particles and the microspheres distributed equally effectively. On average, 27% of the ^{99m}Tc-labelled plain drug particles and 50% of the ^{111}In-labelled microspheres were retained at the initial site of deposition 30 min after inhalation. Thus, the removal of microspheres from the nasal cavities was clearly impeded by polyacrylic acid.

Introduction

Nasal delivery of drugs is affected by rapid mucociliary clearance that sweeps foreign material towards the pharynx (Pennington et al., 1988). Mucoadhesive preparations have been developed to increase the contact time between a dosage form and mucosal layers of nasal cavities thus enhancing drug absorption (Illum, 1987). Mucoadhesive properties are conventionally studied with several in vitro methods using either artificial or animal membranes (Marvola et al., 1982; Ch'ng et al., 1985; Ranga Rao and Buri, 1989; Saettone et al., 1989). Although these in vitro techniques give valuable information about the behaviour of mucoadhesive dosage forms, interactions of a formulation with physiological processes cannot be predicted reliably. In vitro observations which show association of materials with mucus should be regarded with caution, since in vivo mucosal turnover is high and mucoadhesion for drug delivery can only be utilized if the mucus is still attached to the epithelium (Wilson, 1990). At present there exist, however, only a few accepted methods for the determination of in vivo mucoadhesion (Wilson, 1990). The

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aim of this study was to apply the double-labelling technique to study nasal mucoadhesion. Deposition patterns of simultaneously administered mucoadhesive microspheres and normal drug particles were evaluated by a gamma camera.

Materials and Methods

The plain disodium cromoglycate (DSCG) (BP 1988, Fermion, Finland) particles were labelled with ^{99m}Tc modifying the spray-drying technique previously described by Vidgren et al. (1987).

The mucoadhesive microspheres were prepared by dissolving polyacrylic acid (PAA) (Carbopol 934, Goodrich Chemicals, U.K.) in 120 ml water to give a 1% w/w solution. An equal amount of DSCG was added. Just before spray-drying ^{111}In in 0.9% w/w sodium chloride solution, 0.25 ml in volume, was mixed with the drug polymer solution. The spray-drying took place (Buchi 190 Mini Spray Dryer, Germany) at a feed rate of 250 ml/h. The air inlet and output temperatures were 218 and 152°C, respectively.

Particle shape and particle size distribution were evaluated from scanning electron micrographs (Jeol Scanning Electron Microscope, type 35, Japan). Feret's diameter of 200 particles was measured.

Equivalent weight amounts (1 : 1 : 1) of ^{99m}Tc-labelled DSCG particles, ^{111}In-labelled PAA-DSCG microspheres and α-lactose monohydrate (325 mesh, DMV, The Netherlands) were mixed. Each dose contained 20 mg of the powder mixture with activities of 140–171 μCi (^{99m}Tc) and 60–76 μCi (^{111}In).

Four healthy non-smoking volunteers, two female and two male, took part in the in vivo test. None of the subjects had nasal problems and all were free of upper respiratory diseases. The volunteers were fully informed about the nature of the study which was approved by the Ethical Committee of Kuopio University Central Hospital, Kuopio, Finland. The study was carried out under medical supervision.

Each dose was spread on a glass plate as a thin stripe. A specially designed straw with a diameter of 4 mm and a length of 80 mm was used to inhale the powder. The straw was placed 0.5 mm deep in the nostril and the other nostril and the mouth were held closed while the inhalation took place. The volunteers blew their noses before the experiment and avoided snuffling, sneezing and coughing during the experiment.

The radiotracers were monitored using a gamma camera. The camera had a 40 cm field of view and it was fitted with a medium-energy parallel-hole collimator. The equipment was tuned to detect simultaneously the 171 and 245 keV radiation of ^{111}In and 140 keV radiation of ^{99m}Tc. The two images were recorded separately by a computer. During the measuring period the head of the subjects was supported under the chin and over the forehead to keep the examined area immobile. At the end of the monitoring period a profile of the subject's head was obtained by outlining the head with a radioactive point source. This was carried out in order to display the distribution of the powder preparation. Lateral images of 60 s duration were recorded over a period of 30 min. In addition, for one subject, images were recorded 150 min after administration. The recorded images were analyzed by drawing a region of interest around the initial site of deposition. The data were collected to the Gamma-11 system with a PDP 11/34 computer (Digital Equipment Corp., MA, U.S.A.) with a 64×64 collection matrix. The counts in each image were determined and corrected for the cross-talk resulting from the scattering of high-energy photons of ^{111}In into the energy window of ^{99m}Tc. The results were also corrected for background radiation and for time decay.

The statistical significance of differences in retention of plain DSCG particles and PAA-DSCG microspheres in the initial site of deposition was tested using a Student's t-test. A P-value < 0.05 was considered to be statistically significant.

Results

According to the scanning electron micrographs the plain spray-dried DSCG particles were nearly spherical, whereas the microspheres were

more irregular and partly shrunken (Fig. 1). The mean particle size (±S.D.) of the plain DSCG particles and the PAA-DSCG microspheres was 5.4 ± 3.2 and 5.0 ±2.8 μm, respectively. About 50% of the particles were larger than 4 μm.

After the administration of the powder dose the plain DSCG particles and the PAA-DSCG microspheres covered initially a relatively small and similar surface of the nasal cavities (Fig. 2A). The gamma camera pictures illustrated clearly the removal of the labelled particles from the initial site of deposition by mucociliary clearance (Fig. 2B). The removal of the plain drug particles was fastest during the first 5 min (Fig. 3). This indicated the initial deposition of the drug particles at the ciliated areas of the nasal cavities. After the 5 min measuring period, on average 30% of the ^{99m}Tc-labelled plain drug particles were removed from the initial site of application. The calculated first-order half-time of clearance (±S.D.) of the plain DSCG particles was 14.4 ± 3.8 min. At the end of the recording period only about 28% of the initial activity was detected at the initial site of deposition.

Mucoadhesion was clearly detected when the removal of the PAA-DSCG microspheres was monitored (Fig. 3). The initial deposition at the ciliated parts of nasal cavities resulted in the loss of activity of 20% during the first 5 min. The first-order half-time of clearance of the PAA-

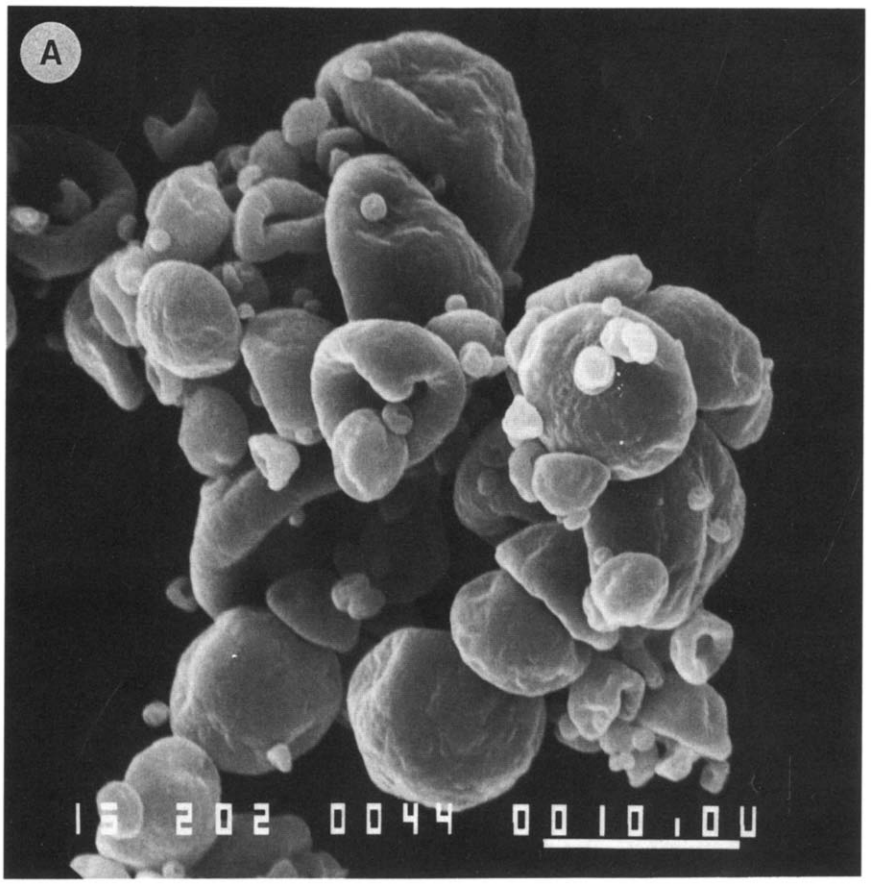

Fig. 1. Scanning electron micrographs of ^{99m}Tc-labelled plain disodium cromoglycate particles (A) and ^{111}In-labelled microspheres containing disodium cromoglycate and polyacrylic acid (50:50) (B). Bar = 10 μm.

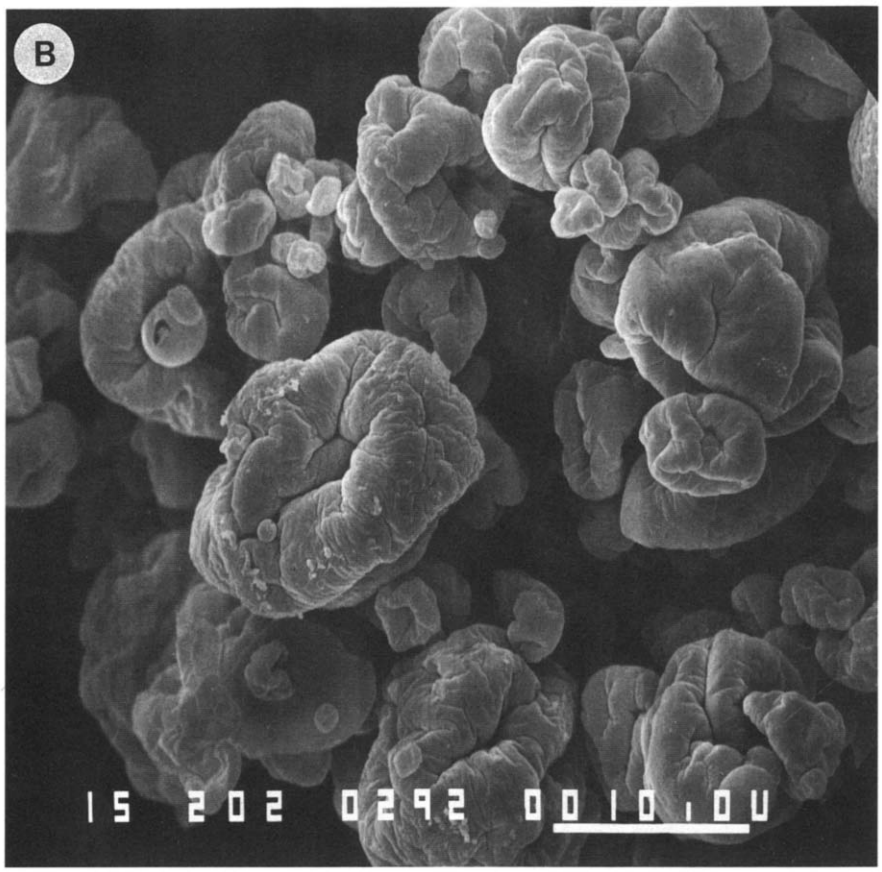

Fig. 1B.

DSCG microspheres was 27.8 ± 2.0 min. Thus, about 50% of the microspheres remained at the initial site of deposition at the 30 min recording point. For one person retention of activity at 150 min was detected. A 4-fold greater amount of PAA-DSCG microspheres (36%) than plain DSCG particles (8%) remained at the initial site of deposition in nasal cavities.

Discussion

The double-labelling technique has previously been used in disposition and transition studies of oral dosage forms (Daly et al., 1982; Davis, 1986). One radiotracer is usually used to visualize the gastrointestinal tract, and the other to monitor the disposition of the swallowed dosage form. To the best of the authors' knowledge, the double-labelling technique has not previously been applied to in vivo mucoadhesion studies in human nasal cavities.

The benefit of this technique in comparison studies of intranasally delivered dosage forms is that the effects of physiological changes and the mode of administration can be minimized. The ciliary activity varies at different parts of the nose (Newman et al., 1987). Therefore, the differences in depth and insertion angle of the delivery system (Aoki and Crawley, 1976; Pennington et al., 1988), as well as in the inspiration volume, may result in different initial deposition patterns and thus different drug removal rates of the inhaled drug particles. Also, the clinical status of healthy nasal cavities may change at different times (Newman et al., 1987), and thus cause additional

variation in comparison studies. Furthermore, pathological changes may affect ciliary activity and rheological properties of mucus and thus change the removal rate of the inhaled particles (Hardy et al., 1985; Paludetti et al., 1988). Most of these problems, which can occur even though the volunteers are used as their own controls, can be overcome by dosing the examined particles simultaneously. According to the results of this study, the double-labelling technique can be applied to overcome the above-mentioned intra-individual variation when studying the in vivo mucoadhesion of differently formulated nasal preparations.

According to the gamma camera evaluation, the spray-dried particles were suitable for testing the usefulness of the double-labelling technique in nasal mucoadhesion studies. Rabbe and coworkers (1977) have suggested that 4 μm is a sufficient particle size for intranasally administered drugs. On the other hand, Illum (1987) has considered that 10 μm particle size is the most suitable for nasal administration. Particles smaller than 1 μm pass the nasal cavities with the inspired air, whereas particles larger than 10 μm deposit at the anterior parts of the nose and thus avoid ciliated absorption areas (Mygind, 1978).

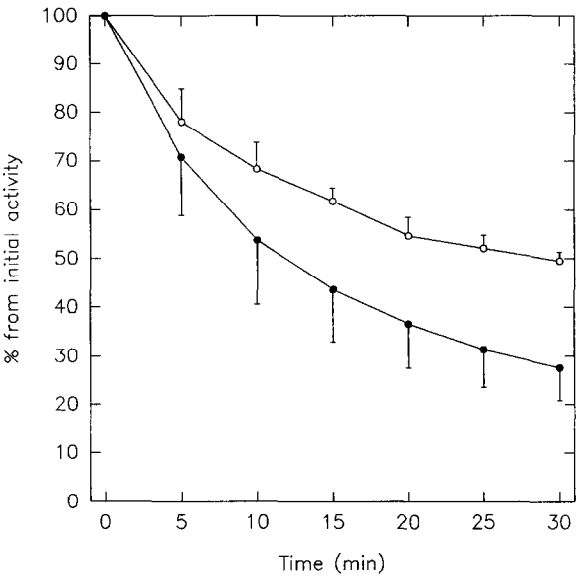

Fig. 3. Removal of the ^{99m}Tc-labelled plain disodium cromoglycate particles (●) and ^{111}In-labelled microspheres containing disodium cromoglycate and polyacrylic acid (50:50) (○) from their initial site of deposition as a function of time.

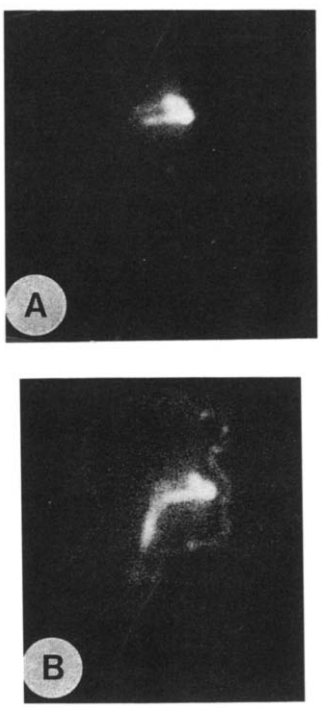

Fig. 2. Typical gamma camera pictures illustrating the removal of the intranasally administered ^{99m}Tc-labelled plain disodium cromoglycate particles and ^{111}In-labelled microspheres containing disodium cromoglycate and polyacrylic acid (50:50) (A, initial deposition; B, after 30 min).

The plain DSCG particles and PAA-DSCG microspheres were distributed equally effectively in the nasal cavities. Thus, the slight difference in the particle size as well as in the particle shape did not have a significant effect on the aerodynamic behaviour of the inhaled particles. The plain DSCG particles and the PAA-DSCG microspheres were therefore subject to similar physiological effects. The differences observed in the removal of the components from their initial site of administration were thus mainly due to the properties of the individual particles.

It has been pointed out that only hydrated polymers possess adequate mucoadhesive properties (Bremecker, 1990). The properties of a mucoadhesive preparation may have a great effect

on the initial hydration phase (Illum, 1986). In this study the removal of the PAA-DSCG microspheres immediately after administration was almost as rapid as the removal of the plain DSCG particles. This may be due to the delayed swelling of the polymer. Thus, the in vivo hydration of PAA-DSCG microspheres takes several minutes. After hydration, the clearance rate of the microspheres clearly diminished, which demonstrated the mucoadhesive effect of polyacrylic acid in disodium cromoglycate microspheres.

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