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Morphological examination of rabbit nasal mucosa after exposure to acetylsalicylic acid, glycofurol 75 and ephedrine

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Abstract

The potential local toxicity of glycofurol 75 and 30% acetylsalicylic acid in glycofurol 75 for nasal application has been evaluated in rabbits. The commercially available 1% ephedrine nose drops were used as a clinical relevant standard. Acetylsalicylic acid may be of potential use in acute myocardial infarction, where the cost/benefit of an instant nasal application may be favourable. 4 h after application of nasal solutions, 50 μl into each nostril, the mucosa of septae and conchae were examined with respect to gross and histopathological changes. All solutions tested resulted in lesions that exceeded normal variations. Most of the inflammatory reactions in the acetylsalicylic acid group seemed reversible within 7 days, at which time, however, osteoblast and osteoclast activity and formation of osteoid were pronounced.

Keywords: Nasal administration; Histology; Toxicology; Acetylsalicylic acid; Glycofurol 75; Ephedrine; Rabbit

1. Introduction

Recent studies (Husted et al., 1989) have shown that acetyl salicylic acid (ASA) is beneficial in the treatment of acute myocardial infarction (AMI). An intravenous dose of 100 mg ASA has been shown to inhibit completely the synthesis of thromboxane A_2 (TXA_2), a potent thrombocyte aggregate and vasoconstrictor (Husted et al., 1989). ASA also affects the synthesis of prostaglandin I_2 (PGI_2) in endothelial cells. The effect of PGI_2 is opposite to that of TXA_2, but it demands a higher concentration of ASA to inhibit the synthesis of PGI_2 than that of TXA_2, and endothelial cells are able to synthesize new enzymes necessary for the synthesis, in contrast to platelets.

In addition, low-dose ASA intravenous treatment of patients with AMI has not demonstrated any side effects (Husted et al., 1992), and it is a cheap medication. Unfortunately, the treatment demands the presence of professional staff. In practice, this means a delay in starting the treatment. For this reason a nasal formulation of ASA seems to be beneficial in the instant treatment of AMI.

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To humans it is possible to administer about 50–150 μl into each nostril. Therefore, administration of sufficient ASA (approx. 100 mg) demands a highly concentrated solution (at least 300 mg/ml). Assuming a high bioavailability of the formulation, a 30% w/v solution of ASA might be of clinical relevance.

Glycofurol 75 (GF) was chosen as the solvent, since it was found to be the only solvent fulfilling the requirements with respect to solubility of ASA and low local toxicity. Due to the instability of ASA in water, aqueous solutions cannot be used.

The assumption of a high bioavailability of ASA is justified by pilot studies showing the bioavailability to be about 83% (to be published). Hussain et al. (1992) have also found a high bioavailability (100%) of ASA from the rat nasal cavity, however, the dose was only 2 mg administered in an aqueous solution and the nasal cavity was ligated by surgical procedures to prevent clearance of the formulation from the cavity.

As ASA is known to cause gastric bleeding and stomach upset, it gives reason for concern as to the toxicity of a 30% w/v ASA solution administered nasally. Three times daily application of 30% GF in polyethylene glycol 200 to rabbits and a single application of 10% GF in polyethylene glycol 200 to humans have been found acceptable (Bechgaard et al., 1991).

Due to the above-mentioned factors, the toxicity of a 30% w/v ASA solution in GF, pure GF, and of the commercially available ephedrine nose drops was assessed. The latter was included in order to have some kind of control, in which the effects on the nasal tissue were officially accepted.

To study the toxicity of nasal formulations, various in vitro and in vivo techniques have been used including measurements of the mucociliary clearance (Gizurarson et al., 1990; Schipper et al., 1991) measurements of electrophysiological properties (Ussing chamber technique) (Wheatley et al., 1988; Bechgaard et al., 1993), erythrocyte haemolysis measurements (Hirai et al., 1981), influence on the viability of cell cultures (Jørgensen et al., 1993), and pathological studies (Chandler et al., 1991).

A combination of macroscopic observations of the rabbit nasal cavity and a histological examination of the rabbit nasal septae and conchae have been used in the present study.

2. Experimental

2.1. Materials

Ephedrine nose drops 1 mg/ml were from Nycomed DAK, (Copenhagen, Denmark), glycofurol 75 was purchased from Roche (Basle, Switzerland) and acetylsalicylic acid (Ph. Eur. 2nd Edn) was obtained from Nomeco (Copenhagen, Denmark).

2.2. Animals

Male and female New Zealand White rabbits were obtained from Hvidesten (Denmark). They were housed individually with free access to water and given approx. 100 g of Altromin no. 2123 from Chr. Petersen, Ringsted (Denmark) daily. The rabbits were 2–3 months old and weighed 2.3–2.6 kg at the beginning of the experiments.

2.3. Experimental design

The experiments were conducted in conscious rabbits, as pilot studies have shown no need for anaesthesia.

All preparations were administered with the rabbits in a supine position. The rabbits were kept in this position for 1 min after administration. Nasal solutions (50 μl) were administered with an Eppendorf Multipipette into each nostril.

2.4. Nasal solutions

The nasal solutions used were pure GF, 30% ASA in GF, and the commercially available ephedrine nose drops containing 1% ephedrine hydrochloride.

2.5. Post-mortem procedures

The rabbits were sedated by an injection of sodium pentobarbital solution 50 mg/ml (2–3

Table 1
Score of gross lesions in the nasal mucosa of untreated rabbits (control, $n = 4$) and rabbits inoculated intranasally with 1% ephedrine (ED, $n = 4$), glycofurol 75 (GF, $n = 4$) and 30% acetylsalicylic acid in GF (ASA, $n = 3$)

Treatment	Observation time	Exudation	Congestion
Control	4 h	0	0
–	–	0	+ +
–	–	0	0
–	–	0	+ +
ED	–	0	0
–	–	0	0
–	–	0	0
–	–	0	0
GF	–	0	0
–	–	+ +	+
–	–	+ +	+
–	–	+ +	+
ASA	–	+ +	+
–	–	+ +	+ +
–	–	+ + +	+ +
–	24 h	+	+ +
–	–	+ + +	+ + +
–	–	+ + +	+ + +
–	3 days	+	+
–	–	+ + +	+
–	–	+ +	+ +
–	7 days	0	0
–	–	0	0
–	–	+	+ +

Exudates: 0, none; +, small strands or clumps; + +, moderate local clumps; + + +, widespread clumps. Congestion: 0, none; +, slight; + +, moderate; + + +, intense.

ml), and bled to death by an incision in the carotid artery. Immediately after death, the nasal cavity was opened by removing the nasal and maxillary bones. The nasal mucosa was inspected and recordings were made of the gross appearance. The septum and the ventral nasal conchae were removed separately and fixed in 10% neutral buffered formalin. After fixation the conchae were decalcified in a 14% solution of ethylenedinitrilotetraacetic acid for at least 2–3 days. The fixed nasal septae and the decalcified conchae were then routinely processed by imbedding in paraffin.

The septae and conchae were sectioned at 5 μm longitudinally as close to the middle as possible. The sections were stained with haematoxylin-eosin, and selected slides were stained with Luna's stain for eosinophilic granulocytes, with periodic acid-Schiff reagent (PAS) for ground substance, and with Van Gieson's stain for collagen.

3. Results

3.1. Gross lesions

A detailed score of the gross lesions appears in Table 1.

In two of the control rabbits a slight, bilateral congestion of the conchal mucosa was observed. No gross changes were observed in the rabbits killed 4 h after inoculation with ephedrine. All the rabbits inoculated with GF displayed redness of a normal character 4 h after inoculation. Slight, greyish exudates, however, were found bilaterally in two of these animals between the septum and concha, and in a third animal a unilateral exudate was detected.

Table 2
Score of main histopathological features in the septae of rabbits inoculated intranasally with 1% ephedrine, glycofurol 75 (GF), and 30% acetylsalicylic acid in GF (observation time indicated between parentheses)

Histopathological features	Control (4 h)	Ephedrine (4 h)		Glycofurol 75 (4 h)		Acetylsalicylic acid			
	$n = 4$	$n = 2$	$n = 2$	$n = 1$	$n = 3$	(4 h) $n = 3$	(24 h) $n = 3$	(3 days) $n = 3$	(7 days) $n = 3$
Epithelial eosinophilia	+	+	+ + +	+	+ + + +	+ + + +	+ + +	+ + +	+
Inflammation	0	0	+ +	+ +	+ + + +	+ + + +	+	+	0
Exudates on mucosa	0	0	0	0	0	+ + +	+ + +	+ + +	0
Epithelial vacuolation and swelling	0	0	+	0	+ +	+ + + +	+	+	0

0, not present or negligible; +, normal (epithelial eosinophils and activated osteoblasts) or slight (other features); + +, moderate; + + +, marked; + + + +, very marked.

Table 3
Score of main histopathological features in the conchae of rabbits inoculated intranasally with 1% ephedrine, glycofurol 75 (GF), and 30% acetylsalicylic acid in GF (observation time indicated between parentheses)

Histopathological features	Control (4 h)	Ephedrine (4 h)		Glycofurol 75 (4 h)		Acetylsalicylic acid			
	$n = 4$	$n = 2$	$n = 2$	$n = 1$	$n = 3$	(4 h) $n = 3$	(24 h) $n = 3$	(3 days) $n = 3$	(7 days) $n = 3$
Epithelial eosinophilia	+	+ +	+ +	+ +	+ + +	+ + + +	+ + +	+ + +	+
Inflammation	0	0	+ +	+ +	+ + + +	+ + + +	+ + + +	+ + + +	+ +
Exudates on mucosa	0	0	+ + +	0	+ + +	+ + + +	+ + + +	+ + + +	+ +
Epithelial vacuolation and swelling	0	0	+ +	0	+ +	+ +	+ +	+ +	+
Epithelial necrosis	0	0	0	0	+ + +	+ + +	+ + +	+ + +	+ +
Activated osteoblasts	+	+ +	+ +	+ +	+ +	+ +	+ + +	+ + +	+ + + +
Osteoclasts	0	0	+	0	+	+ +	+ + +	+ + +	+ + +
Osteoid	0	0	0	0	0	0	0	0	+ +

0, not present or negligible; +, normal (epithelial eosinophils and activated osteoblasts) or slight (other features); + +, moderate; + + +, marked; + + + +, very marked.

Slight to heavy, bilateral, septal and conchal congestion and exudation were observed in almost every rabbit killed 4 h, 24 h and 3 days after inoculation of ASA. It was not possible to see whether part of the redness was due to haemorrhages in cases where the mucosa was heavily congested. It was almost constantly noted that the predominant changes were found in the anterior two-thirds, approximately, of the conchae. One of the rabbits killed 7 days after inoculation with ASA showed unilateral, moderate congestion and slight, bilateral exudation.

3.2. Light microscopy

A detailed score of the main histopathological features in septae and conchae appears in Tables 2 and 3.

3.2.1. Controls (4 h)

In all four control animals, the septae (Fig. 1) and conchae (Fig. 2) displayed no pathological changes. A few eosinophils and small aggregates of lymphocytes which are normally present in the

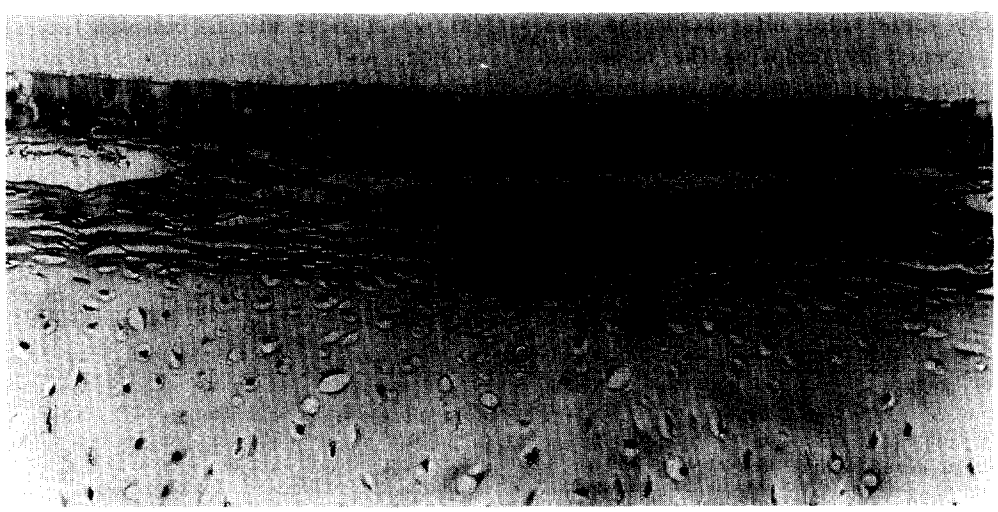

Fig. 1. Nasal septum of control rabbit. Epithelium (E), lamina propria (P), and septal cartilage (C). HE. ×250.

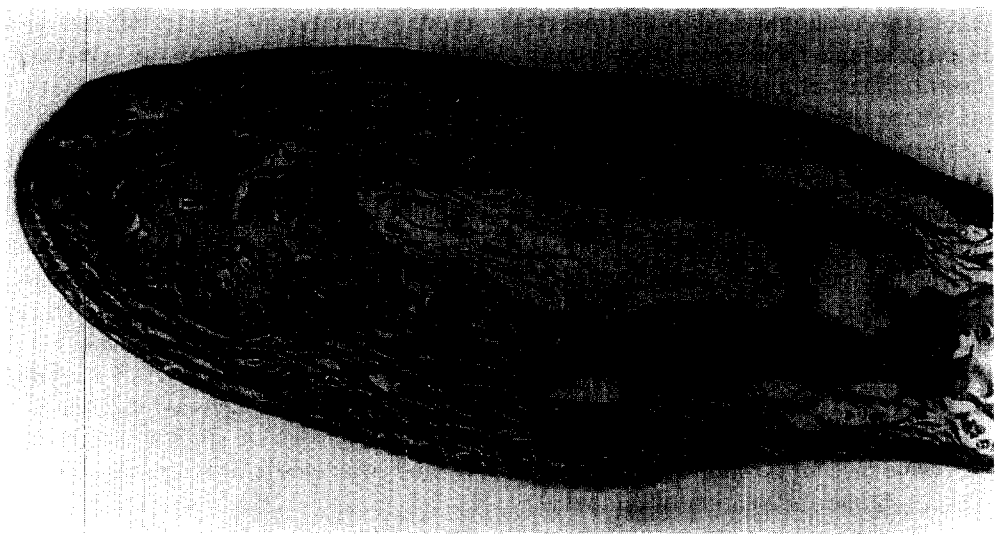

Fig. 2. Nasal concha of control rabbit. Epithelium (E), lamina propria (P), bony spicules (S), and a narrow brim of scattered, inactive osteoblasts (arrow heads). HE. ×250.

nasal mucosa of animals were occasionally observed. The moderate degree of hyperaemia, visible on gross inspection in two of the rabbits, was reflected histologically in a slightly dilated vascular plexus in the lamina propria and submucosa.

Osteoblasts lining the bony spicules of the conchae were mostly inactivated, i.e., the cells were flattened with indistinct cytoplasm and with a position parallel to the surface of the spicules. A few osteoblasts were activated, i.e., they were plump, cuboidal to columnar cells with basophilic cytoplasm towards the bone surface and with the nuclei located at the end of the cell away from the bone surface.

3.2.2. Ephedrine (4 h)

In two rabbits, the septae and conchae showed histological features very similar to those of the controls except for the fact that there was a slight increase in the number of eosinophils and activated osteoblasts in the conchae of the rabbits inoculated with ephedrine.

In the other two rabbits, most parts of the septae showed normal features, but at local areas of the respiratory region vacuolation and hypertrophy of the epithelial cells were seen. In the same areas a moderate inflammatory reaction was noted in the soft tissues dominated by eosinophils, oedema, and hyperaemia. Eosinophils were present in the lumen of many small blood vessels. Extravascular eosinophils were mainly found near the epithelium. The vestibular regions were normal. Widespread exudates with eosinophils were seen on the mucosa of the conchae from both rabbits. Throughout the approximate two-thirds of the rostral part of the conchae, the epithelium was moderately infiltrated by eosinophils. Moderate inflammation dominated by eosinophils, oedema, and hyperaemia was seen in the lamina propria and submucosa. Many small blood vessels contained eosinophils. Epithelial vacuolation and hypertrophy were conspicuous in some areas, and aggregates of lymphoid cells were frequent in one of these rabbits. The number of osteoclasts and activated osteoblasts were slightly increased compared to the controls, and the vascular congestion was marked. The aboral one-third, approximately, of the conchae were without any essential changes.

3.2.3. Glycofurol (4 h)

In one rabbit, a slight infiltration by eosinophils was seen in the epithelium of the respiratory and

olfactory regions of the septum and in the underlying lamina propria and submucosa, where they formed an essential part of a moderate inflammatory reaction. There were more eosinophils in the superficial layers below the epithelium than deeper close to the septal cartilage. An almost identical inflammatory reaction was observed in the anterior two-thirds, approximately, of the conchae, and the number of activated osteoblasts were slightly increased.

In the other three rabbits, the epithelium of the septae was heavily infiltrated by eosinophils which were also found in the lumina of many small blood vessels. A heavy inflammatory reaction with an abundance of eosinophils was seen in the soft tissues. Some vacuolation of epithelial cells was seen in the respiratory and olfactory regions. The vestibular region, however, was almost free of cellular infiltrations and any other changes.

In the above-mentioned three rabbits, exudates with eosinophils and desquamated epithelial cells were frequently observed covering eroded epithelium in part of the anterior two-thirds, approximately, of the conchae, but were also detected lying free without any epithelial attachment. Eroded epithelium and epithelium with vacuolated, hypertrophic cells were seen with and without association with exudates. Lamina propria and submucosa – particularly in the anterior two-thirds of the conchae – were heavily infiltrated by inflammatory cells dominated by a great number of eosinophils. Fibrin, haemorrhages, hyperaemia, and oedema were frequent findings, and made up part of the inflammatory reaction. Lymphoid cells arranged in follicles were occasionally seen. A slightly increased osteoblastic and osteoclastic activity was observed.

3.2.4. Acetylsalicylic acid (4 h)

The septae from all three rabbits showed an almost identical picture. There was a pronounced infiltration by eosinophils in the epithelium of the respiratory and olfactory regions. A heavy inflammation dominated by eosinophils was noted in the underlying soft tissues, but with the greatest

Fig. 3. Concha of rabbit 4 h after intranasal application of acetylsalicylic acid. Cellular inflammatory reaction (I) dominated by eosinophilic granulocytes. Cellular and fibrinous exudate on the mucosal surface (X). Similar changes were seen in the septae and conchae of rabbits 4 h after application of glycofurol 75 and ephedrine. HE. ×250.

concentration of the eosinophils in the superficial layers. In several places there was a pronounced vacuolization and swelling of the epithelial cells in the regions mentioned. Exudates containing eosinophils and desquamated epithelial cells were present covering eroded epithelium or lying free on the mucosa. The cellular infiltrations and the epithelial changes commenced abruptly at the point of transition between the vestibular and respiratory regions, i.e., the vestibular region was normal in all three rabbits.

The conchae from the three rabbits also showed an almost identical picture (Fig. 3 and 4). The most pronounced lesions were found in the anterior two-thirds of the conchae. A massive infiltration by inflammatory cells, particularly eosinophils, was almost universally present in the epithelium. Exudates with eosinophils, fibrin, desquamated epithelial cells, and cellular debris were frequently seen on the mucosa or free between the scrolls of the conchea. Congestion, haemorrhages, oedema, and deposition of fibrin with a pronounced mixture of eosinophils made up the main ingredients of a very marked inflammatory reaction in the lamina propria and submucosa. In addition, some increased osteoblastic and osteoclastic activity was evident on the surface of the lamellar bone plates.

3.2.5. Acetylsalicylic acid (24 h)

The septae from all three rabbits were generally normal, but in the respiratory and olfactory regions a few small areas with epithelial vacuolation and swelling were seen in association with a rather intense infiltration by eosinophils in the epithelium and underlying soft tissues, particularly in the superficial layers close to the epithelium.

The conchae exhibited changes similar in histopathological features to those in the conchae from rabbits killed 4 h after exposure to ASA. Only was the osteoblastic and osteoclastic activity around the bone spicules more pronounced.

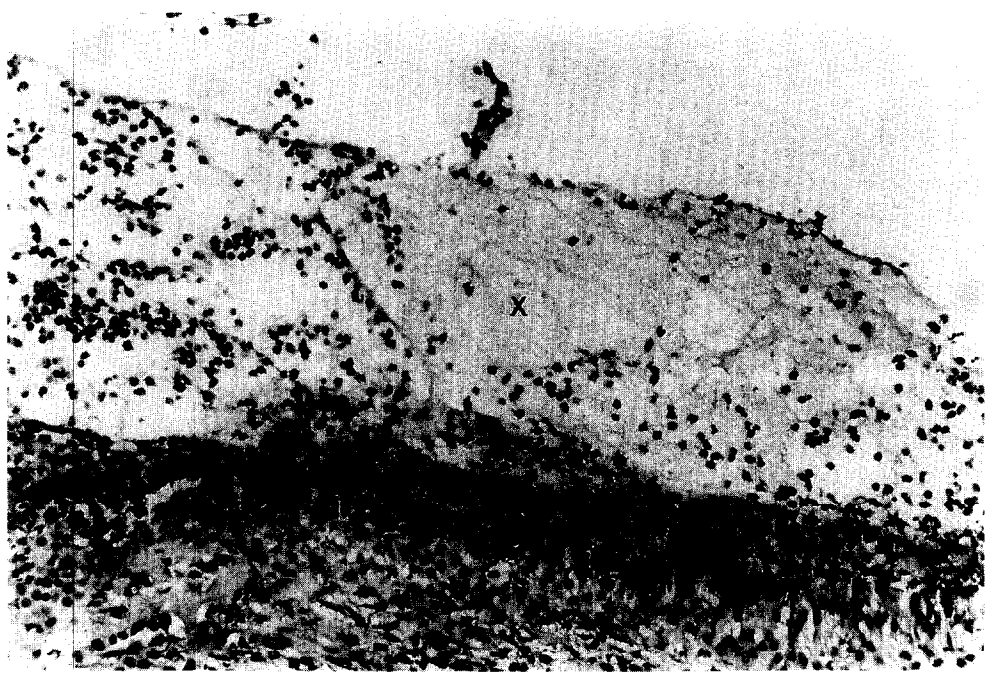

Fig. 4. Concha of rabbit 4 h after intranasal application of acetylsalicylic acid. Cellular and fibrinous exudate (X) on the mucosa inflamed and partially necrotic epithelial lining (arrow heads). Similar changes were seen in the septae and conchae of rabbits 4 h after application of glycofurol 75 and ephedrine. HE. ×250.

3.2.6. Acetylsalicylic acid (3 days)

The septae and conchae from all three animals had lesions of the same character and to the same degree as was seen in the preceding group of rabbits observed 24 h after application of ASA.

3.2.7. Acetylsalicylic acid (7 days)

The septae from all three animals showed a degree of epithelial and subepithelial infiltration by eosinophils not exceeding what was found in the control animals. The conchal epithelium and the lamina propria and submucosa was less affected than in the rabbits killed 3 days after exposure to ASA as regards epithelial vacuolisation, swelling and necrosis, eosinophilia, inflammation, and formation of exudates.

The most characteristic features of the conchae of these three rabbits were (1) a widespread osteoblastic and osteoclastic activity including a conspicuous re-modelling of bone spicules, (2) a proliferation of mesenchymal cells lying in a matrix of oedematous ground substance, and (3) a spotwise formation of osteoid in relation to the increased osteoblastic activity and the mesenchymal proliferation (Fig. 5).

4. Discussion

The macroscopical examination (Table 1) revealed that the nasal mucosa of half the control rabbits showed a moderate congestion. It was only surpassed by two of the three rabbits inoculated with ASA killed 24 h after inoculation. When that is compared to the degree of congestion observed in the other groups of rabbits, it appears that the degree of congestion of the nasal mucosa is not a reliable parameter from which to judge the possible local effect of an intranasal application of drugs.

Exudation on the nasal mucosa is evident in rabbits inoculated with ASA and killed 4 h, 24 h, and 3 days after inoculation. However, GF – the vehicle for the ASA formulation – has produced

Fig. 5. Concha of rabbit 7 days after intranasal application of acetylsalicylic acid. Newly formed osteoid (O) surrounded by a brim of activated osteoblasts (arrow heads). HE. ×400.

moderate exudation in three out of four rabbits which indicates that the exudation in the rabbits treated with ASA is most likely to be a combined effect of the ASA and GF.

The histological examination of the septae (Table 2) showed a rather stereotype pattern of reaction. Regardless of the treatment, there was epithelial eosinophilia and inflammation with features quite similar from group to group, but varying in intensity – most intense in rabbits inoculated with GF and ASA and killed 4 h after inoculation. The great similarity between the latter two groups again suggests that the effect observed in the rabbits inoculated with ASA is a combined effect as mentioned above. This suggestion is supported by the results of the histological examination of the conchae (Table 3).

The fact that exudates were only found on the septae of rabbits inoculated with ASA strongly indicates that, after all, the damage to the septal mucosa in these rabbits was more severe than in the other groups.

It is worth noting that half the rabbits inoculated with the commercially available ephedrine nose drops had a moderate inflammation in the septae.

The observation that the vestibular regions in all the rabbits were free of essential changes, even in the most affected animals, indicates that the vestibular epithelium (keratinized stratified squamous) is more resistant to external stimuli than the respiratory and olfactory epithelia (ciliated pseudostratified columnar).

The histological examination of the conchae (Table 3) revealed the same stereotype pattern of reaction as seen in the septae. Exudation was present not only in the groups treated with GF and ASA, but also in the group inoculated with the ephedrine nose drops, thus demonstrating that the application of the commercially available ephedrine nose drops has provoked a marked inflammatory reaction in half the treated animals not only in the conchae, but also in the septae as shown above. The reaction in the conchae even included an increased osteoblast activity.

The fact that only the approximate two-thirds of the rostral part of the conchae displayed major changes may be linked to the experimental procedures which included the application of the drugs in the rostral part of the nasal cavity.

ASA is known to inhibit osteoblast activation (Palmer, 1993), so when activated osteoblasts are seen in the conchae in all groups including the group treated with ASA, it is then possibly a side effect of the inflammatory reaction (Palmer, 1993).

The occurrence of osteoid in the conchae of the rabbits killed 7 days after application of ASA showed in combination with the activated osteoblasts and osteoclasts that the late effect of the administration of ASA was dominated by a marked influence on the bony fundament of the conchae. Future studies may show whether this influence can lead to permanent malformations of the bony structures of the conchae, or whether the changes are reversible.

A general problem with all predictive in vitro/in vivo tests is to extrapolate to the clinical situation. Therefore, the commercially available 1% ephedrine nose drops was also initially tested. As seen from Table 1, no gross lesions were observed after 4 h, but various histopathological changes were observed, especially inflammation and activated osteoblasts, indicating that long-term studies of both reversibility and repeated dosage of ephedrine may be of interest. Clinically, ephedrine may be dosed up to five times a day for a maximum of 1 week.

Relative to 1% ephedrine, GF resulted in more gross and histopathological changes. However, these changes were moderate and may partly be due to hyperosmolarity.

Most of the inflammatory reactions in the ASA group seemed reversible within 7 days, at which time, however, osteoblast and osteoclast activity and formation of osteoid were pronounced. Except for some initial snuffling all animals behaved normally, and had a normal food and water consumption. It is therefore concluded that nasal application of ASA may be relatively acceptable in AMI where a fast onset of fibrinolytic treatment is important. However, additional studies (more than 7 days) are necessary to provide more information on the reversibility os the changes in the bony structures of the conchae after a single application of the ASA formulation.

In other acute situations where the cost/benefit ratio is also favourable, GF may be used as a vehicle.

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