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Delay in migration of the corneal epithelium after incorporation of [^{3}H]-mannose in organ cultures

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PURPOSE. *Corneal epithelial cell surface carbohydrate moieties influence migration during wound healing. The purpose of the present study was to investigate the incorporation of galactose and mannose sugar during corneal epithelial migration and their effect on the rate of wound healing.*

METHODS. *Organ cultures of non-migrating (n=6) and migrating (n=30) corneal epithelia were prepared. The incorporation of [^{3}H]-galactose and [^{3}H]-mannose was followed in the migrating samples after 18, 24, 48 and 72h incubation and at 18h in the non-migrating samples. Wound size was documented at the same intervals and the rate of healing was established.*

RESULTS. *The migrating corneal epithelium of the galactose and mannose treated samples incorporated more radioactive sugar than the non-migrating samples. Mannose incorporation decreased with time whereas the rate of healing was significantly delayed (41±0.01 µm/h) compared to galactose treated samples (63±0.01 µm/h) and controls (61±0.01 µm/h) during the active healing phase (18-48h). Galactose incorporation increased with time.*

CONCLUSIONS. *Incorporation of mannose sugar has an inhibitory effect on the rate of migration during the linear healing phase of the corneal epithelium. (Eur J Ophthalmol 2003; 13: 360-4)*

KEY WORDS. *Corneal epithelium, Migration, Mannose, Galactose*

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INTRODUCTION

Carbohydrate moieties on the surface of corneal epithelial cells influence migration during wound healing (1-4). The expression of cell surface glycosylation of non-migrating and migrating corneal epithelia has been studied with lectin-binding profiles (5, 6). Using fluorescein-labeled lectin, soybean agglutinin that binds to N-acetyl-D-galactosamine, Ahmed and Rahi (5) found very strong fluorescence in preparations of normal corneal epithelia. Blockage of the N-acetyl-galactosamine delays corneal epithelial migration. The study also demonstrated that blocking glucose, mannose and glucosamine moieties inhibited corneal epithelial cell migration (6).

Increased binding of lectins to cell membranes of migratory corneal epithelia has been attributed not only to increased glycosylation of already present glycoconjugates but also to the synthesis of new glycoconjugates required for the normal migratory process (7-11). In view of these findings one would assume that increased extracellular concentrations of sugars could help speed up wound healing. However, Takahashi et al (12) recently reported that elevated extracellular glucose levels significantly delayed the migration of corneal epithelial cells but it is still not clear whether various other carbohydrates facilitate or retard wound healing in this tissue.

The present study focused on the utilization of man-

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nose and galactose moieties during migration and investigated whether incorporation of these sugars affects the rate of migration during wound healing of the corneal epithelium.

METHODS

Radioisotope incorporation in rabbit corneal epithelial organ cultures

All chemicals were purchased from Sigma Ltd. unless otherwise stated. Rabbit corneas (n=36) were cultured for 18, 24, 48 and 72h to prepare the migrating and non-migrating corneal epithelial samples under sterile conditions (Gelaire Flow Laboratories), as described previously (13). Each experiment was run in triplicate. The integrity of the corneal epithelium was checked with fluorescein before processing the cornea. Intact eyes were held with sterile gauze by the optic nerve and posterior globe and the epithelial surface was marked out with a 7-mm trephine. The epithelium within the trephined region was mechanically removed with a sterile scalpel blade (#10) under a dissecting microscope (Olympus M081). A 1-2 mm slit was made just outside the limbus with the scalpel blade and the cornea was excised along the limbus with a pair of corneal scissors. For non-migrating epithelium, corneas were excised similarly without scraping the epithelium.

The excised corneas were then placed in a sterile Petri dish (60 mm) containing sterile Hanks Balanced Salt Solution (HBSS) and washed three times in this solution. The HBSS was then removed and the corneas were disinfected with antibiotic-antimycotic solution (50 µl/cornea) for 5-7 minutes. The corneas were again rinsed in HBSS and transferred to modified Supplemental Hormonal Epithelial Media (SHEM) (1 ml/cornea). The migrating and non-migrating corneas were then placed in a CO_2 incubator at 37°C (95% O_2, 5% CO_2) (Queue) and the media was replaced after 24-36 h. Radiolabeled precursors were added in equal amounts during the last three hours of incubation using 3 µCi/ml of D-[6-^{3}H]-galactose (18.5 Ci/mmol; 1 mCi/ml) (ICN Pharmaceuticals, Inc) and D-[2-^{3}H]-mannose (30 Ci/mmol; 1 mCi/ml) (ICN Pharmaceuticals, Inc). Migrating and non-migrating corneal epithelia were harvested after 18, 24, 48 and 72h and 18h incubation respectively, using a scalpel blade (#10); 100 ml water was added to each sample, and the tissue was sonicated for 2 minutes. An equal volume of 15% (w/v) trichloroacetic acid (TCA) was then added, vortexed, and allowed to precipitate overnight at 4°C. After centrifugation (1600xg) (Sorvall), the supernatants were drawn off, the pellets were washed three times with 100 ml TCA (7.5 %) and dissolved in 200 µl of NaOH (0.2M). The total protein concentration for each preparation was determined by the micro-method of bicincomimic acid (BCA) protein assay (Pierce, Rockford, IL) using an ELISA reader (Dynatech Laboratories). The radioactive counts were taken in a LS-6500 Scintillation Counter (Beckman).

Wound healing

The progress of healing was monitored after 18, 24, 48 and 72h incubation. The corneas were removed from the medium, placed with the epithelial side down in one drop of Richardson stain for 1 min, then washed by repeated dipping in phosphate buffered saline. The wound diameter (stained region) was measured with a ruler (14). Linear regression analysis was done and the rate of healing was calculated.

RESULTS

Figure 1 shows the incorporation of [^{3}H]-mannose and the healing pattern in the migrating and non-migrating and control samples. Incorporation declined with time. Non-migrating corneal epithelium incorporated 54 ± 2.08 cpm of [^{3}H]-mannose/mg of protein, and the migrating corneal epithelium incorporated 265 ± 8.66 cpm 18h after wounding. A sharp decrease was seen by 24h (86.5 ± 0.76 cpm/µg of protein). Mannose treated samples showed average mean wound areas of respectively 29.91 ± 1.64 and 17.25 ± 2.35 and 8.89 ± 1.83 mm^2, at 18, 24 and 48h (Fig. 3). The [^{3}H]-mannose treated samples showed a slight non-linear healing curve (r^2=0.69; $p<0.01$) during the active healing phase, i.e. 18-48 h (Fig. 1). In addition, the rate of healing was markedly delayed in the [^{3}H]-mannose treated samples and controls (Tab. I).

Figure 2 shows the uptake of [^{3}H]-galactose in the non-migrating (378 ± 44 cpm/µg of protein) and migrating corneal epithelia. The migrating corneal epi-

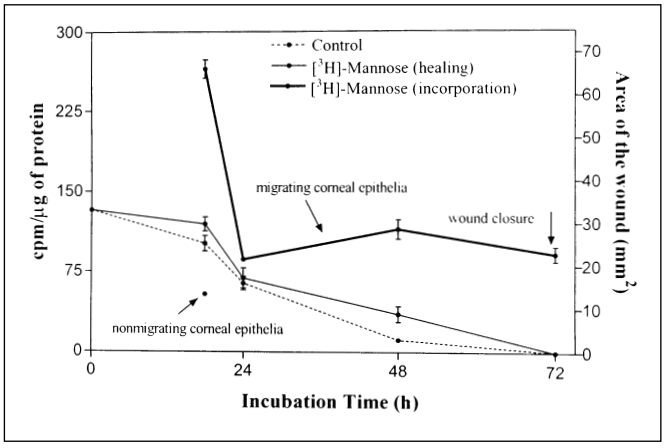

Fig. 1 - *Migrating and non-migrating rabbit corneal epithelia in organ culture were incubated with radiolabeled [^{3}H]-mannose. The rate of incorporation and the healing pattern of the treated migrating corneal epithelia are shown after 18, 24, 48 and 72 h incubation. Uptake was higher in the migrating than the non-migrating corneal epithelia at 18h. Wound healing in the active migrating phase (18-48 h) was delayed in the treated samples. By 72 h the wounds had closed. Vertical bars indicate standard error of the mean (SEM). The results are representative of three separate experiments.*

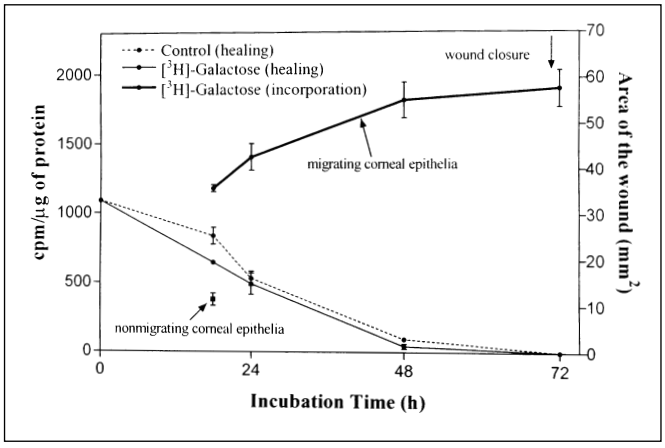

Fig. 2 - *Migrating and non-migrating rabbit corneal epithelia in organ culture were incubated with radiolabeled [^{3}H]-galactose. The rate of incorporation and the healing pattern of the treated migrating corneal epithelia are shown after 18, 24, 48 and 72 h incubation. Uptake was higher in the migrating than the non-migrating corneal epithelia at 18h. Wound healing in the active migrating phase (18-48 h) was slightly enhanced in the treated samples. By 72 h the wounds had closed. Vertical bars indicate standard error of the mean (SEM). The results are representative of three separate experiments.*

thelium incorporated 1177 ± 26, 1403 ± 97, 1811 ± 128 and 1893 ± 131 cpm/µg of protein 18, 24, 48 and 72h after wounding. The galactose treated samples showed an average mean areas of respectively 19.6 ± 0.0, 14.91 ± 2.34 and 1.32 ± 0.54 mm^2 after 18, 24 and 48h (Figs. 2 and 3). In the 18-48h interval, the pattern of wound healing was linear in the galactose treated samples (r^2=0.96; p<0.01) (Fig. 2) whereas the rate of healing was slightly higher than the controls (Tab. I).

TABLE I - RATE OF WOUND HEALING, WOUND SIZE AND INCORPORATION OF [^{3}H]-GALACTOSE AND [^{3}H]-MANNOSE AFTER 48H OF CORNEAL EPITHELIAL MIGRATION OVER THE ORIGINAL WOUND (33.72 ± 0.53 mm^2) IN ORGAN CULTURE

Sample	Mean wound area after 48h (mm^2 ± SEM)	Rate of healing from 18-48h (µm/h ± SEM)	Incorporation after 48h (cpm/mg of protein)
Control (n=18)	3.15 + 0.50	61 + 0.01	-
[^{3}H]-Mannose (n=9)	8.89 + 1.83*	41 + 0.01*	115 + 9.00
[^{3}H]-Galactose (n=9)	1.32 + 0.50**	63 + 0.01†	1811 + 128

*p<0.01; **p>0.05; †p>0.01

DISCUSSION

Sugar moieties on cell surfaces play a role in cell adhesion, migration and proliferation (15-19). The present study was undertaken to determine the relation between incorporation of galactose and mannose sugar and the rate of healing of the corneal epithelium. Both sugars were incorporated in greater amounts in the migrating than the non-migrating corneal epithelia. However, galactose uptake was significantly higher than mannose, suggesting an increase in its utilization. This is consistent with the fact that the galactose moiety appears to be required in the synthesis of new glycoconjugates during migration (9). To date no such study on mannose sugar has been reported.

The effects of galactose and mannose uptake were followed, and the rate of migration was lower after

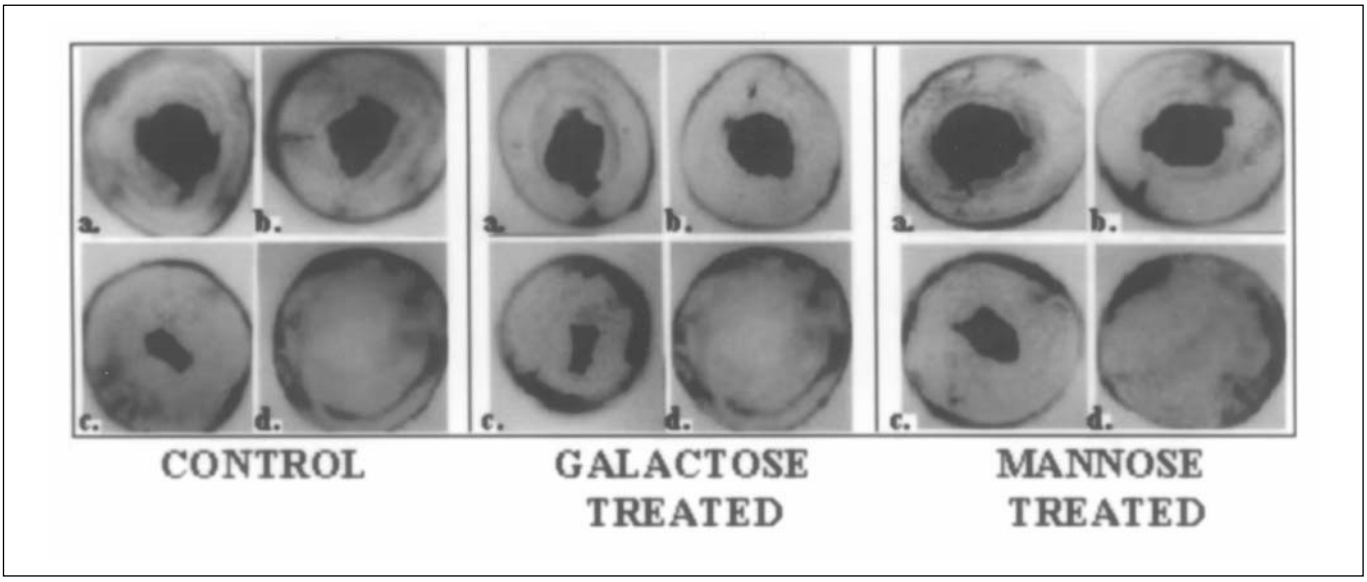

Fig. 3 - *Rabbit corneas showing comparison of the corneal epithelial wound areas of control, [^{3}H]-galactose and [^{3}H]-mannose treated samples after (a) 18h, (b) 24h, (c) 48h and (d) 72h of incubation. The stained region (Richardson stain) indicates the size of the remaining wound. At 48h, wound diameter was larger in the [^{3}H]-mannose treated samples than the [^{3}3H]-galactose treated samples and controls.*

mannose incorporation than with galactose and controls (Tab. I). Thus, although galactose is essential for normal migration, the healing rate remains unaffected by the low concentration used in this study. However, mannose uptake seems to exert an inhibitory effect, slowing migration. Earlier studies showed that blocking glucose, mannose and glucosamine sugar moieties of glycoproteins on the cell surface or basement membrane inhibited epithelial cell migration during healing of rat corneal abrasions (6). The diverse effects of the same sugar may be explained on the basis of lectin studies that have shown sugar moieties of glycoconjugates to be blood group-specific (19) and to differ in different cell types (14).

A major finding in the mannose treated samples is that, although incorporation was greater after 18h than 48h incubation, the wound area was significantly different only at 48h. Recent studies have reported that corneal injury exposes carbohydrate moieties of glycoconjugates that provide additional attachment sites to bacteria, rendering the cornea susceptible to infection (1, 2, 20). Latkovic and Nilsson (21) found significantly more wheat germ agglutinin (WGA) receptors were exposed with contact lens wear than without.

Thus, the greater mannose incorporation at 18h may be attributed to the increased availability of pilus binding sites in the newly injured corneal epithelium more than to the rate of wound healing. But at 48h, when fewer sites are exposed, the sugar is more likely to play a role in the delay of the healing process.

In summary, mannose has an inhibitory effect on the migratory rate during the linear healing phase of the corneal epithelium. Whether this is due to the addition of mannose moieties to existing glycoconjugates or synthesis of new glycoconjugates remains to be clarified.

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