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An estimate of turnover time of intestinal mucus gel layer in the rat in situ loop

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Summary

A method is described to estimate the turnover time of the mucus gel layer in chronically isolated intestinal loops in the rat during perfusion with isotonic saline. Measuring the concentration of a marker substance (total hexose) in the perfusion solution allowed calculation of the volume of produced mucus (V_p) to be in the range between 0.03 and 0.16 μl min^{-1} cm^{-1}. The volume of the adhering mucus gel layer (V_a) was calculated using anatomical data from the literature to be 7.78 ± 0.32 μl cm^{-1}. Hence, the turnover time of the mucus gel layer as given by the quotient of V_a/V_p could be estimated to vary in the range between 47 and 270 min. This time scale is well comparable with the mean residence time found for mucoadhesive microspheres in earlier experiments using the same animal model. It is concluded that mucus turnover probably represents a crucial physiological factor for the concept of mucoadhesive dosage forms.

Introduction

So-called mucoadhesive drug delivery systems are expected to stick onto the mucus gel layer and hence to provide a prolonged and intensified contact with the underlying mucosal tissue (Junginger, 1990). With respect to the development of oral dosage forms, the concept of bioadhesion offers the possibility of controlling their gastrointestinal (GI) transit to improve the bioavailability of the drug (Ch'ng et al., 1985). Irrespective of the intrinsic mucoadhesive performance of the polymers used for these dosage forms, the maximal residence time of such a drug delivery system at the site of adhesion is probably limited by the time scale during which the mucus gel layer is being renewed. The presence of such a distinct gel layer is thought to be the result of a steady state determined by synthesis, secretion and degradation of the mucus constituting glycoproteins (Allen et al., 1984). Many efforts have been made to investigate structure and composition of GI mucus. A comprehensive recent review has been given by Neutra and Forstner (1987). Surprisingly, how-

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ever, no concise data or even estimates seem to be available in the literature about the turnover time of the mucus gel layer.

The appearance of GI mucus can be classified in three states: (i) native mucin as it is stored within goblet cell granules prior to exocytosis and gel formation; (ii) gel state mucus which adheres to the mucosal surface as a continuous, translucent layer of measurable thickness (Bickel and Kauffman, 1981; Kerss et al., 1982; McQueen et al., 1983) and (iii) sloughed and solubilized mucus in the form of coagulated clots or as a viscous, mobile solution in the lumen (Allen et al., 1984). A direct method to determine the amount of gel-state mucus produced per unit time depends on the possibility of isolating this mucus fraction quantitatively. Such a technique, however, has yet to be developed. Probably all approaches to separate the adherent mucus gel and the underlying tissue — either by solubilization or by scraping — would also embrace the intracellularly stored mucins. Furthermore, it must be noted that such manipulations have influence on mucin release unlike the 'normal' situation of the used test model, irrespective whether this is a living animal, an isolated organ, or a tissue culture system.

In this study, an indirect approach was chosen which relies upon the experimental determination of mucus output in an in situ perfused intestinal loop in the rat as developed by Poelma and Tukker (1987). In the following, a method is described showing how the amount of mucus collected in the perfusate can be related to other physicochemical and anatomical data reported in the literature in order to obtain a first estimate about the turnover time of the intestinal mucus gel layer. Whether data obtained from such a particular animal model can be transferred to the normal physiological situation or even to man is not known. Nonetheless, they will be directly comparable to transit studies with mucoadhesive microspheres which have been performed using the same model. (Lehr et al., 1990). Comparing the time scale of the transit process on the one hand with that of mucus turnover on the other, however, might indicate a possible interrelation between both processes. If this interrelation does exist, it can be assumed to hold also for other models or species.

Methods

Animal model

A chronically isolated intestinal loop in the rat as described earlier (Poelma and Tukker, 1987) was used to study the intraluminal release of mucus as indicated by the amount of hexoses detectable in the homogenized perfusate. Briefly, an intestinal segment of 6–9 cm (approx. 15 cm proximal to the ileo-caecal junction) was isolated with its intact blood supply. The loop remained in the peritoneal cavity and was attached to two stainless-steel cannulae, inserted in and running through the abdominal wall. Loop length was measured with a thread immediately after its installation at an accuracy of ± 0.5 cm. The continuity of the remaining intestine was restored by end-to-end anastomosis.

For this study, 20 male Wistar rats were used. Average body weight was 243 ± 21 g (mean $\pm$ SD) in the range of 210–300 g. The loops had an average length of 7.4 ± 1.2 cm (mean $\pm$ SD) in the range of 5.0–9.0 cm. Perfusion experiments were performed between 3 and 30 days (12 ± 8; mean $\pm$ SD) after surgery.

Determination of mucus output

Before starting the experiments, the intestinal loop was cleaned by rinsing manually and subsequently by perfusing with isotonic saline of 37 °C at a constant flow of 1.0 ml min^{-1} for 30 min. The intraluminal release of hexoses was determined in the perfusate after recirculating perfusion (volume, 60 ml; flow rate, 1.0 ml min^{-1}; temperature, 37 °C) for 3 h. Hexoses in the perfusate were determined according to a slightly modified version of the orcinol method of François et al. (1962), as described earlier (Poelma et al., 1989). Glucose monohydrate was used as standard and the release of hexoses was expressed as glucose equivalents/unit intestinal length per unit time (μg cm^{-1} min^{-1}).

Results

Total hexose in the perfusate, expressed as glucose equivalents, was found to be 0.40 ± 0.25 μg

min^{-1} cm^{-1} (mean $\pm$ SD, $n = 30$ experiments on 20 animals). The detected sugar may have its origin in both the solubilized and the mucus shed from the gel layer. However, if perfusion is performed during a longer period of time, mucus output is a direct measure for the mucus production in steady state.

Volume of mucus produced during perfusion

In order to estimate the amount of native mucus from the output of a marker substance (hexose), two essential pieces of information are required: (1) the content of the marker in the native mucus glycoprotein (mucin); (2) the mucin concentration in the rat intestinal mucus gel.

Carbohydrate composition of rat small intestinal mucin has been reported by several authors (Forstner et al., 1973; Fahim et al., 1983; Shub et al., 1983). In view of the different techniques of isolation and analysis, for the total hexose content a range between 25 and 30% (m/m) of mucin dry weight is assumed.

The greatest uncertainties are met with respect to the mucin concentration in the rat intestinal mucus gel. Rheology of gastrointestinal mucus appears to have been investigated extensively for pig mucus. Along the gastrointestinal tract, the mucus glycoproteins vary in intrinsic viscosity and hydrodynamic volume. This is also reflected in different glycoprotein concentrations in the native mucus gels. For the pig, these have been reported to be 55 mg/ml for gastric mucus (Allen et al., 1982), 50 mg/ml for duodenal mucus (Allen et al., 1982), 10.5 for small intestinal mucus (Mantle and Allen, 1981) and 20 mg/ml for colonic mucus (Allen et al., 1982), respectively. Comparable data for the rat, however, are not available. Hence, a possible concentration range between 1 and 5% (m/v) will be assumed for our estimation.

According to the assumptions above, the hexose output as found in the perfusion experiments corresponds to an amount of mucin between 1.3 and 1.6 μg min^{-1} cm^{-1} or a mucus volume (V_p) between 0.03 and 0.16 μl min^{-1} cm^{-1} produced in steady state.

Volume of adherent mucus gel-layer

Geometrically, the small intestine can be described as a hollow cylinder. In the cross-section

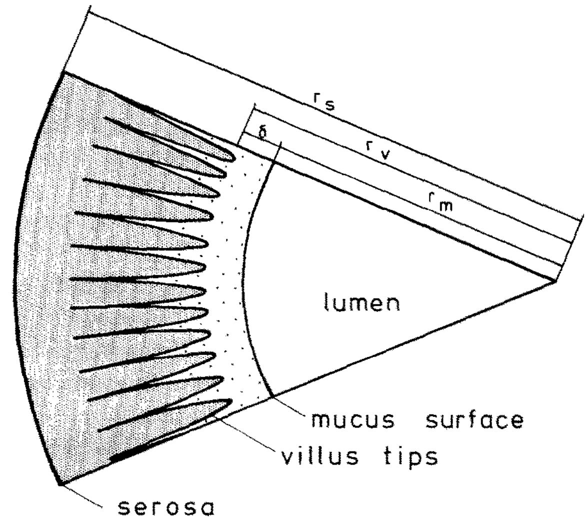

Fig. 1. Schematic cross-section of an intestinal segment (see text).

of a gut segment, three distinct borders are important for the following considerations (Fig. 1). These are the outer serosal tissue surface, the smooth mucosal tissue surface formed by the tips of the villi and the luminal surface of the mucus gel layer. By each of these borders a corresponding radius can be defined in order to describe the dimensions of the cylindrical cross-section. These are the serosal radius (r_s), the villous radius (r_v) and the mucous radius (r_m). As the mucus gel layer has the thickness δ, the relation between villus radius and mucus radius is

$$r_v = r_m + \delta \quad (1)$$

According to this cylinder model, the volume of the adherent mucus gel layer (V_a) for an intestinal loop of the length l is given by the volume difference of the inner luminal cylinder and the mucosal cylinder:

$$V_a = r_v^2 \pi l - (r_v - \delta)^2 \pi l \quad (2)$$

which can be further simplified to

$$V_a = (2r_v - \delta) \pi l \delta \quad (3)$$

Holzheimer and Winne (1989) developed a method which enabled them to calculate the vil-

lous radius ('intraluminal radius ρ' in their terminology) of in situ perfused intestinal segments of rats by measuring the serosal circumference with a thread. In previous microscopical studies on cross-sections of loops after in situ fixation, an empirical relation has been established between these two parameters. The average value for the villous radius of jejunal loops is reported to be 1.55 ± 0.05 mm (mean ± confidence limits (CL) for $p \leq 0.05$, SD = 0.31, $n = 180$).

The thickness of the mucus gel layer in the more distal parts of the small intestines seems not to have been measured yet, either in rats or in humans. The most reasonable approximation might be the value for duodenal surface mucus which is reported by McQueen et al. (1983) to be 82 ± 0.6 μm (mean ± CL for $p \leq 0.05$, SD = 7, $n = 532$ readings from six sections on six animals) for normal rats after 24 h fast.

With Holzheimer's value for the villous radius $r_v = 1.55 \pm 0.05$ mm and McQueen's value for the mucus thickness $\delta = 82 \pm 0.6$ μm, the volume of adhering gel mucus V_a according to Eqn 3 for an in situ perfused intestinal segment is estimated to be 7.78 ± 0.32 μl cm^{-1}. The indicated error is obtained by calculating with the upper and lower confidence limits.

Mucus turnover time

The time necessary for complete renewal of the mucus gel layer is given by the ratio of the volume of adherent mucus (V_a) and that of produced mucus (V_p):

$$t = \frac{V_a}{V_p}\left[\frac{\mu\text{l cm}^{-1}}{\mu\text{l min}^{-1}\,\text{cm}^{-1}} = \text{min}\right] \quad (4)$$

This value is called the 'turnover time' of the mucus gel layer and is independent of the length of the perfused segment. Its reciprocal value with the dimension of min^{-1} represents the turnover rate and has the character of a frequency describing how many times the mucus gel layer is renewed during a given period of time.

Dividing the previously deduced volume of the adherent mucus gel layer ($V_a = 7.78 \pm 0.32$ μl cm^{-1}) by the volume of produced mucus per unit time ($V_p = 0.03$–0.16 μl min^{-1} cm^{-1}) yields a renewal time ranging between 47 and 270 min. Hence, mucus turnover time is estimated to be in the order of a few hours.

Discussion

The value for the villous radius as reported by Holzheimer and Winne (1989) seems to provide a good basis for the calculation of the volume of the mucus hollow cylinder as it has been determined from a considerable number of animals. The perfusion rate in these experiments was 0.2 ml/min and hence somewhat slower than that used in the present study. However, as was also the case in our experiments, the height of the outflow tube was at the same level as the loop, which means that there was no further distension of the tissue due to hydrostatic forces. In summa, experimental circumstances are comparable. On the other hand, the villous radius as determined from jejunal loops will certainly deviate from that which would have been found for the ileum due to the proximal-distal decrease in villus height. Furthermore, the inter-villus spaces are neglected. Nevertheless, in comparison to other assumptions which must be made in the context of these calculations, these differences might be negligible. The same holds for a slight difference in average body weight of the animals as well as the fact, that the dimensions of chronically isolated loops might change due to atrophy.

A reliable estimation for the thickness of the intestinal mucus gel layer is more difficult to obtain, as the reported measurements focused especially on stomach mucus (Kerss et al., 1982; Bickel and Kauffman, 1983). The reported value for rat duodenal mucus thickness (82 ± 7 μm, mean ± SD) did not differ significantly from that found for the stomach (73 ± 5 μm, mean ± SD) (McQueen et al., 1983). As these authors noted, there was a large variation (up to 10-fold) between individual measurements from the same mucosal section, but only a small intra- and interindividual variation of the average values. This additional information may justify the assumption to use the

duodenal value also for calculations concerning the ileum.

The question remains how reliably these data obtained from an intact GI tract of normal animals can be transferred to the situation of an in situ perfused loop. Whereas the absence of food might lead to an increase in mucus gel thickness in comparison to the normal physiological situation (Kerss et al., 1982), permanent perfusion of an isolated segment will probably have an opposite effect. However, in vitro studies of the same authors did not demonstrate any effect of stirring on mucus thickness when mucosal specimens were exposed for 60 min to either isotonic or hyperosmolar saline. These observations support the expectation that the equilibrium thickness of the mucus gel layer in an in situ perfused intestinal segment will not largely differ from that of an intact rat.

Total hexose output has been chosen as mucus marker because this assay is easily performed and is described to give the same yields for all relevant glucosides (mannose, galactose, fucose). The level of absorbance is determined solely by the number of saccharide units and not by the configuration of the CHOH groups. This is advantageous, as mucin carbohydrate composition is not identical in different regions of the intestines even within a single species and probably will change as a function of physiological circumstances. The disadvantage of such a nonspecific assay, however, is a possible interference with carbohydrate groups in non-mucin glycoproteins and cell membrane components. Therefore, it is obvious that the most uncertain assumptions in the whole calculation are those which must be made with respect to the content of the hexose marker in native mucin and the concentration of mucin in the mucus gel. A possible improvement in future experiments would be to isolate the macromolecular fractions of the perfusate and monitor the hexose. Furthermore, a direct fluorimetric assay of *O*-linked glycoproteins has been described by Crowther and Wetmore (1987), which could give a direct measure for mucus glycoprotein both in the perfusate as well as in small samples of the native gel from mucosal scrapings.

Conclusions

To our knowledge, the present approach is the first study that allows one to estimate the rate at which the intestinal mucus gel layer is permanently renewed, at least under the conditions of this in situ model. Although the reported estimate for the mucus turnover time is relatively rough, its similarity to the mean residence time as observed for mucoadhesive microspheres in the in situ perfused loop (Lehr et al., 1990) is very intriguing. The latter was found to be 94 ± 18 min (mean $\pm$ SE, $n = 5$).

To prove this interrelation between transit of microspheres and mucus turnover, additional experiments are necessary which are presently being performed. Further support for this hypothesis, however, is already given by the observation that stimulating the mucus output by perfusion with 10 mM sodium taurocholate led to a significant shortening of the mean residence time of microspheres. On the contrary, microspheres did not become detached from dead mucosal tissue in vitro when stirred for more than 18 h (in preparation).

As the present results indicate, mucus turnover is probably that rapid that it could be crucial for the concept of mucoadhesion as a means to control the GI transit of drug delivery systems.

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