THE EFFECT OF AN ENDOGENOUS MUCUS LAYER ON TETRACYCLINE ABSORPTION

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The everted rat gut preparation is a well established method for the in vitro assessment of bioavailability. The technique has been used to study the effects of intestinal mucus on drug absorption by using a solution of mucus as a model for the mucus layer which overlies the mucosal epithelium in vivo (Braybrooks et al 1975; Lovering & Black 1974). However no work has been done which takes into account the endogenous mucus which is secreted, in vitro, by the intestine itself. This work describes a method which utilizes this intact native mucus layer and looks at its effects on the absorption of tetracycline hydrochloride.

The jejuno-ileal section of intestine from male rats (250 g) was taken and four consecutive sections 10 cm in length were cut and everted on glass rods. Each section was thoroughly washed in buffer then mounted on a single cannulated perfusion apparatus of our design and placed in a 50 ml gut tube containing isotonic Tris-HCl nutrient buffer, pH 7.4. This was maintained at 37°C and bubbled with a O_2/CO_2 mixture. After 1½ hours a mucus gel developed on the epithelial surface of the gut. This secretion was either removed or left intact and the segments transferred to fresh mucosal buffer: removal of adhered mucus was readily achieved by gently blotting the gut with tissue. Drug was injected into the mucosal solution to give a final concentration of 2 mM and the serosal solution washed out every 10 minutes with 6 ml of buffer. The perfusate was acidified to pH 1.0 with concentrated HCl and drug content assayed spectrophotometrically at 354 nm. Data for proximal (jejunal) and distal (ileal) sections were analysed separately. Lag times were calculated from extrapolation of the linear portion of cumulative amount transferred versus time plots. Values obtained with the intact mucus layer were 12.5 ± 5.5 (16) minutes and 7.9 ± 2.2 (10) minutes for proximal and distal sections respectively; the figures in brackets indicate the number of determinations. For segments with the mucus layer removed the lag times were 1.3 ± 0.7 (12) and 2.5 ± 0.9 (8) minutes. These latter sections gave equilibrium drug absorption rates of 20.6 ± 2.1 (8) and 18.4 ± 2.6 (8) μg/mM mucosal concentration/min/10 cm gut length.

Experiments were also carried out to determine the percentage increase in drug transfer rate after removal of the mucus layer. These gave values of 87 ± 15% (6) for proximal sections and 60 ± 13% (6) for distal sections. The dry weight of mucus removed from gut segments was 63.5 ± 6.9 mg (jejunal) and 39.4 ± 13.2 mg (ileal) which would correspond to a concentration of some 0.1% w/w if dispersed in the mucosal medium. Solutions of purified mucus however produced a suppression of drug transfer of only 8.6 ± 2.5% (3) at a concentration of 1.5% w/w.

It can be seen therefore that mucus in the form of a gel layer on the gut surface is not only much more effective in reducing tetracycline transfer than when it is dispersed in solution but also can impart a substantial lag time to the process. It is interesting that the thickness of this gel layer, calculated from its wet weight after removal and the dimensions of the gut, was 0.58 ± 0.05 mm for jejunal and 0.54 ± 0.14 mm for ileal sections which compares favourably with values obtained elsewhere in in vivo studies (Smithson et al 1981). It is therefore concluded that the presence of native mucus on the surface of everted rat intestine can substantially affect drug transport measurement and this should always be considered when studying drug absorption in vitro.

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Smithson, K.W. et al (1981) Science 214:1241

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