Absorption of Etoposide (VP-16-213) from the Small Intestine of the Rat. The Potential Role of Mucus as an Absorption Rate Limiting Barrier

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Abstract: The absorption of etoposide (VP-16-213) was investigated in a perfused intestinal loop. The absorbed amount of drug was determined by collecting the blood draining the loop via cannulation of the efferent jejunal vein. The absorption rate of VP-16-213 strongly depended on the composition of the perfusion medium. The addition of taurocholate to an aqueous etoposide solution enhanced the absorption rate. When N-acetylcysteine was added to an aqueous solution, the absorption rate dropped significantly.

The absorption of etoposide in man after oral administration is erratic (1). In rats relatively high concentrations of etoposide were found in the intestine. This finding may indicate binding of etoposide to intestinal mucus. In the literature, mucus binding has been described for various drugs (e.g. 2-7). In most cases, the transport rate of the drug from the intestinal lumen to the blood was decreased if a drug-mucus interaction occurred. Only 10-20% of benzomethamine, a monoquaternary compound, was taken up over 3-4 hours; absorption mainly occurred during the first 45 minutes. It was shown, that binding to mucus was responsible for the slow absorption rate (8, 9). Meli et al. (10) observed that ethynylestradiol cyclopentyl ether disappeared from the intestinal lumen by 85% within 7.5 minutes. Almost the entire amount that disappeared was bound to the intestinal tissue from where it was released very slowly. The absorption of some water soluble dyes was linearly related to the binding of these substances to the intestinal mucus (11-14). The binding to intestinal mucus was essential for the absorption of these dyes, because digestion of the mucus glycoproteins, and even the glycosalyx, caused a severe decrease in the absorption of these dyes.

If the intestinal mucus is a significant barrier to the absorption of a drug, attempts can be made to promote absorption by reducing the influence of the mucus. A possible way to increase the diffusion rate through the mucus might be to decrease the apparent viscosity of the gel. Organic compounds, containing thiol groups such as dithiothreitol, 2-mercaptoethane-sulfonate, glutathione and cysteine derivatives can reduce the disulfide bridges in the core of the glycoprotein molecule, thereby, breaking up the native structure of the glycoproteins (15-17). This results in a decreased elasticity and an increased ciliary transport rate (18-20). The addition of 2% N-acetylcysteine (N-ac) causes a 40% reduction in consistency of the mucus after a contact time of 60 minutes (17). The optimal pH for the action of N-acetylcysteine (N-ac) lies around 7 (21, 22). N-acetylcysteine also enhances the binding of some antibiotics to mucus (2).

The resistance of the mucus layer in the absorption process can also be changed by bile salts. It was shown that bile salts affect mucus viscosity and elasticity (23); therefore, these compounds might influence the absorption kinetics of mucus interacting drugs. The benificial effect of bile salts on the absorption process of a number of drugs has been reported (24-26). In general, the mechanism behind the observed phenomena is not clear. An increase in the “apparent” solubility, or a change in membrane or mucus characteristics might play a role.

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In this study an attempt was made to enhance the absorption rate of the antineoplastic agent etoposide. N-Acetylcysteine was added to the aqueous solution as it was expected that this compound would decrease the viscosity of the mucus. A concentration of 4% was used, since it was reported by Schiller et al. (27) that this concentration did not damage the intestinal mucosa of the rat. Taurocholate (TC) micelles were used to increase the "apparent" solubility of the drug and thereby the concentration gradient over the intestinal barrier. Besides, these micellar dispersions might affect the resistance of the absorption barrier. The concentrations of taurocholate were 10 and 40 mM. These concentrations are in the physiological range (28). The experimental technique enabled the determination of the drug immediately after absorption. The blood draining the perfused loop was collected by cannulating the efferent jejunal vein. With this technique whole body distribution and metabolism was avoided.

Materials and Methods

Animals

Male Wistar rats, 285 ± 26 g (mean ± S.D.) were used. The animals were not fasted prior to the experiments.

Absorption Experiments

The preparation and perfusion technique is described elsewhere (29). Briefly, after intraperitoneal injection of urethane (1 g/kg body weight) the abdomen was opened and a suitable segment of the jejunum was exposed. Loops of 6.7 ± 1.3 cm (mean ± S.D.) were cleared from food material and rinsed with an isotonic saline solution until the effluent was clear. The amount of etoposide absorbed was measured in the blood draining the perfused segment via cannulation of the efferent jejunal vein. The total blood volume of the animal was kept constant by an infusion of heparinized donor blood via a jugular vein. All perfusion solutions were prepared one hour prior to the experiment. A volume of 1.0 ml perfusate was oscillated at a flow rate of 0.88 ml/min. The standard perfusion solution contained (g/l): NaCl 5.2, KCl 0.5, MgSO4·7H2O 0.29, CaCl2·2H2O 0.27, NaHCO3 1.50, KH2PO4 0.06, mannitol 13.0. All ingredients were of pharmacopeial grade. Etoposide concentrations were 0.1 and 2.0 mg/ml. The pH was 6.5. N-Acetylcysteine (OPG, Utrecht) solutions were prepared by dissolving 4 g N-acetylcysteine in 100 ml demineralized water; the pH was adjusted to 6.5 with a sodium hydroxide solution. Micellar solutions were prepared by dissolving sodium taurocholate (98% pure, Sigma Chemicals, St. Louis, MO) and etoposide (Bristol Myers, New York, NY) in methanol. The solvent was evaporated with a Rotavapor RE 120 (Büchi, Flawil, Switzerland) and was maintained under reduced pressure for two hours to completely remove the solvent. Then the standard perfusion solution was added to obtain the desired concentration of micelles and the film was dispersed using glass beads.

Determination of Etoposide

Plasma was obtained by centrifugation of the blood samples at 2000 rpm for 10 minutes. The plasma samples were either extracted immediately or were frozen until extraction. They were analyzed according to the method of Holtzuis et al. (30) – a reversed phase HPLC analysis with electrochemical detection after extraction from plasma – with two modifications. First, the structural analogue tenoposide was used as an internal standard and second, all organic extracts were filtered through a phase separating filter (Whatman FS-1) prior to evaporation.

The mucus layer was separated from the intestinal tissue using a method recommended by Marriott (personal communication). A piece of Kleenex tissue was gently pressed on the opened loop and removed from the tissue after a few seconds. The amount of etoposide in the intestinal tissue was measured by extracting the tissue after grinding with 5 g of dried sodium sulphate. 4.0 ml Dichloroethane (DCE) was added, and the mixture was vortexed for 1 minute. After standing overnight at 4°C, the mixture was filtered through a swab of cotton wool, and the extract was filtered through a phase separating filter. Another 3.0 ml DCE was poured over the powder and filtered in the same way. The organic solvent was evaporated at room temperature under nitrogen and the residue was dissolved in 1.0 ml eluent for the HPLC determination. The Kleenex tissue was extracted twice with 3.0 ml DCE. After evaporation of the organic solvent at room temperature under nitrogen, the residue was dissolved in the HPLC eluent. The presence of taurocholate or N-acetylcysteine did not affect the assay.

Histological Investigations

In separate experiments, intestinal loops were perfused for 1 hour with the test solutions. After removing the loop, a thin ring was immersed in Bouin's solution (saturated picric acid solution (150) + 40% formaldehyde solution (50) + glacial acetic acid (10)). After 24 hours, the Bouin solution was replaced by 70% alcohol. After drying with pure alcohol, the tissue was imbedded in glycolmethacrylate and 5 μm coupes were cut. The coupes were stained with P. A. S. (Periodic Acid Schiff) – Haemalun (Mayer). After drying, they were imbedded in malinol and examined at a magnification of 40 and 100x.

Data Analysis

The flux of etoposide (ng/min-cm) was calculated by multiplication of the plasma concentration (ng/ml) and the bloodflow (ml/min-cm). The length of the intestinal segment was determined at the end of the experiment. The segment was excised and stressed with a 10 gram weight for 60 seconds. No correction was made for the decreasing concentration of etoposide in the lumen because the fluxes were rather low (see Results and Discussion). The total amount of etoposide in the intestinal tissue and in the mucus layer was expressed as μg/cm intestinal length. No correction for bloodflow variation was applied in the different experiments, because the flow was kept constant during the experiment, and the inter-experimental variation was small. For statistical analysis of the etoposide fluxes Student's t-test was used. Differences were considered significant when P < 0.05.

Results and Discussion

Flux of Etoposide

The penetration of drugs through the epithelium might lead to an impaired absorption because of a damaging effect on the epithelial cells (31). Figure 1 shows a typical plot of the cumulative amount of etoposide in the blood draining the perfused jejunal segment as a function of time. A standard perfusion solution with a concentration of 0.1 mg/ml etoposide