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# The enhancement of pipemidic acid permeation into the pig urinary bladder wall

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**Abstract**

The influence of interactions between polycarbophil and calcium on a model drug permeation into the pig urinary bladder wall was investigated. Pipemidic acid was used as a model drug. One percent w/v polycarbophil dispersion significantly increases the permeation of pipemidic acid into the urinary bladder wall. The enhanced absorption of pipemidic acid caused by polycarbophil is significantly less pronounced in polycarbophil dispersions containing calcium. The enhancement of pipemidic acid permeation into the urinary bladder wall could be due to the opening of tight junctions, which causes higher paracellular permeability. In the case of polycarbophil dispersion with calcium some carboxylic groups of polymer are already occupied with calcium, present in the dispersions. As a consequence extracellular calcium binds to polycarbophil in lower extent if compared with polycarbophil dispersion without calcium and transport is increased to a lesser degree. We concluded that the mechanism of drug absorption enhancement caused by polycarbophil could be similar for urinary bladder as described in the literature for intestinal mucosa. 

*Keywords:* Polycarbophil; Calcium; Enhanced absorption; Urinary bladder wall

Polycarbophil, homopolymer of acrylic acid crosslinked with divinyl glycol, is one of the most potent mucoadhesive polymers in use. In addition to mucoadhesive properties, polycarbophil as well as some other poly(acrylic acid) derivatives also has the ability to enhance the intestinal absorption of drugs (Borchard et al., 1996; Lueβen et al., 1996). Numerous carboxylic groups attached on the polycarbophil backbone are the main functional groups of this polymer, which are able to bind calcium ions. The chelation of calcium by polycarbophil could explain the increase of intestinal drug absorption observed in the presence of polycarbophil (Kriwet and Kissel, 1996). Calcium ions are essential for cell to maintain intercellular contacts. The removal of extracellular calcium in confluent cell monolayers prevents not only the formation of new tight junctions but also causes the opening of previously formed tight junctions. Both effects result in enhanced paracellular transport (Lacaz-Vieira, 1997).

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The ability of polycarbophil to enhance the drug absorption is usually studied on intestinal mucosa. However, it has been recently shown that polycarbophil increases the permeability of the pig urinary bladder wall for pipemidic acid as well (Grabnar et al., 1999). The purpose of this work is to evaluate the influence of interactions between polycarbophil and calcium on the permeation of

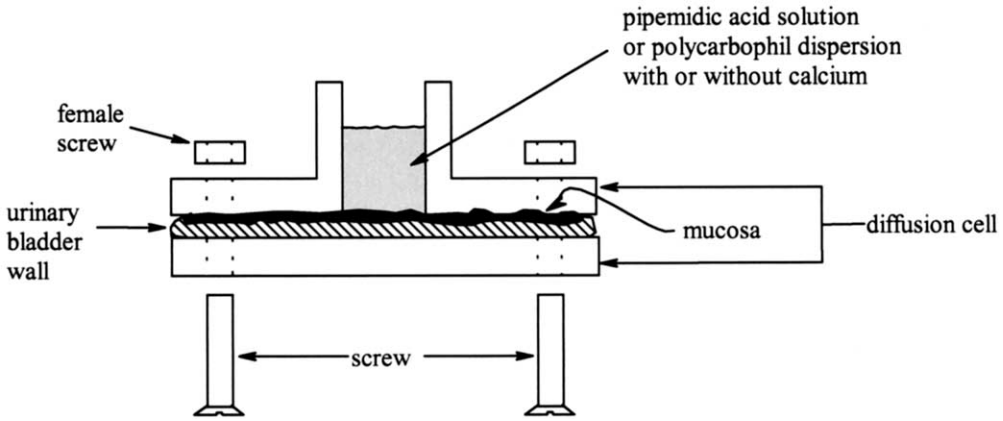


Fig. 1. Schematic presentation of a diffusion cell.

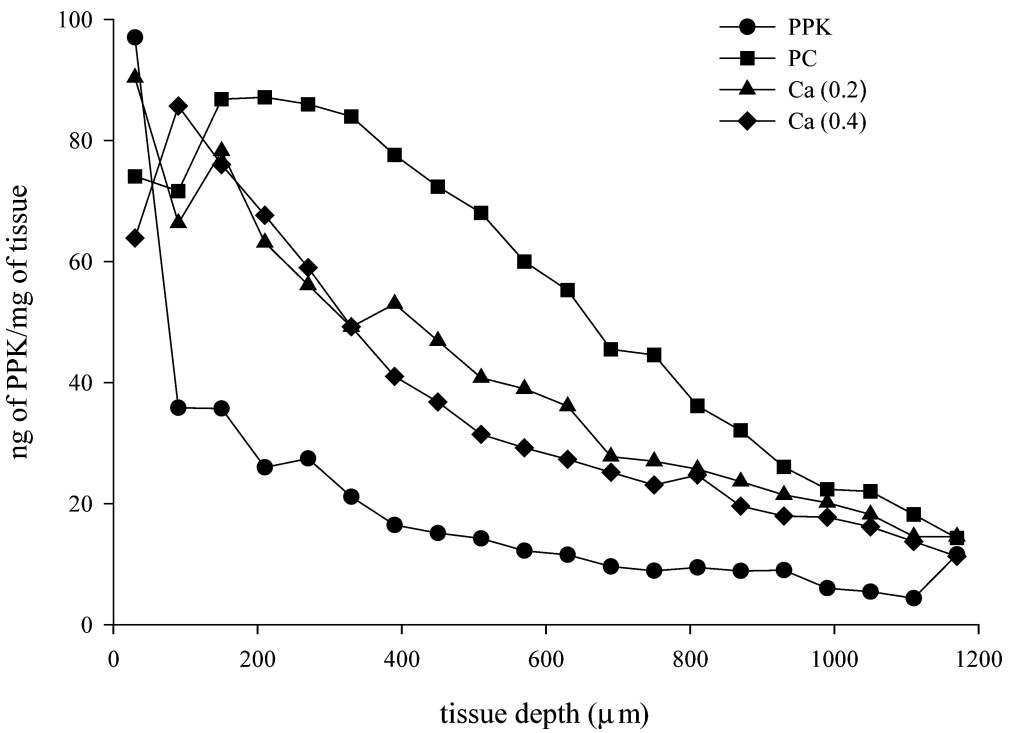


Fig. 2. Amounts of pipemidic acid which permeated into the urinary bladder wall as a function of the tissue depth (mean, $n = 5$). PPK is solution of pipemidic acid, PC polycarbophil dispersion without calcium, Ca (0.2) and Ca (0.4) are dispersions of polycarbophil with calcium where molar ratio of calcium to carboxylic groups of polycarbophil was 0.2 and 0.4.

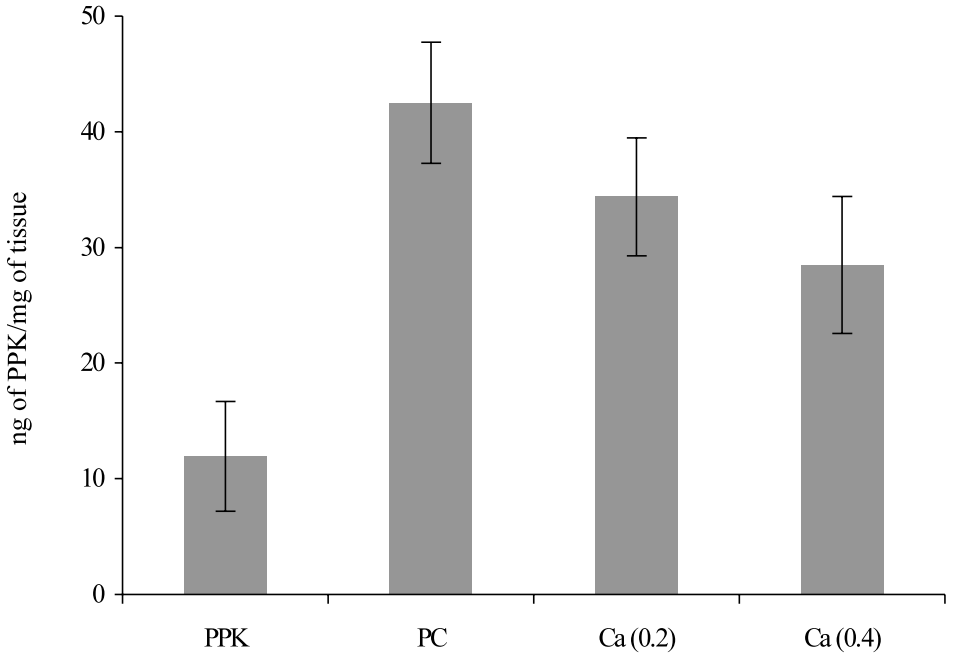


Fig. 3. Cumulative amounts of pipemidic acid that permeated into the urinary bladder wall (mean ± S.D., $n = 5$). PPK is solution of pipemidic acid, PC polycarbophil dispersion without calcium, Ca (0.2) and Ca (0.4) are dispersions of polycarbophil with calcium where molar ratio of calcium to carboxylic groups of polycarbophil was 0.2 and 0.4.

pipemidic acid into the pig urinary bladder wall, which could give us the first insight in the mechanism of polycarbophil permeability enhancement in the urinary bladder.

First the pipemidic acid solution with concentration 0.014% w/v was prepared in phosphate buffer. The buffer consisted of 0.472 g $Na_2HPO_4$, 0.095 g $KH_2PO_4$ and 1.6 g NaCl in 1 l of deionised water. The dispersions of polycarbophil (Noveon AA-1®, BFGoodrich, OH, USA) with or without calcium were prepared in the solution of pipemidic acid. All dispersions had the same concentration of polycarbophil (1% w/v) and pipemidic acid (0.014% w/v). For polycarbophil dispersions with calcium, stock solution of calcium chloride in phosphate buffer was added. Molar ratio of calcium to carboxylic groups of polycarbophil was 0.2 or 0.4. pH of all dispersions was adjusted to 4.

The urinary bladders were obtained from a local slaughterhouse. After the pigs were sacrificed the urinary bladders were obtained and kept in carbogen saturated phosphate buffer saline (Ph. Eur. III) at 5 °C until used. All experiments were performed within 5 h of sacrifice. The mucosa of the middle part of the urinary bladder was cut into four pieces (approximately 25 × 25 mm) and each piece was mounted into a diffusion cell, developed at the Faculty of Pharmacy, Ljubljana (Fig. 1). Luminal side of the bladder wall was exposed to a solution of pipemidic acid or polycarbophil dispersion with or without calcium for 50 min. After that the bladder wall was rapidly frozen with liquid nitrogen and sectioned by cryostat (Cryocut E, Reichert-Jung Inc./Cambridge Instruments Co., Keene, USA) in segments of 20 μm thickness parallel to luminal surface up to 1.2 mm of depth. After extraction of pipemidic acid from the tissue segments, drug concentration was determined by high performance liquid chromatography and cumulative amounts of pipemidic acid that permeated into the bladder wall were calculated. All dispersions were tested on the urinary bladders of five different animals.

Amounts of pipemidic acid as a function of the tissue depth are shown in Fig. 2, while the

cumulative amounts of pipemidic acid that permeated into the urinary bladder wall are presented in Fig. 3. Pipemidic acid permeation into the urinary bladder wall is significantly increased in the presence of 1% w/v polycarbophil ($t$-test, $P < 0.05$). This could be explained by the chelation of calcium by carboxylic groups of polycarbophil. Decreased extracellular calcium concentration results in opening of tight junctions, higher paracellular permeability and increased cumulative amounts of pipemidic acid in the bladder wall. Moreover, both polycarbophil dispersions with calcium significantly increase the cumulative amounts of the drug in the tissue if compared with the solution of pipemidic acid alone ($t$-test, $P < 0.05$) and significantly reduce these amounts if compared with the polycarbophil dispersion without calcium ($t$-test, $P < 0.05$). In polycarbophil dispersions with calcium some carboxylic groups of polymer are already occupied with calcium, present in the dispersions. As a consequence extracellular calcium binds to polycarbophil in lower extent if compared with polycarbophil dispersion without calcium and paracellular transport is increased to a lesser degree. There is no significant difference in the cumulative amounts of pipemidic acid that permeated into the urinary bladder wall if polycarbophil dispersion with molar ratio 0.2 or 0.4 was used ($t$-test, $P > 0.05$). The influence of these two calcium concentrations could not be distinguished due to the high variability.

We can conclude that the mechanism of drug absorption enhancement caused by polycarbophil could be the same for urinary bladder as it is for the intestine. The removal of extracellular calcium by binding to carboxylic groups of polycarbophil increases the paracellular transport of the model drug probably through opening of tight junctions. This effect is less pronounced when a part of carboxylic groups is already blocked by calcium before exposure to the tissue.

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