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# Buccal delivery of thiocolchicoside: in vitro and in vivo permeation studies

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**Abstract**

Thiocolchicoside, a muscle-relaxant agent, is administered by the oral, intra-muscular and topical route. After oral administration the extent of bioavailability compared with intra-muscular administration is low, due to a first pass effect. In this paper, the delivery of thiocolchicoside through oral mucosa is studied to improve the bioavailability. Thiocolchicoside in vitro permeation through porcine oral mucosa and in vivo buccal transport in humans were investigated. Two dosage forms, a bioadhesive disc and a fast dissolving disc for buccal and sublingual administration of thiocolchicoside, respectively, were designed. The in vitro permeation of thiocolchicoside through porcine buccal mucosa from these dosage forms was evaluated and compared with in vivo absorption. Results from in vitro studies demonstrated that thiocolchicoside is quite permeable across porcine buccal mucosa and that permeation enhancers, such as sodium taurocholate and sodium taurodeoxycholate, were not able to increase its flux. The in vivo thiocolchicoside absorption experiments, in which the drug loss from oral cavity was measured, indicated that both formulations could be useful for therapeutic application. The fast dissolving (sublingual) form resulted in a quick uptake of 0.5 mg of thiocolchicoside within 15 min whereas with the adhesive buccal form the same dose can be absorbed over an extended period of time.


*Keywords:* Thiocolchicoside; Buccal; Formulation; Permeability; Enhancers; In vitro/in vivo

## 1. Introduction

Thiocolchicoside (Muscoril®, Coltramyl®; Fig. 1) is a semi-synthetic sulfur derivative of colchicoside, a naturally occurring glucoside of Colchicum.

Thiocolchicoside is an agonist of the GABA receptors in the central nervous system, exhibiting muscle relaxation, analgesic and local anesthetic activities. Therefore, thiocolchicoside is prescribed for the treatment of orthopedic, traumatic and rheumatologic disorders (Janbroers, 1987). At present, it is administered parenterally (i.m.), orally (tablets or capsules) and topically (cream and ointment). The oral relative bioavailability,

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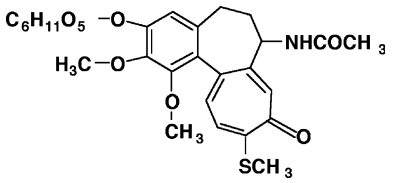


Fig. 1. Structural formula of thiocolchicoside ($C_{27}H_{33}NO_{10}S$).

compared with intramuscular administration, is approximately 25%, mainly due to hepatic first pass-effect, with a high intra- and inter-subject variability (Sandouk et al., 1994). It is hypothesized that alternative routes could improve the bioavailability of this drug.

Analgesic drugs are very often formulated as buccal dosage forms, for accelerating the drug absorption and consecutively, the appearance of analgesic effect. The buccal mucosa, with a wide surface (150 $cm^2$), is quite permeable and easily accessible (Squier and Wertz, 1996). Drug absorption could be very fast also for hydrophilic compounds like thiocolchicoside. In addition, in the case of thiocolchicoside, buccal delivery could also avoid pre-systemic metabolism and hepatic first pass-effect.

Buccal mucosa consists of a stratified squamous epithelium supported by a connective tissue lamina propria (Wertz and Squier, 1991). The major routes involved in drug transport across buccal mucosa are the transcellular and paracellular pathways, but it was supposed that, for many hydrophilic drugs, the buccal permeation was mainly through paracellular way by passive diffusion (Zhang and Robinson, 1996a). In addition, the superficial layers of the epithelium (approximately the uppermost 25–30%) are reported to be the major barrier for penetration of substances (Squier, 1973; De Vries et al., 1991). In case of low drug permeability across buccal epithelium, penetration enhancers were successfully used in order to overcome the major limitation to drug delivery.

Bile salts have been often used to increase drug permeation across mucosal membranes. Their effect is supposed to be reversible and dependent on their concentration. Hoogstraate et al. demonstrated that glycodeoxycholate enhanced buserelin permeation across buccal mucosa (Hoogstraate et al., 1996a) and in vivo buccal delivery of fluorescein isothiocyanate-dextran 4400 in pigs (Hoogstraate et al., 1996b). Sodium taurocholate was used to increase buccal absorption of peptides as alpha-interferon (Steward et al., 1994) or insulin (Ritschel et al., 1989; Zhang et al., 1994). Sodium taurocholate and sodium taurodeoxycholate are also reported to be able to increase permeability of porcine buccal mucosa for fluorescein isothiocyanate-dextran in vitro and in vivo (Junginger et al., 1999).

The aim of this work was to study the in vitro and in vivo buccal permeation of thiocolchicoside. Because of the similarity to the non-keratinized human mucosa, the porcine buccal mucosa was chosen for in vitro permeation study (Beckett and Triggs, 1967; Hoogstraate and Boddè, 1993; Zhang and Robinson, 1996b). The effect of buccal permeation enhancers, such as sodium taurocholate and sodium taurodeoxycholate (10, 100 mM), on thiocolchicoside transport was also investigated. Moreover, by means of thin slicing technique (Fuchs and Green, 1980), the concentration profile of thiocolchicoside within the buccal epithelium, after the transport experiments, was measured as well.

Two thiocolchicoside dosage forms were designed to either dissolve under the tongue or to adhere to the cheek or gingival mucosa. The in vivo buccal uptake of thiocolchicoside from the dosage forms was measured in humans and compared with the in vitro transport through porcine buccal mucosa.

## 2. Materials and methods

### 2.1. Reagents

Thiocolchicoside (MW 563) was kindly supplied by Sanofi-Synthélabo S.p.A. (Limito, MI, Italy). Thiocolchicoside shows an aqueous solubility of $17.1 \pm 0.5$ mg/ml at 37 °C. Measured octanol/water distribution coefficient of thiocolchicoside is $0.45 \pm 0.02$ at pH 7.0. Sodium taurocholate (NaTC) and sodium taurodeoxycholate (NaTDC) were purchased from Sigma. The excipients used in the preparation of dosage forms were: gelatin powder (ACEF, Fiorenzuola d'Arda, PC, I),

carboxymethylcellulose (Blanose CG 7 MFD, Aqualon, Eingenmann e Veronelli S.p.A., Milano, I), aspartame (Fine Granular, Nutra Sweet AG, Zug, CH), glycine (Carlo Erba, Milano, I) and pharmacopoeia grade mannitol. All chemicals were of analytical grade.

### 2.2. Drug assay

The thiocolchicoside assay was performed with a reverse phase high performance liquid chromatography (HPLC) using a Shimadzu LC 10 AS system (Kyoto, Japan), equipped with a Waters Nova-Pak C8 column (Waters, Milford, USA) and an UV detector at wavelength of 370 nm; the mobile phase was acetonitrile:water (15:85), pumped at 1 ml/min.

### 2.3. Manufacturing of buccal dosage forms

Two dosage forms were manufactured: one fast dissolving and the second as a bioadhesive delivery system. Each dosage form contained 4 mg thiocolchicoside. Their composition is reported in Table 1.

#### 2.3.1. Bioadhesive dosage form manufacturing

Gelatin powder was dissolved in a beaker containing distilled water under stirring at a temperature of 65 °C. After cooling, to the obtained solution, slowly and under stirring, carboxymethylcellulose, thiocolchicoside, and aspartame were added. Amounts of solution, corresponding to 4 mg of drug, were dropped in plastic hollow moulds. The moulds were firstly frozen (−20 °C), then plunged into nitrogen and lyophilized (Edwards Modulyo, UK).

Table 1
Composition of buccal dosage forms containing thiocolchicoside (mg)

| | Bioadhesive form | Fast dissolving form |
|---|---|---|
| Thiocolchicoside | 4 | 4 |
| Gelatin | 14 | 14 |
| Sodium carboxymethylcellulose | 12 | – |
| Aspartame | 0.3 | 0.24 |
| Glycine | – | 14 |
| Mannitol | – | 20 |

#### 2.3.2. Fast dissolving dosage form manufacturing

Gelatin powder was dissolved in a beaker containing distilled water under stirring at a temperature of 65 °C. After cooling, under stirring, thiocolchicoside, mannitol, glycine and aspartame were added to the obtained solution. Amounts of solution, corresponding to 4 mg of drug, were dropped in plastic hollow moulds. The moulds were frozen (−20 °C), and plunged into nitrogen and lyophilized (Edwards Modulyo).

### 2.4. Dosage forms dissolution test

Dissolution experiments were carried out in the USP 24 dissolution Apparatus 2 at 37 °C (paddle rotation 100 rpm). The dissolution medium was distilled water. Drug release was monitored spectrophotometrically at 370 nm.

### 2.5. In vitro permeation experiments through porcine buccal mucosa

#### 2.5.1. Permeability coefficient determination

A slaughterhouse (Schiedam, The Netherlands) kindly provided buccal tissue (cheek) from pigs weighing 70–100 kg. After removal, the tissue was placed in cold Krebs buffer (pH 7.4) and immediately transported to the laboratory. The buccal mucosa, with a part of sub-mucosa, was carefully separated from fat and muscles using a scalpel. Then, using an electro-dermatome (Padgett, Kansas City, MO, USA), the epithelium was isolated from the underlying tissue. The thickness of samples was about 500 μm. Because of the time dependent viability decline, the buccal epithelium was used within 2 h upon removal.

Buccal mucosa was mounted in Using chamber (FMA Gorleasus Laboratory, Leiden, The Netherlands, diffusional area 1.1 $cm^2$) with the mucosal side facing the donor compartment. Then, donor and acceptor compartments were filled with 6 ml of Krebs buffer (pH 7.4). Carbogen gas (95% $O_2$, 5%$CO_2$) was circulated through both compartments in order to maintain tissue viability and to

provide mixing. After 1 h equilibration period at 34 ± 0.5 °C, the receptor was replaced with fresh Krebs buffer and the donor side was filled with the donor solution. The donor solution used to measure the permeability coefficient was a saturated solution of thiocolchicoside in distilled water (pH 6.8). The donor solutions used in order to investigate the potential enhancement effect were saturated solution of thiocolchicoside in distilled water containing sodium taurocholate (NaTC; 10, 100 mM), and sodium taurodeoxycholate (NaTDC; 10 mM).

Diffusion experiments were conducted for 3 h. Samples of 300 μl were collected from the receptor side at 30 min intervals and replaced with the same amount of fresh Krebs buffer solution. The thiocolchicoside concentration was determined by HPLC.

### 2.5.2. Thiocolchicoside concentration gradient determination in porcine buccal mucosa

The buccal tissue of pigs (10–11 months old, Landrace × Large White × Duroc or Landrace × Large White), purchased from a local slaughterhouse (Macello Sassi, Colorno, Parma, I) was kept in ice-cold Krebs buffer solution (pH 7.4) during transport. The underlying connective tissue was removed carefully with a surgical scalpel. The obtained epithelial membranes had a mean thickness of 1.85 mm and were used within 2 h after slaughter.

Vertical, Franz-type diffusion cells (DISA, Milan, I) with a diffusional area of 0.6 $cm^2$ were used. The mucosa was mounted with the epithelium facing donor chamber. The receptor compartment was filled with saline (NaCl 0.9%), magnetically stirred in a water bath at 37 ± 0.5 °C. After equilibration for 30 min with saline in both compartments, the donor was filled with 1 ml of 8 mg/ml thiocolchicoside solution in saline. At 15 min time intervals, 20 μl of the donor solution were sampled to measure the disappearance rate of drug. Every 30 min, samples (300 μl) were collected also from the receptor compartment, and analyzed for thiocolchicoside content by HPLC. At the end of the diffusion experiment, the mucosa membrane was punch biopsed and immediately frozen in liquid nitrogen. The membrane was cut in such a way to get slices of 40 μm parallel to surface. The investigated thickness from the surface was 600 μm.

Thiocolchicoside was extracted from each slice with 200 μl of distilled water for 60 min at room temperature. Adding 200 μl of 1N $HClO_4$ the proteins were precipitated. The obtained suspension was centrifuged (12000 rpm, 20 min) and filtered (nylon 0.45 μm). The filtered solution was assayed by HPLC. The analytical recovery of thiocolchicoside, calculated in experiments in which a known amount of drug was added to a blank slice of mucosa, was 99%.

### 2.5.3. Permeation of thiocolchicoside from dosage forms

The porcine tissue preparation was carried out as described for the determination of thiocolchicoside concentration gradient (see Section 2.5.2). In these experiments, four vertical Franz-type diffusion cells (Disa, MI, I), with a diffusional area of 3.91 $cm^2$ were placed in a water bath (37 ± 0.5 °C). After the membrane equilibration (30 min), the dosage forms (each containing 4 mg of drug) were deposited on the mucosal surface in the donor compartment and 20 μl of saline solution were added. After fixed intervals of contact time (15, 30, 45, 60 min), the respective experiment was stopped. The residual formulation was collected and the donor compartment was washed with distilled water. The residue of the formulation recovered and the washing solutions were combined and then analyzed for the drug content. The amount of thiocolchicoside absorbed in the mucosa was calculated as the difference between initial and recovered mass.

### 2.5.4. Thiocolchicoside buccal absorption from dosage forms in humans

The 'buccal absorption test' was performed on healthy volunteers (males and females, age range 25–33) according to Rathbone (1991).

Both the bioadhesive and fast dissolving dosage forms, each containing 4 mg of thiocolchicoside, were tested. Before each buccal administration, the volunteers washed their mouth with 100 ml of distilled water. Then, the dosage form was placed under the tongue (fast dissolving) or in contact

with the gingival mucosa (bioadhesive) and kept there without swallowing during a fixed period of time. Then, the residue of dosage form was expelled and the mouth was rinsed with distilled water ($3 \times 20$ ml). The residue of dosage form and the washing solutions were combined and analyzed for the remaining drug content. The amount of thiocolchicoside absorbed from the mucosa was determined as difference between initial and recovered mass.

## 3. Results

### 3.1. Thiocolchicoside permeation across porcine buccal mucosa and influence of penetration enhancers

The amount of thiocolchicoside permeated per unit surface area is plotted versus time in Fig. 2. The curve is characterized by an unexpected initial high flux, as, without any time lag, a prompt transport of drug was observed. After 30 min a lower steady state flux was reached as the linear part of the profile shows.

The linear part of the curve was used for the determination of the steady state flux ($J$) and the permeability coefficient ($P$), by means of a standard solution of Fick equation for steady state membrane transport:

$$J = PC_d$$

where $C_d$ is drug concentration in the donor solution.

According to the experimental conditions, the permeability coefficient of thiocolchicoside was $1.9 \pm 0.6 \times 10^{-7}$ cm/s. This value indicated interesting permeability characteristics of thiocolchicoside through porcine buccal mucosa.

In buccal administration drug permeation is often facilitated by means of enhancers. We studied the enhancing effect on thiocolchicoside transport through buccal mucosa of two enhancers, i.e. sodium taurocholate (NaTC) and sodium taurodeoxycholate (NaTDC), in different concentrations.

Fig. 3 shows the in vitro permeation profiles of thiocolchicoside obtained from saturated solution of drug containing sodium taurocholate, NaTC (10, 100 mM) and sodium taurodeoxycholate, NaTDC (10 mM). As shown, the bile salt derivatives did not enhance the transport of thiocolchicoside through the pig buccal mucosa. On the contrary, the transport of drug in presence of 100 mM of NaTC was practically suppressed. At concentration of NaTC of 10 mM, we observed basically the same steady state flux as without enhancer, but the initial part of the drug transport was lower, but not significantly different. In case

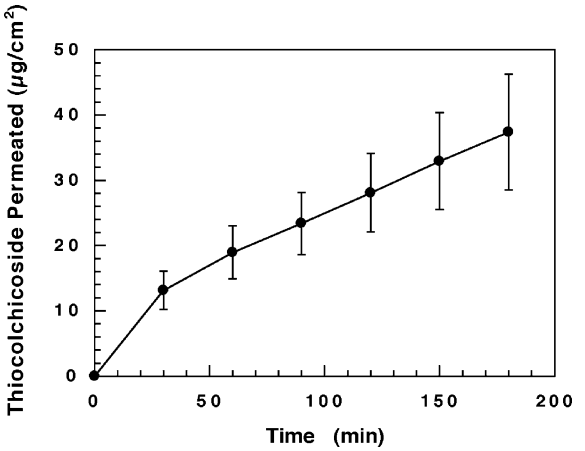


Fig. 2. Permeation profile of thiocolchicoside across porcine buccal mucosa using an aqueous saturated solution as donor (mean value ± S.E.M., $n = 10$).

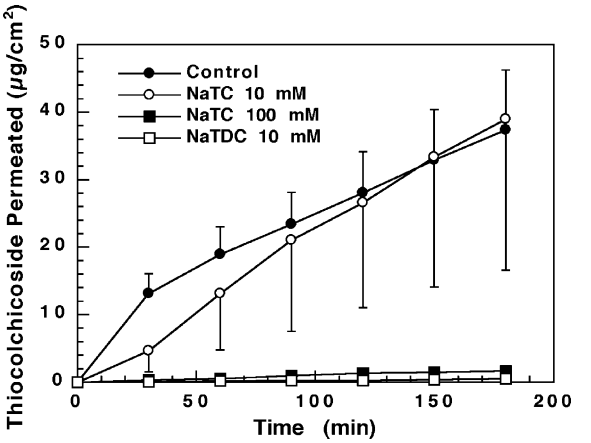


Fig. 3. Permeation profiles of thiocolchicoside across porcine buccal mucosa obtained from saturated solution with or without enhancers (mean value ± S.E.M., $n \geq 5$).

of NaTDC already a concentration of 10 mM completely suppressed the thiocolchicoside flux.

In another transport experiment through the full thickness porcine buccal tissue, with a donor solution of 8 mg/ml of thiocolchicoside, we found a little amount of thiocolchicoside in the receptor after 3 h (0.2 μg/cm$^2$). This could be due to the presence of the lamina propria in the tissue, which is an additional barrier for transport of hydrophilic drugs. Therefore, the disappearance of the drug from donor compartment was monitored during this permeation experiments. Fig. 4 shows the thiocolchicoside loss from donor compartment, expressed as percent remaining of the initial mass plotted versus time. We observed a quick decrease of drug concentration in the donor during the first 30 min, in which about 1.5 mg of thiocolchicoside disappeared. After this initial fast decrease the concentration seemed to remain stable. Fig. 4 also shows the profile of thiocolchicoside loss from a donor phase containing a 8 mg/ml solution of thiocolchicoside together with 100 mM sodium taurocholate. It can be seen that in the presence of enhancer the uptake in the porcine membrane after 20 min is much slower compared with the drug alone ($P < 0.05$).

This experiment confirmed the very quick initial absorption of thiocolchicoside from the buccal mucosa as measured by the disappearance of drug from donor solution. The addition of the enhancer retarded the drug disappearance from donor.

As a result of these experiment, we considered the possibility that there is an immediate uptake of drug in the superficial layer of membrane during the first 30 min, explaining the fast decrease in the donor solution concentration and, thereafter, the constant level of drug in the donor phase.

### 3.2. Thiocolchicoside concentration gradient determination in porcine buccal tissue

In order to get further insight in the permeation characteristic of thiocolchicoside, its distribution inside the buccal epithelium was measured as disappearance of the thiocolchicoside from the donor phase.

Fig. 5 shows the thiocolchicoside concentration profile plotted versus distance from the surface of the porcine buccal tissue obtained after three different contact times (15, 30, 180 min). It was discovered that already after 15 min of contact time, a concentration gradient inside the mucosa existed. At this time the distribution profile showed a peak value around 100–200 μm in depth, followed by a decrease of the drug concentration in the deeper layers. After 30 min, the concentration peak was even more evident at the same depth, followed by an almost flat profile of concentration. With the increase of application time to 180 min, the peak moved to deeper layers

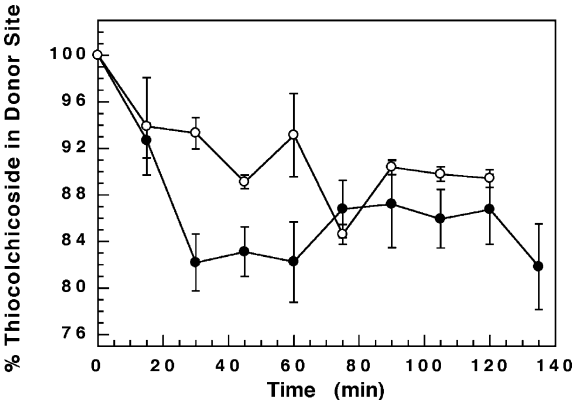


Fig. 4. Thiocolchicoside loss from donor compartment during the permeation experiments (full circle, aqueous saturated solution; empty circle, aqueous solution with sodium taurocholate 100 mM; mean value ± S.E.M., $n \geq 3$).

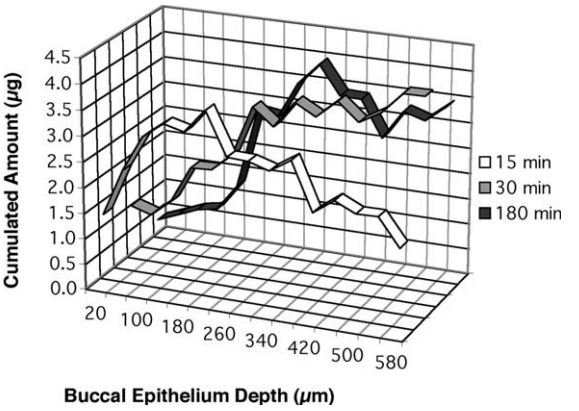


Fig. 5. Thiocolchicoside cumulated amount in the pig buccal mucosa during the permeation experiments as a function of time ($n \geq 2$).

(>250 μm). After 180 min, the amount of thiocolchicoside cumulated in the outer layers was very low even if the drug concentration in donor phase was still high (see Fig. 4).

### 3.3. Buccal dosage forms: dissolution and permeation

For the therapeutic application of thiocolchicoside across the buccal mucosa two different solid dosage forms were developed. The first formulation was a bioadhesive disc intended for cheek or gingival sticking. The adhesion of the formulation to a smooth and relatively immobile surface, like the gingival mucosa, allows a close contact between drug and absorption site for a longer period of time. Moreover, this formulation is suitable for a sustained delivery of drug.

The second dosage form was a fast dissolving disc suitable for sublingual administration. The sublingual region has been extensively used to deliver suitable drugs with a prompt onset of the therapeutic effect. The fast dissolving dosage form, quickly soluble in a small volume of saliva, could rapidly create a high drug concentration in contact with a relatively large absorption surface.

Fig. 6 shows the dissolution profiles of the two dosage forms prepared. The two formulations were clearly different with respect to the drug release rate: about 30 min for the bioadhesive and a few min for the fast dissolving dosage form were required for complete dissolution and release of the drug.

Fig. 7 shows the disappearance of the drug released from the two different dosage forms after application to the surface of freshly isolated buccal mucosa of pigs. In this study, the residual thiocolchicoside concentrations in the donor phase were measured. As shown in Fig. 7, the amount of thiocolchicoside absorbed from the bioadhesive form is lower because of the slower release from the dosage form. The drug loss profile obtained with the fast dissolving dosage form was similar to results obtained from thiocolchicoside solution (8 mg/ml) absorption profile. The quantity of drug permeated into the tissue during the first 30 min is comparable (about 20%), even if the amount of thiocolchicoside in the dosage form is only one half compared with the solution.

In vivo permeation experiments through human buccal mucosa were performed on volunteers as a buccal 'absorption' test (Rathbone, 1991).

Fig. 8 compares the amount of thiocolchicoside absorbed in vitro through porcine buccal mucosa with the amount obtained with eight volunteers in vivo, from the fast dissolving form. The quantity of drug absorbed, calculated by the difference between the initial and the remaining amount of thiocolchicoside, is plotted versus time. In this experiment, the volunteers are advised to maintain

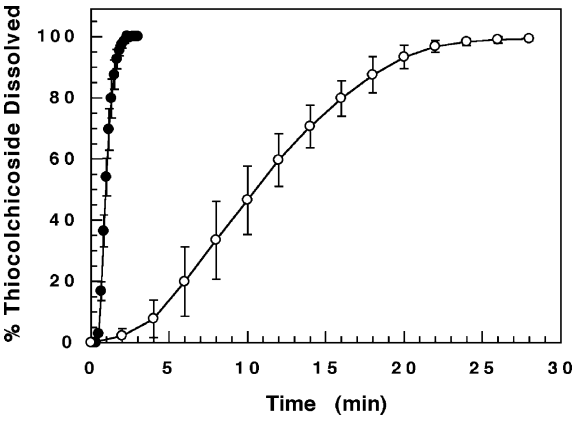


Fig. 6. Dissolution profiles of thiocolchicoside dosage forms (full circles, fast dissolving form; empty circles, bioadhesive form; mean ± S.E.M., $n = 3$).

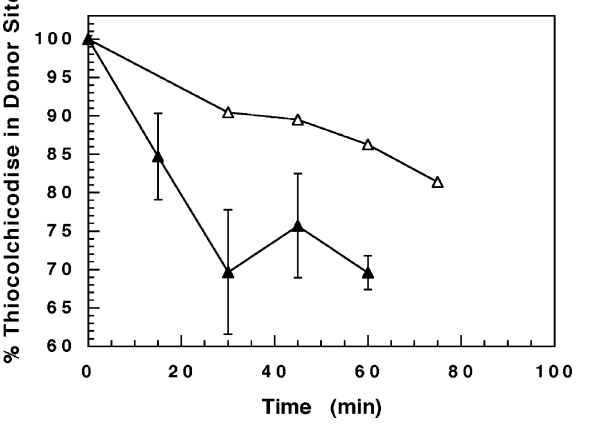


Fig. 7. Thiocolchicoside loss from donor compartment containing bioadhesive (open triangle) and from fast dissolving dosage form (closed triangle), both containing 4 mg of drug.

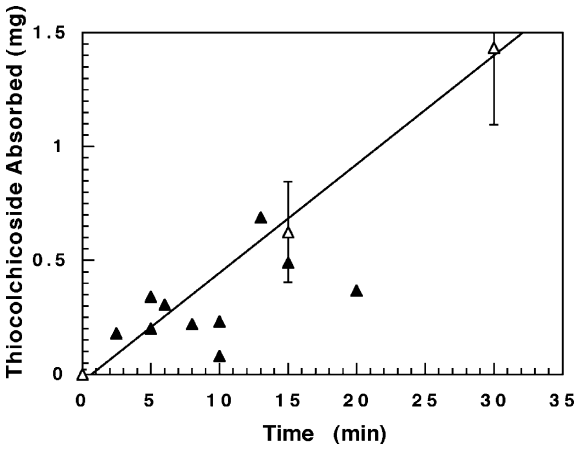


Fig. 8. Comparison between the thiocolchicoside transported in vitro through porcine mucosa (open triangle) and absorbed in vivo in volunteers (closed triangle) from fast dissolving form.

dosage form in the sublingual area for a fixed period of time (up to 20 min). As shown, despite of variability of in vivo data, a quite good correlation between in vivo and in vitro results can be demonstrated. The volunteers reported that, in few seconds after deposition of the dosage form in the area under the tongue, the form dissolved so that allowing prompt drug absorption from sublingual mucosa.

The same experiment was performed with the bioadhesive form. Fig. 9 compares the absorption profile of thiocolchicoside obtained in vitro and in vivo in the buccal absorption test. The curve shows the quantity of drug permeated, measured as difference between the initial and the remaining amount of thiocolchicoside plotted versus time. As reported in Fig. 9, the amount of drug absorbed in vivo and in vitro were comparable, even if an in vivo intersubject variability was noted. As supposed, due to the presence of bioadhesive agent (carboxymethylcellulose), the dosage form can be immobilized at buccal or gingival mucosa for prolonged period of time (up to 60 min) without any inconvenience for the patient.

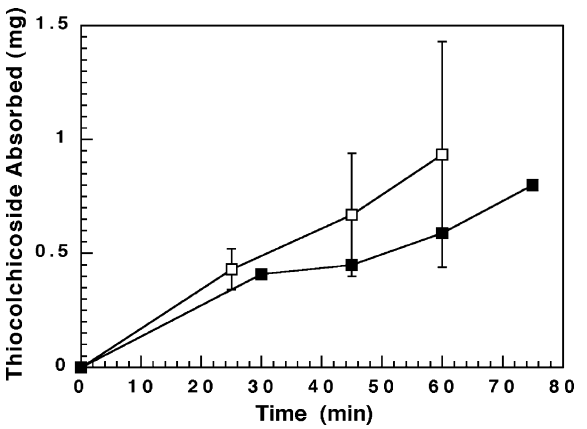


Fig. 9. Comparison between the thiocolchicoside transported in vitro through porcine mucosa (closed squares) and absorbed in vivo in volunteers (open squares) from bioadhesive form.

## 4. Discussion

The results obtained in these studies prove the feasibility of administering thiocolchicoside through the buccal route. Buccal or sublingual administration is a useful alternative to the oral route, avoiding pre-systemic metabolism.

The permeability coefficient calculated shows that thiocolchicoside has quite good permeability characteristics through buccal mucosa. Two penetration enhancers, such as sodium taurocholate and sodium taurodeoxycholate at different concentration (10 and 100 mM), were tested in order to increase thiocolchicoside transport across buccal mucosa. The results obtained showed that the penetration enhancers not only provided no increase, but they suppressed drug permeation across buccal tissue. The lack of enhancement observed with the employed bile salts towards thiocolchicoside was already described for other compounds. The phenomenon depends on the permeant, as mentioned by Senel and Hincal (2001). Moreover, the short time of the permeation experiment could reduce the possibility to evidence differences in permeation, as the comparison with the results of Senel et al. (1997) in the case of morphine sulfate can suggest. Concerning the reduction of the permeation due to the presence of bile salts, the hypothesis of an interaction between bile salts and thiocolchicoside seems to be reasonable. In fact, a DSC analysis on a 1:3 molar mixture of thiocolchicoside and NaTC showed an evident interaction between the two

substances when water was present. The phenomenon will require further investigations.

The concentration profile of thiocolchicoside into the epithelium was determined with a tissue slicing method so that studying thiocolchicoside transport properties became possible. The obtained profile showed that already after 30 min of permeation, thiocolchicoside is cumulated into superficial layers of epithelium. A peak drug concentration at about 150–200 μm depth was created. After 180 min, despite of the drug concentration in the donor phase was still high, the drug decreased from upper and moved to deepest layers.

Similar results were reported by Veuillez et al. (2002), studying the permeation and the profile concentration of a myristoylated dipeptide across the buccal mucosa. In fact, the dipeptide, after 8 h of permeation, was found mainly in the outer layers facing the donor compartment. Increasing the permeation time, the dipeptide moved reaching the deeper layers and after 48 h its amount in the outer layers was very low, even if its concentration in the donor compartment was still high (Veuillez et al., 2002). The thiocolchicoside concentration profiles obtained showed clearly a fast penetration of drug to the deepest strata of the buccal epithelium. In addition, a peak concentration of thiocolchicoside at about 150–200 μm from the surface was identified. The presence of the peak in the mucosa indicated an accumulation of thiocolchicoside probably due to its precipitation inside this tissue level that roughly corresponds to the cell layer of buccal mucosa in which membrane-coating granules (MCG), spherical or oval organelles, 100–300 nm diameter, are located (Wertz et al., 1996). MCG appear to fuse with cell membranes and extrude their content, mainly made of lipids, into the intercellular space. It is believed that the volume of MCG in the intracellular space and the discharged lipids in the intercellular space, form the major barrier through transcellular and paracellular pathways (Squier, 1977). In fact, Lesch et al. showed the existence of a relationship between the relative volume occupied by MCG in human stratified squamous epithelia and permeability to water (Lesch et al., 1989). Nevertheless, Hoogstraate et al., by means of confocal laser scanning microscopy, were able to visualize in porcine buccal epithelium, the region corresponding to MCG as the rate-limiting zone for diffusion of fluorescein isothiocyanate, a hydrophilic marker (Hoogstraate et al., 1996c). This data confirmed that the intercellular lipids extruded by the so-called 'MCG' (150–200 μm deep) represents the major permeability barrier in the buccal mucosa, for hydrophilic compound like thiocolchicoside. Finally, the overall penetration of thiocolchicoside into the buccal pig mucosa was very fast, in particular considering the time lower than 30 min in which thiocolchicoside could already been detected in a depth of 600 μm inside the buccal tissue which is the tissue adjacent to the lamina propria.

Two buccal solid dosage forms were prepared. The first formulation was realized as fast dissolving form intended for sublingual administration. This dosage form was made of thiocolchicoside and excipients promptly soluble in the small volume of saliva underlying the tongue. So, when brought in contact with the sublingual mucosa, the dosage form immediately dissolved allowing the drug penetration across the mucosa.

The second formulation was a bioadhesive dosage form containing thiocolchicoside, gelatin and a bioadhesive agent such as sodium carboxymethylcellulose. This form was projected for a gingival application far from salivary ducts. Due to its adhesive properties the form realized a close contact with absorption site for longer period of time (up to 60 min).

Also, the dosage forms were different in terms of rate of drug release. The fast dissolving dosage form was able to deliver the entire dose in about 2 min while the bioadhesive in about 30 min. The absorption of thiocolchicoside through buccal mucosa both from solution and from fast dissolving dosage form, was similar and linear during the early 30 min, and then decreased. The fast dissolving dosage form allows a high drug concentration at the absorption site, with a good absorption characteristic. The mucoadhesive drug delivery system performed a delayed release resulting in a slower uptake of the drug in the buccal tissue.

Despite the variability of the in vivo obtained results from the buccal absorption tests, in which the drug uptake in the buccal tissue was measured, there is a quite good correlation between 'in vitro' (porcine) and 'in vivo' (human) data for both dosage forms. In fact, the transport of thiocolchicoside through human buccal mucosa was very fast so that in 15 min about 0.5 mg of thiocolchicoside was absorbed from sublingual formulation. In case of gingival formulation, the amount and the rate of thiocolchicoside absorption was lower than sublingual formulation, mimicking the in vitro measured data.

Volunteers reported that the fast dissolving dosage form, designed to obtain a rapid onset of therapeutic effect, dissolved immediately in the sublingual region, and that the bioadhesive form, suitable for a sustained drug release, adhered strongly to gingival mucosa for prolonged periods of time. However, due to the low residence time of fast dissolving formulation after its administration, it can be supposed that, in practice, only a small amount of drug was absorbed from sublingual mucosa. In the case of bioadhesive formulation the buccal absorption of thiocolchicoside could be improved by designing a bioadhesive dosage form directing thiocolchicoside only to the mucosa.

In both cases, none of the volunteers reported local sensibilization or irritation after administration of thiocolchicoside formulation.

Our primary interest was to evaluate the feasibility of a sublingual/buccal delivery of thiocolchicoside in order to improve the bioavailability and faster the therapeutic effect. On the basis of the obtained results, we concluded that the delivery of thiocolchicoside via oral mucosa has good perspectives of therapeutic application. A future work will concern the study of in vivo thiocolchicoside absorption measuring drug blood levels so that supporting the data presented in this paper.

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