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Sodium lauryl sulphate increases tiludronate paracellular transport using human epithelial Caco-2 monolayers

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Abstract

The potential effect of the common pharmaceutical wetting agent, sodium lauryl sulphate (SLS), on the transport of the hydrophilic bisphosphonate, tiludronate, was investigated both by performing physico-chemical determinations of the SLS-tiludronate interaction and by measuring the paracellular transport of tiludronate (Boulenc et al., *Biochem. Pharmacol.*, 46 (1993) 1591–1600) across the in vitro human intestinal epithelium model, i.e., Caco-2. SLS did not affect the contact angles determined with different liquids (glycerin, dioxane, sulphuric acid, water, mercury, heptane and decane) on tiludronate tablets. SLS influenced neither the disintegration of tiludronate tablets nor tiludronate solubility. However, both the efficiency and effectiveness of SLS, in reducing surface tension, were affected by tiludronate. Hence, the presence of 0.48 g/l tiludronate in a water solution changed the efficiency of SLS in reducing the surface tension from 1 to 0.3 g/l. Before evaluating tiludronate transport across Caco-2 monolayers, the reversible (absorption enhancement of orally administered drugs) and irreversible (cell cytotoxicity) effects of SLS on the viability of Caco-2 monolayers were investigated. Following a 1 h exposure of well-differentiated Caco-2 cells to SLS concentrations above 100 mg/l, mitochondrial dehydrogenase activity decreased in a concentration-dependent manner, cytosolic lactate dehydrogenase leakage occurred, mannitol transport was irreversibly increased and a structural separation of the tight junctions was observed by electron microscopy. SLS concentrations up to 80 mg/l did not affect mitochondrial dehydrogenase and cytosolic lactate dehydrogenase activities, while both a reversible increase in mannitol paracellular transport and tight junction opening were observed. Under these incubation conditions, tiludronate paracellular transport was increased in a concentration-dependent manner. These studies demonstrate that SLS increased tiludronate paracellular transport through its specific and transient effect on the permeability of the intercellular space.

Keywords: Intestinal absorption; Paracellular route; Intestinal model; Surfactant; Caco-2 monolayer

Abbreviations: TEER, trans-epithelial electrical resistance; FCS, foetal calf serum; SLS, sodium lauryl sulphate

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1. Introduction

Tiludronate, or (4-chlorophenyl)thiomethylene diphosphonic acid (disodium salt), is a novel bisphosphonate with potential use in the treatment and prevention of osteoporosis and the treatment of Paget's disease. Bisphosphonates are known to be poorly absorbed when given orally (Fogelman et al., 1986; Janner et al., 1991) and their absorption varies extensively between and within individuals. Among various pharmacokinetic studies performed in both animals and humans (Janner et al., 1991; Hyldstrup et al., 1993) which demonstrated their low bioavailability, recent in vitro studies performed with the Caco-2 monolayer model reported that tiludronate was slowly transported across the intestinal epithelium, through the paracellular route (Boulenc et al., 1993).

The use of surfactants in formulations intended for administration to human subjects can lead to changes in the absorption of drugs and hence, in their pharmacological activity, although the surfactant effect on drug absorption does not readily allow generalisations to be made (Florence, 1981). These products were demonstrated to influence the disintegration and dissolution of solid dosage forms; they can be considered as absorption enhancers by increasing membrane permeability and/or affecting membrane integrity (Tomita et al., 1988; Van Hoogdalen et al., 1989). Moreover, it was also suggested that surfactants such as bile acids (Freel et al., 1983; Tomita et al., 1988), caprate and caprylate (Sawada et al., 1991; Anderberg et al., 1993) affected tight junction regulation. Hence, drugs for which dissolution and solubility are the rate-limiting steps for their absorption and drugs for which membrane transport is the rate-limiting step might be affected differently. Surfactants have been shown either to increase, decrease or exert no effect on the transfer of drugs across biological membranes (Attwood and Florence 1983). The influence of surfactants depends on the concentration, degree of ionization and hydrophile-lipophile balance (HLB) of both the surfactant and the drug, as well as drug solubility.

Sodium lauryl suphate (SLS), a hydrophilic anionic surfactant molecule, is a known wetting agent, able to modify the surface properties of solids through the phenomenon of adsorption, and to decrease interfacial tension at solid/liquid interfaces. Moreover, like naturally occurring micelle formers such as bile salts and phospholipids, SLS can also form micelles and leads to the enhancement of dissolution and solubilization. These properties when added to the SLS effect on intestinal membrane permeability and integrity may modify drug absorption (Muranishi, 1990).

The human adenocarcinoma cell line Caco-2 which reproducibly displays a number of the properties of differentiated intestinal cells was shown to be the most relevant in vitro system for investigating transepithelial transport processes (Artursson, 1990; Artursson and Karlsson, 1991; Dantzig and Bergin, 1990; Hilgers et al., 1990; Lundin and Artursson, 1990; Rubas et al. 1993), rapidly evaluating the permeability of a drug, defining the mechanisms of transport (Boulenc et al., 1993), and testing novel strategies for enhancing drug transport (Audus et al., 1990; Wilson, 1990; Artursson, 1991).

This study was carried out in order to evaluate the qualitative and quantitative influence of SLS on the bioavailability of tiludronate, by analysing two different processes such as drug dissolution and its further absorption across the in vitro intestinal epithelium model, Caco-2.

2. Materials and methods

2.1. Chemicals

^{14}C-labelled (spec. act. 33 mCi/mmol) and unlabelled tiludronate were obtained from Sanofi Recherche, Montpellier, France. [^{14}C]PEG_{4000} (Mol. Wt 4000; spec. act. 13 mCi/g), [^{14}C]PEG_{400} (Mol. Wt 400; spec. act. 15.3 mCi/g) and [^{3}H]mannitol (Mol. Wt 182; spec. act. 30 Ci/mmol) were purchased from Amersham International (Bucks, UK) and New England Nuclear Products (Boston, USA), respectively.

SLS (98% sodium lauryl suphate) was purchased from Laserson and Sabetay (Etampes, France). Magnesium stearate was purchased from

Table 1
Tablet composition (mg)

Tablet	A	B
Tiludronate disodium	240.0	240.0
SLS	4.5	–
Magnesium stearate	4.5	4.5
Cross-linked sodium carboxymethylcellulose	24.0	24.0
Microcrystalline monohydrated lactose	177.0	181.5

Atochem, CECA, France. Cross-linked sodium carboxymethylcellulose (Ac-Di-Sol) was purchased from FMC Corp. (Philadelphia, PA, USA) and microcrystallized monohydrated lactose (99.7%) from S.A. Sucre de Lait (Sains du Nord, France). Liquids used were distilled water, glycerin, dioxane, sulphuric acid, heptane and decane and were obtained from commercial sources.

2.2. Tablet composition

The tiludronate tablets studied have the compositions listed in Table 1.

2.3. Wetting measurement

Wetting refers to the process which occurs when a solid-air interface is replaced by a solid-liquid interface. In pharmaceutical technology, wetting has usually been characterized by contact angles (Gissinger and Stamm, 1980; Buckton and Newton, 1986; Lippold and Ohm, 1986). When a drop of liquid is placed on a plane, homogeneous and solid surface, it assumes a shape which corresponds to the minimum free energy for the system. The contact angle formed by a drop of liquid on the horizontal surface is the angle formed by the solid surface and the tangent of the liquid surface at their intersection. The condition for minimum free energy at equilibrium is that given by Young's equation:

$$\gamma_{S/G} = \gamma_{S/L} + \gamma_{L/G} \cos\theta$$

where θ is the contact angle, determined by photographing the drop placed on the surface and $\gamma_{S/G}$, $\gamma_{S/L}$ and $\gamma_{L/G}$ denote the interfacial tensions at the solid/gas, liquid/gas and solid/liquid interfaces, respectively. θ is measured directly by using a microscope fitted with a goniometer eyepiece and by photographing the droplet (Adamson, 1976, Uyama et al., 1990)

Zisman (1964) established a linear relationship between $\cos\theta$ and 1/surface tension of the wetting liquid for many surfaces with low surface energies. The surface tension obtained by extrapolation to $\cos\theta = 1$ is known as γ_c the critical surface tension of the solid. The γ_c value is a characteristic of a solid and may be related to the composition of the solid. The condition for wetting of a solid is that the surface tension of the wetting liquids must not exceed the value of γ_c. Thus, tablets with low γ_c are only wetted by liquids of very low surface tension. Increased γ_c values improve the wetting of the tablet.

2.4. Disintegration and dissolution kinetics

The disintegration and dissolution kinetics were determined on six tablets according to the official methods of the US Pharmacopeia XXII (1990). The medium used was 0.1 M hydrochloric acid to which sodium chloride (2 g for 1000 g) was added. The final pH value was set at 1.2 and experiments were performed at 37° C. The concentration of tiludronate was determined by UV spectroscopy at 261 nm on a Beckman UV spectrophotometer.

2.5. Surface tension measurement

Measurement of the surface tension was accomplished using the Wilhelmy plate method according to the ISO R 304 Method (1978) on a Prolabo [1] N3 tensiometer. For the purpose of comparing the performance of surfactants in reducing the surface tension, it was necessary to distinguish between the efficiency of the surfactant, i.e., the bulk phase concentration of surfactant required to reduce the surface tension to its minimum value whatever the surfactant concentration, and its effectiveness, i.e., the maximum reduction in tension that can be achieved regardless of surfactant concentration (Rosen, 1978). Surfactant efficiency is expressed as a surfactant concentration, when surfactant effectiveness is expressed as a force per length unit.

2.6. Cell culture

Caco-2 cells, originating from a human colorectal carcinoma (Fogh et al., 1977), were obtained from Dr A. Zweibaum (INSERM U-178, Villejuif, France). Cells were cultivated as described elsewhere (Boulenc et al., 1993). All tissue culture media were obtained from Eurobio Laboratories (Paris, France). Cells used in this study were between passages 90 and 120.

For transport studies Caco-2 cells were seeded onto 0.45 μm pore collagen type I-coated inserts (Millicell-CM; pore size, 0.4 μm; diameter, 30 mm; Millipore, Bedford, MA) at 63 000 cells/cm^2. The monolayers used in this study were 12–20 days post-seeding or 7–16 days post-confluence.

2.7. Integrity of the monolayers

The integrity of the monolayers was determined by measurement of the potential difference (TEER, transepithelial electrical resistance) and by following the transepithelial transport of a macromolecular marker, PEG_{4000} as previously described (Boulenc et al., 1993). The potential difference was expressed as transmembrane resistance (Ω/cm^2) after subtraction of the intrinsic resistance of the model (i.e., the resistance obtained over the cell-free inserts). A monolayer with low TEER was assumed to exhibit extensive leakage through imperfect occluding junctions or holes in the monolayer (Hidalgo et al., 1989; Artursson, 1990). TEER values (230 ± 50 Ω/cm^2) remained constant from day 12 to 20 (Hidalgo et al., 1989; Artursson, 1990).

[^{14}C]PEG_{4000} (38 μg/ml) was added to the apical side of the monolayers. The radiolabelled marker transported over Caco-2 cells was evaluated after 3 h at 37° C on a 0.5 ml aliquot part withdrawn from the basolateral chamber. The samples were measured in a liquid scintillation counter from Packard. Inserts without cells were used to determine the maximal transport of the marker during the same time period. The results were expressed as the percentage transported of the dose. The rate of [^{14}C]PEG_{4000} transported was 0.1 ± 0.02% per h. These results are consistent with those previously reported by Hidalgo et al. (1989), who showed that undamaged monolayers are almost impermeable to macromolecules such as PEG_{4000}.

2.8. Preparation for transmission electron microscopic examination

The inserts were washed in PBS buffer (pH 7.2) and fixed in a solution containing 2% glutaraldehyde and 0.1 M sucrose in 0.1 M cacodylate buffer (pH 7.3) for 1 h at 25° C. The cells were rinsed in 0.1 M Hanks buffer and fixed with 1% osmium tetroxide in 0.1 M sodium cacodylate · HCl buffer for 1 h at 25° C. After dehydration, the preparation was embedded in Epon resin, sliced with a Reichert ultramicrotome, stained with uranyl acetate and lead acetate, and examined on a transmission electron microscope (Jeol 100S).

2.9. Measurement of drug transport and radioactive scintillation counting

Drug solutions were prepared from the radiolabelled isotopes and the corresponding unlabelled compounds in Hanks buffer to give final concentrations up to 10^{-3} M in the apical compartment. All transport experiments were performed as described elsewhere (Boulenc et al. 1993).

2.10. Calculations

The apparent permeability coefficient (P_{app}) was determined as previously reported:

$$P_{app} = dQ/[dt \times A \times C_0],$$

where dQ/dt is the transport rate (μg/s) and corresponds to the slope of the regression line determined on at least four different time points, C_0 denotes the initial concentration in the donor chamber (μg/ml or μg/cm^3), and A is the area of the membrane (5.7 cm^2)

2.11. Cytotoxicity tests

2.11.1. Mitochondrial dehydrogenase activity

The intracellular mitochondrial dehydrogenase activity was determined by the MTT method.

Briefly, MTT is a tetrazolium salt cleaved by the mitochondrial dehydrogenase activity in living but not dead cells to a dark-blue product. Cells (approx. 10^5 cells) were seeded in 96-well tissue culture plates and cultured for 5 days. Cells were treated with increasing SLS concentrations for 1 h. The incubation medium was removed, and cells were incubated with MTT (5 mg/ml) for an additional 1 h. The incubation medium was measured in a multiwell scanning spectrophotometer (Multiscan MCC/340, Labsystem).

2.11.2. Lactate dehydrogenase activity

Lactate dehydrogenase is a cytosolic enzyme which is recovered in the extracellular compartment following membrane disruption. Caco-2 monolayers were treated for 1 h with increasing concentrations of SLS and the lactate dehydrogenase activity was determined in the extracellular compartment using the Sigma kit.

2.11.3. Mannitol transport

The integrity of Caco-2 monolayers can also be investigated by its permeability to mannitol, a compound which is transported via the paracellular route. Caco-2 cell monolayers were treated for 1 h with increasing SLS concentrations ranging between 4 and 100 mg/l. The apical compartment containing SLS was removed and replaced by SLS-free fresh medium. After a 24 h period of rest, [^{3}H]mannitol was added to the apical side of the monolayers and its rate of transport across the Caco-2 monolayer was evaluated over a 3 h period at 37° C, by quantification of radiolabel in the basal compartment. Inserts without cells were used to determine the maximal transport of the marker during the same period of time.

2.12. Statistical test

Values are expressed as mean ± standard deviation. Three experiments were performed ($n = 3$), except where indicated otherwise in the figure legends. The results were analysed by one-way analysis of variance. Significance: $^{*}p < 0.1$; $^{**}p < 0.05$; $^{***}p < 0.01$.

3. Results

3.1. Physico-chemical studies

3.1.1. Influence of SLS on tablet wetting

As tablet porosity plays an important role in the variation of the contact angle of a liquid drop on the tablet, contact angle measurements were performed as a function of time with water drops. Table 2 shows the contact angles determined with different liquids (glycerin, dioxane, sulphuric acid, water, mercury, heptane and decane) on tilu-

Table 2
Contact angles and surface tension of various liquids on tiludronate tablets formulated in the absence or presence of SLS

Liquid	Contact angles (°)		Surface tension (mN/m)
	Tablet A (with SLS)	Tablet B (without SLS)	
Glycerin	70	70	63.4
Dioxane	10	10	–
Sulfuric acid	45	47.5	51
50% sulfuric acid	47	–	58
Mercury	136	136	4356.5
Heptane	0	0	18.4
Decane	0	0	23.9
Water			
$t = 0$	55	50	72
$t = 1$ min	45	45	
$t = 2$ min	40	40	
$t = 3$ min	35	35	
$t = 5$ min	30	30	

dronate tablets supplemented or not with SLS. The results reported in Table 2 indicate that no significant modification of the contact angle occurred with the different liquids on tablets supplemented or not with SLS. The kinetics of tablet wetting by water are not affected by the presence of SLS.

Fig. 1 illustrates the linear relationship between $\cos\theta$ and $1/\text{surface tension}$ of the wetting liquid. The equation for this relation is as follows:

$$\cos\theta = 88.7(1/\gamma) - 0.93.$$

The surface tension obtained by extrapolation to $\cos\theta = 1$ is known as γ_c, i.e., the critical surface tension of the solid. The γ_c value obtained for tiludronate tablets irrespective of the presence of SLS is then $\gamma_c = 46$ mN/m, which represents the surface free energy of the tiludronate tablets.

3.1.2. *Influence of SLS on tablet disintegration kinetics*

The disintegration times of tiludronate tablets ranged between 6 and 7 min. SLS had no influence on tiludronate tablet disintegration.

3.1.3. *Influence of SLS on tiludronate solubility and dissolution kinetics*

Tiludronate is soluble in water up to 160 g/l and its solubility was not modified in the presence of SLS concentrations ranging between 0.1 and 10 g/l.

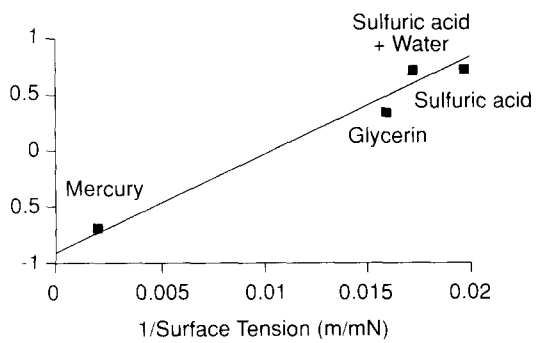

Fig. 1. Effect of SLS on critical surface tensions of tiludronate tablets. Contact angles of different liquids were determined on tiludronate tablets formulated in the absence or presence of SLS.

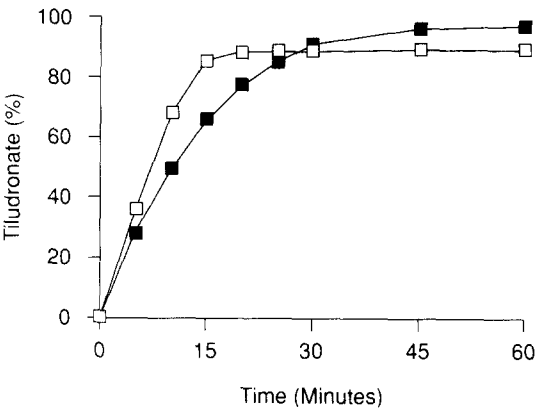

Fig. 2. Effect of SLS on tiludronate solubilisation. Solubilisation of two tiludronate tablets formulated in the absence (□) or presence (■) of SLS was investigated in a USP XXII dissolution test and the precentage of solubilisation was evaluated as a function of time (SD 10%).

Fig. 2 illustrates the amount of tiludronate solubilized during the dissolution test conducted with tiludronate tablets supplemented or not with SLS. These results indicated that tiludronate was solubilized more rapidly from a tablet which did not contain SLS than from a tablet supplemented with SLS. However, after a 1 h period, slightly greater amounts of tiludronate were solubilized from a tablet supplemented with SLS (96.6% vs 88.7% in the presence or absence of SLS, respectively; $n = 6$).

3.1.4. *Influence of SLS on surface tension of tiludronate solutions*

The influence of tiludronate on water surface tension was first investigated for concentrations up to 10 g/l, a concentration higher than that assumed in the gastric juice. Fig. 3 illustrates the solution surface tension obtained for various concentrations of SLS. Both the efficiency and effectiveness of SLS in reducing surface tension were affected by tiludronate. The presence of 0.48 g/l tiludronate in a water solution changed the efficiency of SLS in reducing the surface tension from 1 to 0.3 g/l. This significant change could be compared to the effect of any salt in solution with an anionic surfactant. The ranges of SLS effectiveness in reducing the surface tension were 0.01–0.3 and 0.01–1 g/l when tests were per-

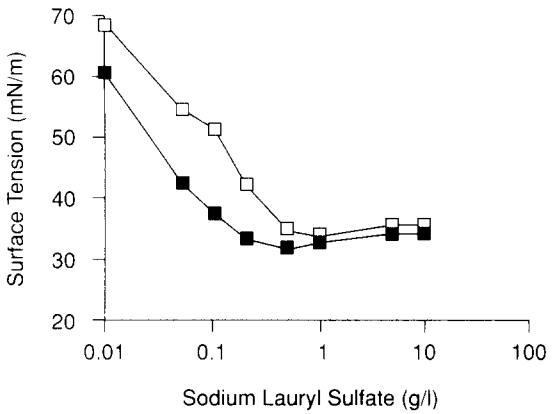

Fig. 3. Efficiency and effectiveness of SLS in reducing surface tension of tiludronate solutions. Surface tension of SLS solutions (4.8 g/l) was evaluated in the absence (□) or presence (■) of tiludronate (SD 10%).

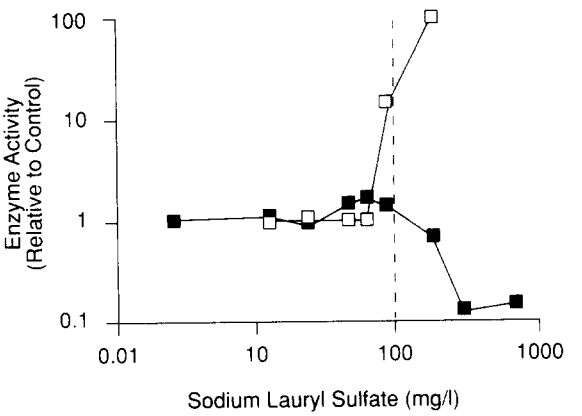

Fig. 4. Effect of SLS on mitochondrial dehydrogenase and cytosolic lactate dehydrogenase activities in Caco-2 cells. Cells were incubated for 1 h with increasing SLS concentrations and the intracellular mitochondrial dehydrogenase activity (■) as well as the extracellular lactate dehydrogenase activity (□) was determined according to the methods described in section 2. Values are expressed as mean ± S.D. ($n = 8$ obtained in two single experiments) (SD 15%).

formed in the presence or absence of tiludronate, respectively.

3.2. Effect of SLS on tiludronate transport

3.2.1. SLS cytotoxicity

In order to evaluate the range of SLS concentrations which could be investigated, Caco-2 monolayers were exposed for 1 h to SLS concentrations ranging between 0.02 and 1500 mg/l (Fig. 4). Mitochondrial dehydrogenase activity remained unaffected up to an extracellular SLS concentration of 47 mg/l. For SLS concentrations ranging between 47 and 100 mg/l, an increase in dehydrogenase activity was observed, which can be associated, at least in part, with an increase in tetrazolium salt transported inside the cells. For SLS concentrations above 100 mg/l, a decrease in enzyme activity was observed, with 50% inhibition of mitochondrial dehydrogenase activity at an SLS concentration of 180 mg/l.

Extracellular lactate dehydrogenase activity was also monitored over the same range of SLS concentration. No significant increase in extracellular enzyme activity was observed up to an extracellular SLS concentration of 80 mg/l. A dramatic leakage of the cytosolic enzyme was demonstrated for SLS concentrations above 100 mg/l (Fig. 4).

Mannitol transport was also determined following a 1 h exposure to SLS concentrations ranging between 4 and 120 mg/l. The permeability coefficient remained constant up to 32 mg/l SLS. A continuous increase was then observed from 64 to 120 mg/l SLS (Fig. 5). The permeabil-

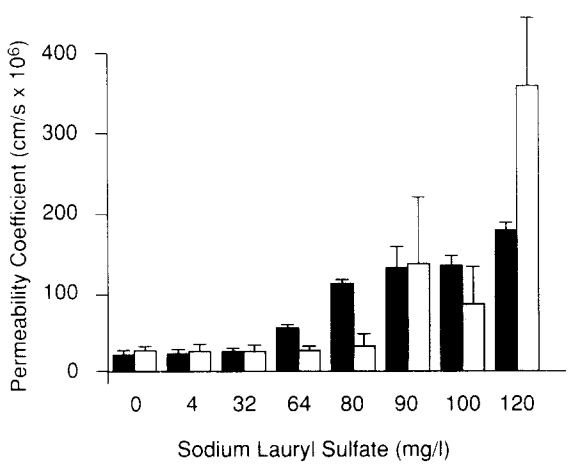

Fig. 5. Effect of SLS pretreatment on mannitol permeability. Caco-2 cells were treated for 1 h with increasing SLS concentrations and [^{3}H]mannitol transport was monitored (■) as described in section 2. [^{3}H]Mannitol transport was reinvestigated following a 24 h period of rest in SLS-free fresh culture medium at 37° C (□).

ity coefficient of mannitol was then redetermined following a 24 h period of recovery in SLS-free fresh medium. Mannitol transport was similar to that observed in untreated cells after exposure of monolayers to SLS concentrations up to 80 mg/l. At higher SLS concentrations, an increase in mannitol transport was still observed, suggesting that monolayer integrity was not recovered following a 24 h period of recovery, subsequent to a 1 h exposure to SLS concentrations above 80 mg/l.

3.2.2. Effect of SLS on monolayer morphology

Caco-2 monolayers were incubated for 1 h with increasing SLS concentrations and monolayer morphology was examined by electron micrography (Fig. 6). Examination of untreated 15-day postconfluence Caco-2 monolayers revealed a

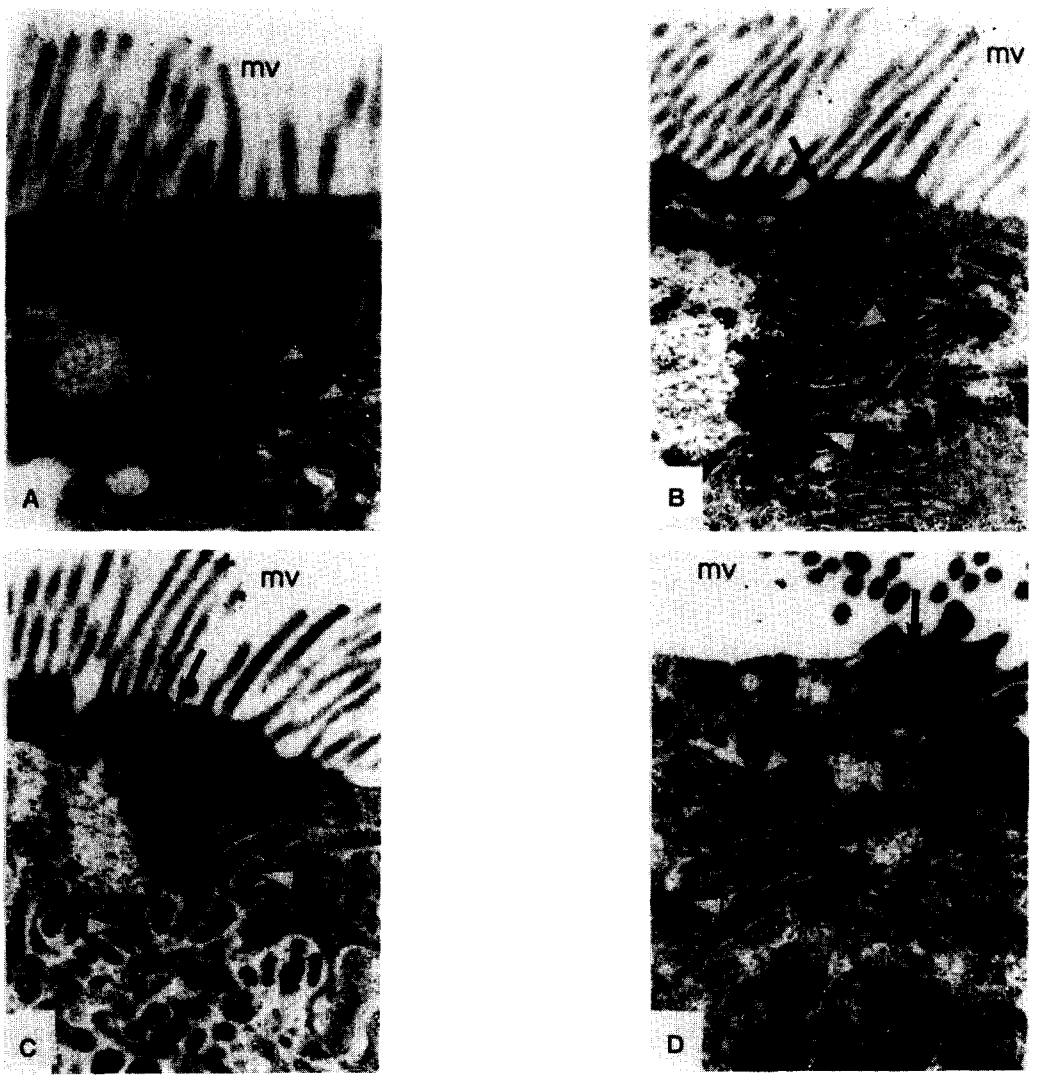

Fig. 6. Electron micrographs of Caco-2 monolayers. Electron micrographs of 14-day-old Caco-2 monolayers cultured over 1 h in the absence (A) or presence of either 24 mg/l (B), 60 mg/l (C), or 180 mg/l (D) SLS. mv, microvilli; open arrowheads, desmosomes; closed arrows, tight junction.

well-differentiated brush border, cells being separated by desmosomes and tight junctions. No major difference was observed following a 1 h treatment with 24 mg/l SLS, although some shortened microvilli appeared. At 60 mg/l SLS concentration, some perturbations at the intercellular level were observed. A widening of intercellular space and of the zonula occludens was observed. Nevertheless, cell integrity was maintained. When cell monolayers were treated with 180 mg/l SLS, most of the microvilli disappeared, nuclei were pycnotic and some extracellular deposits were observed in the cytosol.

3.2.3. Effect of SLS on tiludronate transport

Tiludronate transport across Caco-2 monolayers, was determined following addition of the radiolabelled drug in the apical compartment, in the absence or presence of increasing SLS concentrations, ranging between 1 and 80 mg/l. The rate of appearance of [^{14}C]tiludronate in the basal compartment was monitored over 2 h. The permeability coefficient for tiludronate transport was evaluated following addition of 1 mM tiludronate in the apical compartment (Fig. 7). Under control conditions, i.e., in the absence of SLS, the permeability coefficient for tiludronate was determined to be $4.0 \times 10^{-7} \pm 2.2 \times 10^{-7}$ cm/s ($n = 8$), based on experiments performed on different cell preparations and on 10–16-day post-confluence cells. The apparent large inter-experiment variability was mostly due to the fact that tiludronate was slowly transported by the paracellular route (Boulenc et al., 1993).

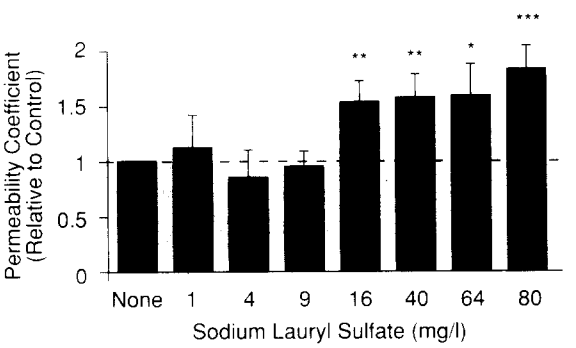

Fig. 7. Effect of SLS on tiludronate transport. Radiolabeled tiludronate (1 μM) was added in the apical compartment of Caco-2 monolayers, in the absence or presence of increasing SLS concentrations ranging between 4 and 80 mg/l. At specified times and over a 1 h period, aliquot parts of culture medium were withdrawn from the basal compartment and the rate of appearance of radiolabel in the basal compartment was determined. Values are expressed as the mean ± S.D. of four experiments performed on different days.

Tiludronate transport remained unaffected in the presence of SLS concentrations up to 16 mg/l. For SLS concentrations ranging between 16 and 80 mg/l, a statistically significant concentration-dependent increase in tiludronate transport was observed compared to control monolayers. The permeability coefficient of tiludronate increased up to $13.0 \times 10^{-7} \pm 1.0 \times 10^{-7}$ cm/s ($n = 4$) in the presence of 80 mg/l SLS. Although higher SLS concentrations led to a greater increase in tiludronate transport (data not shown; the tiludronate permeability coefficient = $21.0 \times 10^{-7} \pm 7.3 \times 10^{-7}$ cm/s ($n = 3$) at 200 mg/l SLS), this phenomenon was mainly due to the cytotoxicity of SLS.

4. Discussion

The human intestinal epithelial cell line, Caco-2, spontaneously differentiated, in culture, to polar cells possessing microvilli and enterocytic properties. Confluent monolayers form tight junctions between cells; thus, monolayers exhibited electrical properties characteristics of an intestinal epithelium. Epithelial cells are joined by junctional complexes which are comprised of three separate structures: tight junctions, intermediate junctions, and desmosomes. The intercellular spaces are sealed by tight junctions which reduce their pore radius to a few ångström units (Madara and Dharmsathaphorn, 1985). The contribution of this paracellular pathway to the total permeability of the epithelial monolayer is only significant for drugs that are transported slowly across the cell membrane, e.g., hydrophilic compounds with a low molecular weight and very low partition coefficients (Artursson and Karlsson, 1991). The integrity of these structures is dependent on free Ca^{2+} ions (Martinez-Paolo et al., 1980).

Various molecules are able to produce a widening of the intercellular space such as surfac-

tants, calcium chelators, fatty acid and palmitoylcarnitines (Muranishi, 1990). Surfactants are generally incorporated into solid dosage forms, so that, when the disintegration process starts, water penetrates and forms a concentrated surfactant layer, lowering the surface tension around the drug particles or granules. Moreover, surfactant adsorption on hydrophobic drug particles below the critical micelle concentration could aid wetting and consequently increase their dissolution rate (Rees and Collet, 1974). Several surfactants are extensively used as pharmaceutical wetting agents. Usually, for lipophilic molecules, surfactants result in an enhancement of the solubilisation rate. This is not the case for bisphosphonates such as tiludronate which are hydrophilic molecules. Nevertheless, a number of authors demonstrated that some surfactants exert an effect on the paracellular route, explaining the effect of this kind of molecule on the absorption of hydrophilic molecules (Freel et al., 1983; Sawada et al., 1991). Recently, Anderberg et al. (1993) showed that SLS induced widening of the intercellular space and tight junctions with Caco-2 monolayers associated with a decrease in monolayer resistivity and an increase in the transport of paracellular marker molecules. Caco-2 monolayers cultured under standard conditions exhibited a transmembrane resistance of 250 Ω/cm^2, i.e., consistent with that observed in the colonic mucosa, this resistance was so high that transport of hydrophilic compounds was reduced to only very low levels. Hence, even small changes in paracellular permeability were therefore readily detectable (Anderberg et al., 1992, 1993; Raeissi and Borchardt, 1993).

The intestinal absorption of various bisphosphonates, i.e., 1-hydroxyethylidene-1,1,bisphosphonate (HEBP) and dichloromethylene bisphosphonate (Cl_2MBP), has already been described (Lamson et al., 1984; Fogelman et al., 1986; Fleich, 1993) as being low and of the order of a few percent in humans. These data are consistent with in vitro studies performed on the Caco-2 model, indicating that tiludronate, a bisphosphonate, was transported by the paracellular pathway (Boulenc et al., 1993).

Since SLS could affect both tiludronate dissolution and disposition, and also membrane permeation, these two parameters were further investigated. SLS affected neither wetting nor disintegration rates of tiludronate tablets. This could be due, at least in part, to the presence of large amounts of hydrophilic substances, such as lactose and disodium tiludronate itself in the tablet. Moreover, due to the considerable hydrophilicity of tiludronate, its water solubility was not affected by SLS. The low solubilization enhancement observed during the dissolution test could be mainly due to the disintegration of small particles. SLS could lower the solution surface tension, since a linear relationship between the dissolution rate and the surface tension of the dissolution medium has already been reported (Finholt and Solvang, 1968). Moreover, these experiments have been carried out in vitro, but the in vivo effect is more complex. It involves a concomitant dilution of the excipients by a complex medium, possible absorption of the surfactant and adsorption of other substances onto the dissolving particles. The surface tension of tiludronate solutions was reduced from approx. 60 mN/m in the absence of SLS, to 30–35 mN/m in the presence of SLS. The surface tension decreased significantly, and as it reflects the concentration of the surfactant at the interface relative to that in the bulk liquid phase, it can be assumed that most of the SLS was adsorbed at the membrane interface after in vivo tablet disintegration.

The effect of SLS on tiludronate transport was investigated on the Caco-2 monolayer model. The range of SLS concentrations used was determined on the basis of cytotoxicity. Four different approaches allowed us to demonstrate that 80 mg/l SLS was the highest SLS concentration which could be used on this cell system. Hence, at 100 mg/l SLS concentration, we observed a decrease in mitochondrial dehydrogenase activity, a dramatic increase in the release of cytosolic lactate dehydrogenase and a irreversible opening of tight junctions as confirmed by mannitol paracellular transport and electron micrography of intercellular spaces.

Hence, tiludronate transport was investigated at SLS concentrations ranging between 4 and 80 mg/l, this latter concentration being considered

as the higher non-toxic concentration. These results are similar to those described by Anderberg and Artursson (1993), who reported that a 20 min exposure of Caco-2 cells to 0.40 mM SLS (or 115 mg/l) resulted in reversible absorption enhancement of mannitol, 1-deamino-8-D-arginine-vasopressin and polyethylene glycol, while a 2 h exposure resulted in irreversible absorption enhancement. Moreover, SLS improves tiludronate absorption at concentrations lower than toxic levels.

SLS concentrations up to 16 mg/l did not affect tiludronate transport. However, for SLS concentrations ranging between 16 and 80 mg/l, a significant enhancement in the permeability coefficient of tiludronate was observed. This increase in tiludronate paracellular transport was associated with an increase in mitochondrial dehydrogenase activity, i.e., a consequence of the SLS effect on membrane permeability, and both a reversible increase in paracellular transport of mannitol and a reversible opening of the intercellular space including tight junctions and desmosomes. These results show that SLS improves tiludronate absorption up to 80 mg/l without toxic effects.

These experiments are in accordance with physico-chemical studies showing a reduction of tension from 1 to 0.3 g/l. This reduction of tension reflects interfacial adsorption of SLS at the lipidic membranes leading to reversible wounds. Hence, such membrane perturbations may lead to a paracellular route for regulation, with a widening of intercellular space via passive entrance of extracellular calcium (Anderberg and Artursson, 1993). These results would suggest that the effect of SLS on tight junctions arises from SLS adsorption at cell membranes.

The interaction of SLS with the intestinal epithelium has previously been described in whole-tissue models (Muranishi, 1990). At SLS concentrations above 2 mM (or 580 mg/l), a partial denudation of the epithelium was observed in situ, as observed in the Caco-2 model. Lower SLS concentrations, i.e., 1 mM, were reported to increase the permeability 2-fold in rat intestinal mucosa (Nadai et al., 1975). Sund (1975) also reported that SLS concentrations lower than 1.7 mM (or 490 mg/l) led to subtle effects on water and sodium secretion in jejunal loops of rats. Among drug solubility, dissolution rate, particle size, density, ionization, chemical stability, etc., the luminal volume played a crucial role in terms of dissolution volume. In agreement with Dressman et al. (1985), the luminal volume was set at 250 ml, based on available information concerning volume, flow rates and transit time.

On the basis of this luminal volume and on in vitro studies performed on the Caco-2 epithelial model, the maximal SLS dosage which could be administered without encountering cell damage would be 30 mg according to Anderberg and Artursson (1993) and 20 mg in this study, while toxicity would occur at 125 mg (Nadai et al.,1975) or 120 mg (Sund, 1975).

In summary, the combination of physico-chemical studies and of transport experiments performed on the Caco-2 monolayer epithelial model allowed us to demonstrate that paracellular transport of tiludronate was maximally increased following treatment of cells with SLS which specifically and reversibly enhances tight junction opening.

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