Ex vivo transport of tamoxifen and its 4-OH metabolite through rat intestine from lecithin/chitosan nanoparticles

S. Barbieri[a,d], F. Buttini[a], A. Rossi[a], R. Bettini[a], P. Colombo[a], G. Ponchel[b], F. Sonvico[a,e,*], G. Colombo[c].

[a]Department of Pharmacy, University of Parma, Parco Area delle Scienze 27/a, 43124 Parma, Italy.
[b]Université de Paris Sud XI, CNRS UMR 8612, Faculté de Pharmacie, 5 rue J.B. Clément, 92296 Châtenay-Malabry, France.
[c]Department. of Life Sciences and Biotechnology, University of Ferrara, Via Fossato di Mortara 17/19, 44121 Ferrara, Italy.
[d]Interdepartmental Center, Biopharmanet-TEC, University of Parma, Parco Area delle Scienze 27/A, 43124 Parma, Italy.
[e]Graduate School of Health Pharmacy, University of Technology Sydney, Broadway, NSW 2007, Australia.

*Corresponding author
Prof. Fabio Sonvico
Department of Pharmacy, University of Parma
Parco Area delle Scienze 27/a, 43124 Parma, Italy
Tel. +39 0521 905088; Fax +39 0521 905006
E-mail: fabio.sonvico@unipr.it

ABSTRACT

The development of formulations for oral administration of anticancer agents would introduce innovative therapies for cancer treatment. Unfortunately, few anticancer drugs are soluble and permeable enough to allow for their administration by the oral route. In this regard, the use of nanocarriers could improve the drug oral bioavailability, therapeutic efficacy and safety profile, since the encapsulated drug is masked within the nanostructure.

Tamoxifen citrate is slightly soluble in water. Administered orally, it shows great intra- and inter-patient variation in bioavailability.

The aim of the present work was to study the transport of lecithin/chitosan nanoparticles loaded with tamoxifen across the rat intestinal wall. Studies were performed *ex vivo* on rat intestinal tissue mounted in an Ussing chamber.

KEYWORDS: Intestinal transport, tamoxifen citrate, oral chemotherapy, chitosan, nanoparticles.

1. Introduction

Formulations for oral administration of anticancer drugs can introduce innovative therapies for cancer treatment [1]. Unfortunately, few anticancer drugs are soluble and permeable enough to allow for their administration by the peroral route. However, innovative drug delivery systems could improve the bioavailability and reduce the heavy administration schedule of such active agents, thus increasing activity and patient compliance [2]. At the scope, the use of nanocarriers could improve the drug oral bioavailability, therapeutic efficacy and safety profile, since the encapsulated drug is masked in the nanostructure. Nanoparticles could prevent the direct contact of the drug with the mucosa, protect the molecule from degradation in the gastric environment [3], or by-pass the cell efflux pumps, key players in multidrug resistance of tumours [4].

We previously described lecithin/chitosan nanoparticles loaded with tamoxifen citrate intended for oral administration in the treatment of estrogen-dependent breast cancer [5]. Lecithin and chitosan self-assembled leading to nanoparticle formation. Chitosan played the role of bridging the phospholipid negative polar heads of formed phosphatidylcholine liposomes, strengthening the vesicle structure [6]. The release of tamoxifen citrate from these lecithin/chitosan nanoparticles was triggered by enzymes acting on the nanoparticle constituents, in particular lipase and lysozyme, thus destabilizing the nanoparticle structure. The drug remained protected from gastric pH and started being released in intestinal fluid in presence of pancreatin, lysozyme or lipase alone or combinations thereof [7].

Tamoxifen citrate is slightly soluble in water. Administered orally, it shows great intra- and inter-patient variation in bioavailability [8]. The mechanisms underlying the variable response to tamoxifen have been the object of a lot of investigation, but remain obscure. However, it is now known that *in vivo* the overall pharmacological action of tamoxifen is due in part to its transformation into active metabolites. As tamoxifen is converted to more potent anti-estrogenic metabolites, one hypothesis is that individual and/or altered patterns of tamoxifen metabolism might contribute to inter-individual variability in the elicited effects [9]. These tamoxifen metabolites are generated mainly from isoform CYP2D6 of the CYP-450 present in the intestinal wall [10]. Beverage et al. showed that 4-hydroxy-tamoxifen (4-OH-TAM; MW 387.5), one of the human metabolites of tamoxifen (TAM; MW 371.5), is about 100 times more potent than tamoxifen. Its erratic appearance could support the inter-individual variability of tamoxifen effect [11].

The aim of the present work was to study the transport of tamoxifen through the rat intestinal wall from donor formulations containing tamoxifen citrate (i.e., free non encapsulated drug) or lecithin/chitosan nanoparticles loaded with tamoxifen. Experiments were performed *ex vivo* using rat intestinal tissue in an Ussing chamber. The appearance of 4-OH-tamoxifen in the receptor phase was monitored during the TAM transport. The influence of pancreatin or lipase on tamoxifen release from lecithin/chitosan nanoparticles during transport experiments was studied. Finally, the effect of the nanoparticle bioadhesion to the intestinal mucosa on permeation of tamoxifen was investigated as well.

2. Material and methods

2.1. *Material*

Chitosan with a deacetylation degree of 95% and a viscosity of 103 cP, as determined by the supplier on a 1% solution (w/v) in acetic acid 1%, was provided by Primex (Chitoclear FG, Haugesund, Norway). Soybean lecithin used was Lipoid S45 (Lipoid AG, Ludwigshafen, Germany). Tamoxifen citrate (MW. 563.6) produced by Plantex Ltd. (Netanya, Israel) was a kind gift from Lisapharma S.p.A. (Erba, Italy). Pancreatin from porcine pancreas and lipase from *Pseudomonas fluorescens* were purchased from Sigma-Aldrich (St. Louis, USA).
All other chemicals of analytical grade were from Carlo Erba (Milan, Italy). Purified Milli-Q water (Millipore, Billerica, MA, USA), degassed and filtered through 0.45 µm regenerated cellulose filters (Sartorius, Barcelona, Spain), was used in all experiments.

2.2. *Preparation of tamoxifen citrate-loaded lecithin/chitosan nanoparticles*

Nanoparticles were produced according to the previously described method [5]. Briefly, 8 ml of a methanol solution containing 200 mg of lecithin and 60 mg of tamoxifen citrate were injected under mechanical stirring at 11000 rpm (Ultraturrax TP 18/10-10N, IKA-Werke GmbH Staufen, Germany) in 92 ml of an aqueous solution containing 10 mg of chitosan prepared by diluting 1 ml of chitosan solution 1% (w/v) in HCl 0.1 N. Injection rate (40 ml/min) was controlled using a mechanical syringe pump (Model 200, KD Scientific, Holliston, MA, USA), pumping through a glass pipette with a 0.5 mm tip orifice. The TAM nanoparticle suspension obtained had a pH value of 2.7.

2.3. *Ex vivo experiments with Ussing chamber*

2.3.1. *Preparation of the intestinal tissue*

The jejunum from small intestine of sacrificed male Wistar rats (200-250 g) (Charles River, Paris, France) was excised, washed with chilled physiological saline solution (NaCl 0.9% w/v) and longitudinally cut into segments of 2-3 cm in length. After visual examination of the tissue, sections containing Peyer's Patches were discarded from the studies. The studies were approved by the Ethical Committee of the University of Paris Sud XI (agreement n° A92-019-01) in strict accordance with the European legislation on animal experiments.

2.3.2. *Transport experiments*

Jejunum segments were mounted in the Ussing chamber with mucosal side facing the donor and the serosal side facing the receptor. The intestinal surface exposed to the transport (1 cm^2) was washed with Ringer solution at pH 6.8. The chamber was maintained at 37 °C and continuously oxygenated with a mixture of O_2 and CO_2 (95-5%). After 30 min of equilibration, the medium in the donor chamber was replaced by 5 ml of preheated (37 °C) Ringer solution containing non-encapsulated drug in suspension or nanoparticles (160 µg/ml of tamoxifen citrate). In the experiment with enzymes, the donor also contained 1% (w/v) of pancreatin or 1000 U/ml of lipase. The receptor chamber was filled with Ringer solution (5 ml) containing 1 % (w/v) of hydroxypropyl-β-cyclodextrin (HPCD). At pre-determined time points (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 h), aliquots of 200 µl were sampled from the receptor chamber and replaced with the same volume of the preheated (37 °C) Ringer solution containing HPCD.

2.3.3. *Drug and metabolite assay*

Tamoxifen and 4-OH-tamoxifen were assayed using the HPLC method reported in the tamoxifen citrate monograph of the Ph.Eur. 6.0 Ed. [12]. A Shimadzu (Kyoto, Japan) HPLC apparatus, equipped with a Spherisorb® ODS2 column (4.6 x 250 mm, 5 µm) (Waters Corporation, Milford, MA, SA), was used. The mobile phase was a mixture (40:60) of acetonitrile and a solution of 0.9 g/l sodium dihydrogen phosphate and 4.8 g/l N,N-dimethyloctylamine, adjusted to pH 3.0 with orthophosphoric acid. Flow rate was set at 1.2 ml/min and injection volume was 10 µl. UV detection was performed at 240 nm. External standard of tamoxifen citrate (10 µg/ml as tamoxifen) and 4-OH-TAM (11 µg/ml) were used. Retention times were 5 min and 8 min, respectively for 4-OH-tamoxifen and tamoxifen. Method suitability for tamoxifen was carried out with the following results: linearity between 0.010 and 110.000 µg/ml, relative standard deviation for repeatability 0.63% (n=6, solution concentration 10 µg/ml), theoretical plates 7154, peak symmetry 0.87.

2.4. *Statistical analysis*

Data were expressed as mean ± standard deviation (SD) of at least three replicates. Statistical significance analysis was processed using the nonparametric Mann–Whitney U-test (p value < 0.05). All calculations were performed using the KaleidaGraph® software program.

3. Results and discussion

3.1. *TAM transport through intestinal tissue from a saturated drug solution*

A first transport experiment was performed to determine the permeability of tamoxifen itself across the intestinal tissue from an aqueous saturated solution of tamoxifen citrate as donor. The donor was a suspension of tamoxifen citrate in Ringer solution at 160 µg of solid per ml. The pH of the suspension was 6.8 and the measured tamoxifen solubility was 27 µg/ml at 37 °C. Both tamoxifen and 4-OH-tamoxifen concentrations were determined in the samples collected from the receptor chamber, given that the alive intestinal tissue contains various enzymatic systems, including CYP450, able to transform TAM into 4-OH-TAM.

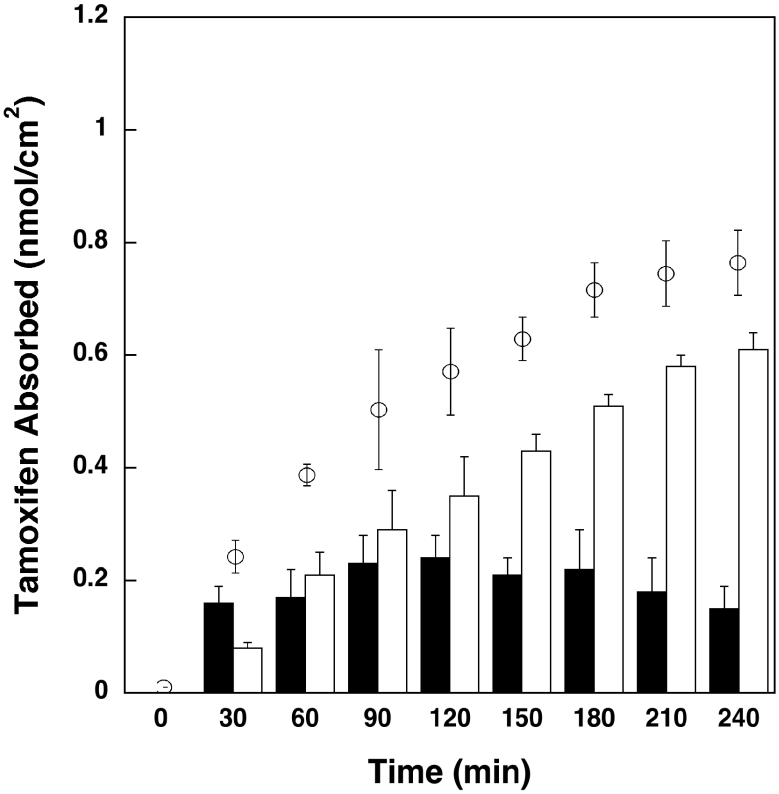

Fig. 1. Total tamoxifen (TAM + 4-OH-TAM) transported through the intestinal tissue from TAM suspension (160 µg/ml of tamoxifen citrate in Ringer solution) (open circles). The bars represent the actual amounts of intact drug and metabolite: black bars, tamoxifen; white bars, 4-OH-tamoxifen.

The amount of TAM absorbed and transported through the intestinal tissue in 4 hours was 0.76 nmol/cm^2 (Figure 1). Unexpectedly, after 60 minutes most tamoxifen in the receptor was

present as the metabolite 4-OH-tamoxifen. This amount increased linearly over time, whereas intact (i.e., non metabolized) TAM molecule did not accumulate in the donor. In fact, no increase in TAM concentration was evidenced after 90 minutes. As a result, the ratio between the metabolite and the transported intact drug increased with time and, in 4 hours, the amount of 4-OH-TAM in the receptor was fourfold that of TAM. Thus, the intestinal tissue metabolized the drug absorbed establishing a concentration gradient of the metabolite in the barrier thickness. Unfortunately, the metabolite concentration in the donor chamber was not measured at the end of this experiment, because the observation that tissue metabolism caused an important metabolite excretion in the donor, was made during later experiments. The metabolism could justify why, despite the constant drug activity in the donor phase containing a drug suspension, the permeation profile for total TAM was not in steady state. In fact, after an initial faster transport rate, a continuous decrease during the four hours of experiment was observed.

3.2. *TAM transport through intestinal tissue from loaded nanoparticles*

A second transport experiment studied the absorption of TAM when nanoparticles loaded with the drug (LCN-TAM) were introduced in the donor. The concentration of tamoxifen citrate as nanoparticles in the donor was maintained at 160 µg/ml. The nanoparticle suspension prepared according to the previous paper [5] was used. It contained approximately 40% of non encapsulated TAM together with the nanoparticles. Figure 2 shows the tamoxifen permeation profile from TAM-loaded lecithin/chitosan nanoparticles. The transported drug profile with the nanoparticles was higher than the one measured from drug suspension, but data variability did not allow claiming significant differences. After 4 hours, the total amount of drug (TAM + 4-OH-TAM) found in the receptor (1 nmol/cm^2) was about 1.5 times as higher as the value obtained with TAM suspension ($p<0.01$).

However, using the nanoparticles, a substantial difference in the metabolite/intact drug ratio was observed, since the amount of intact TAM in the receptor significantly increased over time paralleling the metabolite amount. Thus, when the nanoparticles were used, more TAM passed through the intestinal tissue without being transformed by the CYP450 enzyme. A paracellular transport, due to the chitosan present in the nanopreparation, seemed likely. In fact, it was the contribution of intact TAM that increased the total TAM absorbed, having determined that the metabolite amount cumulated in the receptor was approximately the

same as in the previous experiment. At the end of this experiment, an important intestinal extrusion into the donor of the intracellularly formed metabolite was also determined, since 24 ± 6 nmol of 4-OH-TAM were found in the donor compartment. Thus, tamoxifen absorbed and transported through the rat intestine was at a great extent metabolized and excreted as metabolite in both compartments of the Ussing Chamber, but predominantly into the donor phase. Tamoxifen excretion is not mediated by P-glycoproteins, since the compound is not a substrate for transporters, but no clear data on the polarized excretion of the metabolite are available [13-15]. The donor polarization of the metabolite indicated a short distance from the cytochrome enzyme to the apical membrane, since the enzyme is polarized towards the apical side in the intracellular space [16-18].

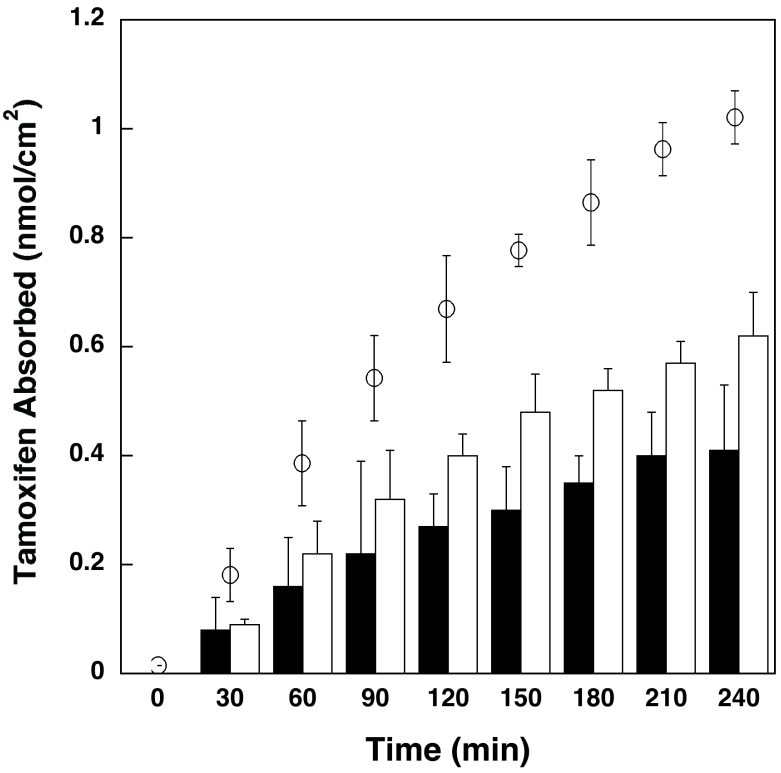

Fig. 2. Total tamoxifen (TAM + 4-OH-TAM) transported through the intestinal tissue from loaded nanoparticles (open circles). Black bars, intact tamoxifen; white bars, 4-OH-tamoxifen.

3.3. *TAM transport through intestinal tissue from loaded nanoparticles in presence of pancreatin.*

It was shown that *in vitro* tamoxifen citrate was released very slowly from the nanoparticles, unless enzymes capable to dismantle the nanostructure, such as pancreatin or lipase, were added to the release medium. In the presence of pancreatin, 50% of the encapsulated TAM was released in 24 hours [5]. Therefore, another permeation experiment was carried out in presence of pancreatin, studying the transport of tamoxifen and its metabolite from TAM nanoparticles or TAM suspension. Figure 3 illustrates the transport of TAM from nanoparticles and pancreatin across the intestinal tissue into the donor phase. The cumulative amount of TAM transported was similar to the experiment without pancreatin in the first two hours, then the transport rate burst after this time. It is undisputable that, starting from 180 minutes, a large amount of intact drug passed through the intestinal tissue, reasonably as a consequence of the nanoparticle degradation by the enzyme and ensuing drug release.

After the burst of intact drug transport, there was a much higher amount of TAM than 4-OH-TAM in the receptor chamber. Again, the amount of metabolite, due to transcellular transport, was similar to that quantified in the previous experiments. Thus, it could be said that now a “door for TAM” had been open in the tissue.

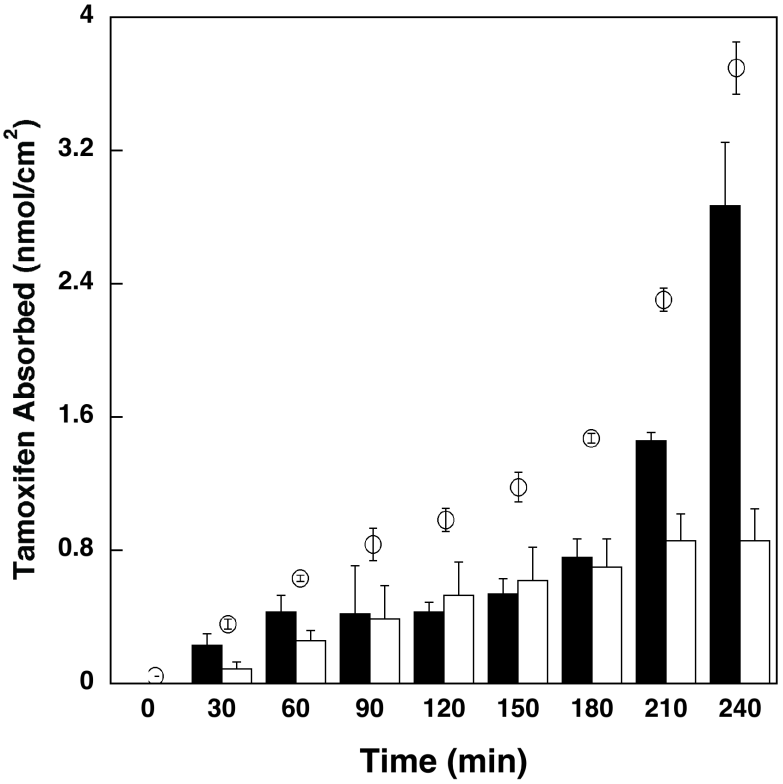

Fig. 3. Total tamoxifen (TAM + 4-OH-TAM) transported through the intestinal tissue from loaded nanoparticles in presence of pancreatin (open circles). Black bars, intact tamoxifen; white bars, 4-OH-tamoxifen.

The improvement in TAM transport rate could be assigned to an important increase of TAM chemical activity due to triggered drug release from the nanoparticles, in concomitance with the activation of a paracellular pathway for TAM through the intestinal epithelium.

This effect is typical of chitosan action on epithelial tight junctions. We already hypothesized a paracellular transport in the previous experiments with nanoparticles without pancreatin, despite the fact that in that case chitosan was strongly engaged in the nanoparticle structure and unable to act intensely on tight junction opening. Here, as the nanoparticles were degraded by the enzyme, the chitosan chains, disengaged from the nanostructure, could better interact with the intestinal cells by opening a paracellular way. Comparing the timing of the events, it was justified that the TAM transport accelerated with the nanoparticle enzymatic degradation, given that the *in vitro* release data showed that the effect of the degrading enzyme on the nanoparticle structure required 1-2 hours to become relevant. The tight junction pathway paralleled the intracellular transport of TAM, but following the paracellular

way, TAM metabolism by the intestinal cells was avoided. In addition, figure 3 shows that the amount of metabolite in the receptor was the same with TAM suspension or LCN-TAM without enzyme. Also in this experiment with pancreatin, an important intestinal extrusion of intracellularly formed metabolite of 23.8 ± 6.3 nmol was measured in the donor. Finally, the total TAM transported to the receptor phase through the intestinal tissue over four hours was 6 and 20 times higher than with LCN-TAM without enzyme and with TAM suspension, respectively.

In order to ensure that pancreatin did not alter the permeability of the intestinal tissue, a transport experiment using TAM suspension and pancreatin in the donor chamber was also carried out. The transport profile in 4 hours resulted superimposed to the one obtained without pancreatin (data not shown). It was concluded, as shown by other authors [19], that pancreatin did not modify the permeability of the intestinal tissue.

3.4. *TAM transport through intestinal tissue from loaded nanoparticles in presence of lipase*

The lipase enzyme was not largely represented in the pancreatin mixture used in the previous experiment (6 U/mg). During the *in vitro* release experiments, we showed that pure lipase was much more efficient than pancreatin in releasing TAM from the lecithin/chitosan nanoparticles (up to 80% in 24 h). To confirm the contribution of the enzyme-triggered degradation of the nanoparticles to the transport of the drug and its metabolite through the intestinal tissue, a transport experiment was carried out from a donor containing nanoparticles and lipase as degrading enzyme.

Figure 4 shows that the transport of TAM from nanoparticles increased of one order of magnitude compared to the experiments without lipase. Practically, the entire amount of drug transported in the receptor was unmodified tamoxifen.

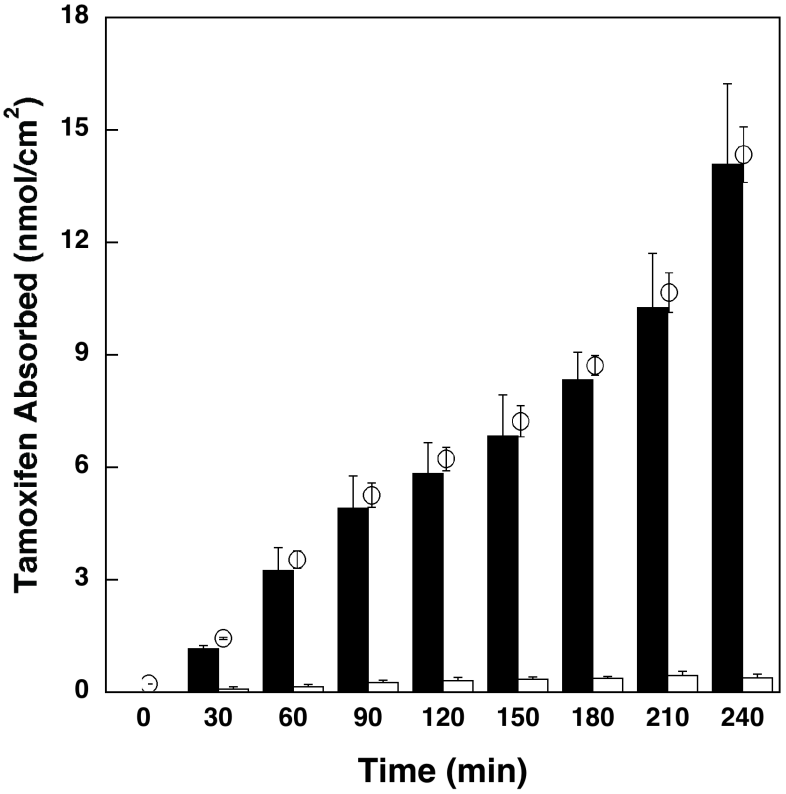

Fig. 4. Total tamoxifen (TAM + 4-OH-TAM) transported through the intestinal tissue from loaded nanoparticles in presence of lipase (open circles). Black bars, intact tamoxifen; white bars, 4-OH-tamoxifen.

Summarizing, the amount of tamoxifen transported through the rat intestinal tissue after 4 hours from LCN-TAM in the presence of lipase, was 4.5 times higher than with the LCN-TAM with pancreatin, 26 times higher than with LCN-TAM without enzymes and 90 times higher than with non encapsulated TAM in suspension. Now, the fraction of unmodified drug transported was prevalent. It must be underlined again that the amount of 4-OH-TAM measured in the receptor was not statistically different from the transport experiments where LCN-TAM with and without pancreatin were tested. This experiment confirms that the enzymatic degradation of lecithin/chitosan nanoparticles played a decisive role in the transport of TAM through the intestinal tissue.

3.5. *TAM transport through intestinal tissue coupled with a semipermeable membrane, from loaded nanoparticles in presence of pancreatin*

Being the increased transport of tamoxifen attributed to the degradation of nanoparticles on the intestinal mucosa, the transport of drug from the nanoparticles was investigated when the

luminal side of intestinal tissue was not accessible to the nanoparticles. The questions to answer were: “Does the adhesion of the nanoparticles to the intestinal tissue play a role in TAM transport through intestinal tissue? Is it the increased chemical activity of TAM or the presence of chitosan that enhanced the absorption of the drug?”.

The study was carried out under the same conditions as for the previous experiments (section 3.3), but avoiding nanoparticle contact and consequently bioadhesion to the mucosa. To do so, a semipermeable membrane (cut-off 100,000 Da) was placed on the mucosal side, separating the donor content from the tissue, according to Bravo-Osuna et al. [20]. The membrane allowed for the passage of drug, but blocked enzymes, chitosan and nanoparticles. The experiment was performed testing the transport from LCN-TAM with pancreatin in the donor. Figure 5 shows the amount of TAM transported during four hours in presence of the semipermeable membrane in comparison with the same experiment without membrane. It is clear that the membrane significantly decreased the amount of TAM transported through the intestinal tissue from the nanoparticles.

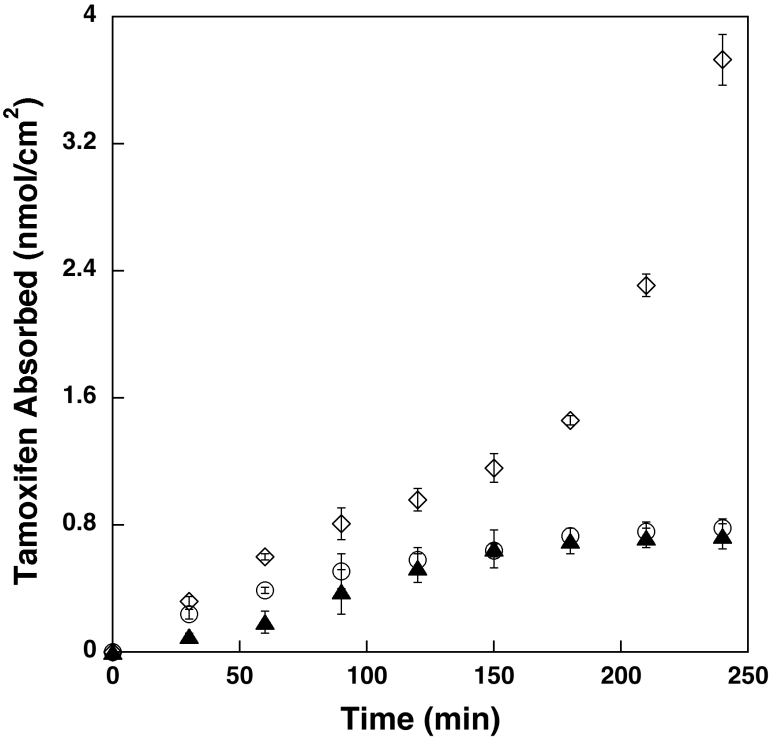

Fig. 5. Total tamoxifen (TAM + 4-OH-TAM) transported through the intestinal tissue from nanoparticles in presence of pancreatin without (diamond) and with (triangle) the semipermeable membrane and from TAM suspension with the membrane (circle).

Preliminarily, it was assessed whether the semipermeable membrane did not affect the transport of the drug. No difference in TAM transport from drug suspension existed between the presence or absence of the semipermeable membrane, as it can be seen comparing the profiles of Figure 5 and Figure 1.

The amount of drug transported from the TAM suspension through the intestinal tissue covered by the semipermeable membrane was similar to the transport from nanoparticles in presence of enzymes through the same barrier. The transport from the suspension was somehow faster up to 120 minutes, but as the nanoparticles degraded, the two profiles become superimposed. The membrane was not an obstacle for TAM molecules to access to the intestinal tissue. Thus, when the mucoadhesion of nanoparticles to intestinal mucosa was prevented, the transport profile of tamoxifen resulted similar to the one determined from the TAM suspension.

4. Conclusions

The obtained results allow to concluding that during the transport through the rat intestinal mucosa, in the conditions applied for the experiments, tamoxifen is heavily metabolized. The metabolite 4-OH-tamoxifen is excreted into both the receptor and the donor compartments. The amount of metabolite in the receptor phase during the experiments did not significantly change with the test conditions, i.e. using tamoxifen-loaded nanoparticles or a drug suspension, but the donor phase contained ten times more metabolite. A cell efflux mechanism able to reduce the amount of tamoxifen absorbed through metabolite formation must be present.

Considering the intact drug transported, the encapsulation of tamoxifen in lecithin/chitosan nanoparticles improved the non-metabolized drug transport through the rat intestinal tissue. When the nanoparticles were degraded by enzymes such as pancreatin or lipase, the intact drug transported amount increased of one order of magnitude compared to the transport from the free drug suspension, likely due to the promoting effect of chitosan molecules deriving from the dismantled nanoparticles. If the contact between the nanoparticles and the mucosa was prevented by the interposition of a semipermeable membrane, the TAM transported from

340 nanoparticles was similar as from tamoxifen suspension. This assigns to the lecithin/chitosan
341 nanoparticle structure a decisive role in tamoxifen intestinal absorption. Hence, the intimate
342 contact or mucoadhesion of nanoparticles to the mucosa is crucial to increase the transport of
343 TAM through the intestinal tissue via a paracellular way.
344

345 References

346

347 [1] S. Mazzaferro, K. Bouchemal, G. Ponchel, Oral delivery of anticancer drugs I: general
348 considerations, Drug Discov Today, 18 (2012) 25–34.

349 [2] K. Thanki, R. Gangwal, A.T. Sangamwar, S. Jain, Oral Delivery of Anticancer Drugs:
350 Challenges and Opportunities, J Control Release, 170 (2013) 15-40.

351 [3] R. Shankarayan, S. Kumar, P. Mishra, Differential permeation of piroxicam-loaded PLGA
352 micro/nanoparticles and their in vitro enhancement, J Nanopart Res, 15 (2013) 1496.

353 [4] M.A. Deli, Potential use of tight junction modulators to reversibly open membranous
354 barriers and improve drug delivery, BBA - Biomembranes, 1788 (2009) 892–910.

355 [5] S. Barbieri, F. Sonvico, C. Como, G. Colombo, F. Zani, F. Buttini, R. Bettini, A. Rossi, P.
356 Colombo, Lecithin/chitosan controlled release nanopreparations of tamoxifen citrate: Loading,
357 enzyme-triggered release and cell uptake, J Control Release, 167 (2013) 276-283.

358 [6] F. Sonvico, A. Cagnani, A. Rossi, S. Motta, M.T. Di Bari, F. Cavatorta, M.J. Alonso, A.
359 Deriu, P. Colombo, Formation of self-organized nanoparticles by lecithin/chitosan ionic
360 interaction, International Journal of Pharmaceutics, 324 (2006) 67–73.

361 [7] M. Thanou, J.C. Verhoef, H.E. Junginger, Chitosan and its derivatives as intestinal
362 absorption enhancers, Adv Drug Deliv Rev, 50 Suppl 1 (2001) S91–101.

363 [8] C.K. Osborne, Tamoxifen in the treatment of breast cancer, N Engl J Med, 339 (1998)
364 1609–1618.

365 [9] M.P. Goetz, Pharmacogenetics of Tamoxifen Biotransformation Is Associated With Clinical
366 Outcomes of Efficacy and Hot Flashes, Journal of Clinical Oncology, 23 (2005) 9312–9318.

367 [10] R. Ferraldeschi, W.G. Newman, The Impact of CYP2D6 Genotyping on Tamoxifen
368 Treatment, Pharmaceuticals, 3 (2010) 1122–1138.

369 [11] J.N. Beverage, T.M. Sissung, A.M. Sion, R. Danesi, W.D. Figg, CYP2D6 polymorphisms
370 and the impact on tamoxifen therapy, J Pharm Sci, 96 (2007) 2224–2231.

371 [12] Council of Europe. European Pharmacopoeia 6 ed. Strasbourg, 2001.

372 [13] R. Callaghan, C.F. Higgins, Interaction of tamoxifen with the multidrug resistance P-
373 glycoprotein, Br J Cancer, 71 (1995) 294–299.

374 [14] M. Werle, Polymeric and low molecular mass efflux pump inhibitors for oral drug delivery,
375 J Pharm Sci, 97 (2008) 60–70.

376 [15] N.A. Colabufo, F. Berardi, M. Contino, C. Inglese, M. Niso, R. Perrone, Effect of some P-
377 glycoprotein modulators on Rhodamine-123 absorption in guinea-pig ileum, Bioorg Med
378 Chem Lett, 18 (2008) 3741–3744.

379 [16] L.Z. Benet, C.L. Cummins, The drug efflux-metabolism alliance: biochemical aspects,
380 Adv Drug Deliv Rev, 50 Suppl 1 (2001) S3–11.

381 [17] S. Berggren, P. Lennernäs, M. Ekelund, B. Weström, J. Hoogstraate, H. Lennernäs,
382 Regional transport and metabolism of ropivacaine and its CYP3A4 metabolite PPX in human
383 intestine, Journal of Pharmacy and Pharmacology, 55 (2003) 963–972.

384 [18] S. Berggren, J. Hoogstraate, U. Fagerholm, H. Lennernäs, Characterization of jejunal
385 absorption and apical efflux of ropivacaine, lidocaine and bupivacaine in the rat using in situ
386 and in vitro absorption models, Eur J Pharm Sci, 21 (2004) 553–560.

387 [19] S.S. Mazzaferro, K.K. Bouchemal, R.R. Skanji, C.C. Gueutin, H.H. Chacun, G.G.
388 Ponchel, Intestinal permeation enhancement of docetaxel encapsulated into methyl-β-
389 cyclodextrin/poly(isobutylcyanoacrylate) nanoparticles coated with thiolated chitosan, J
390 Control Release, 162 (2012) 568–574.

391 [20] I. Bravo-Osuna, C. Vauthier, H. Chacun, G. Ponchel, Specific permeability modulation of
392 intestinal paracellular pathway by chitosan-poly(isobutylcyanoacrylate) core-shell
393 nanoparticles, Eur J Pharm Biopharm, 69 (2008) 436–444.

394