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Expression of transporters potentially involved in the targeting of cytostatic bile acid derivatives to colon cancer and polyps

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Abbreviations:
ASBT, apical sodium-dependent bile salt transporter
MDR, multidrug resistance protein
MRP, multidrug resistance-associated protein
OATP, organic anion transporting polypeptide
OCT, organic cation transporter
OST, organic solute transporter
TC, taurocholate

ABSTRACT

Drug targeting might help to overcome resistance to chemotherapy. Here we investigated whether colon cancer and polyps do express functional carriers involved in the uptake of cytostatic bile acid derivatives, in this case Bamet-UD2 [*cis*-diammine-bisursodeoxycholate-platinum(II)], which has been reported to be taken up by colon cancer cells ''in vitro'', efficiently induce apoptosis and overcome resistance to cisplatin. Although at lower levels than in ileum, a detectable expression of ASBT, OATP8/1B3, OCT1 and OSTα in colon tissue was found, which was not impaired in colon cancer or polyps. The expression of OATP-A/1A2 and OSTβ was also found in colon, but this was markedly decreased in neoplastic colon tissue. In contrast, the expression of OATP-C/1B1 was low in colon but significantly enhanced in neoplastic colon tissue. MDR1 and MRP2 were poorly expressed in colon as compared with ileum, whereas MRP3 expression was higher in colon than in ileum. The abundance of mRNA for these ABC proteins was not changed in colon cancer or polyps. When RNA from different tissues was injected to *Xenopus laevis* oocytes their ability to take up taurocholate and Bamet-UD2 was enhanced (healthy ileum $>$ healthy colon $\approx$ neoplastic colon tissue). In all cases, uptake was lower for taurocholate than for Bamet-UD2, probably due to that ASBT mediates sodium-dependent uptake of both substrates, whereas additional transporters expressed in these tissues can participate in Bamet-UD2 uptake. In conclusion, our results suggest that the use of cytostatic bile acid derivatives might be a good pharmacological strategy for the treatment of colon tumors.

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1. Introduction

A large proportion of all cancers is constituted by those affecting tissues included in the enterohepatic circuit, i.e., the liver, the gallbladder, the biliary tree and the intestine [1–3]. Moreover, these tumors usually have a poor prognosis, aggravated by the lack of response to the available chemotherapy. Among the attempts made to overcome resistance by gastrointestinal tumors to chemotherapy are investigations aimed to evaluate the possibility of using cytostatic bile acid derivatives to enhance intracellular drug concentrations in liver tumors [4]. In this line of research, several groups have synthesized different compounds and have explored whether they have the required characteristics, i.e., to inhibit tumor cell growth, to be less toxic than the parent drug, and to be efficiently taken up by transporters involved in the uptake of cholephilic organic anions by the liver [4]. One of the most interesting compounds fulfilling these requirements is *cis*-diammine-bisursodeoxycholate-platinum(II) (Bamet-UD2), which was obtained by chemical coupling of cisplatin to ursodeoxycholic acid [5], and which has been recently shown to be taken up by colon cancer cells ''in vitro'', efficiently induce apoptosis and overcome resistance to cisplatin [6]. Thus, this compound has been used in the present study as a model drug to evaluate the possible interest of using bile acid derivatives in drug targeting to colon tumors.

The major transporter involved in active bile acid uptake by the intestinal mucosa is the apical sodium-dependent bile salt transporter or ASBT (gene symbol *SLC10A2*) [7]. The ability of this carrier to transport bile acid derivatives obtained by coupling the active agent to the hydroxyl group at C3 of the bile acid moiety has been suggested previously [8]. Here, we investigated whether bile acid derivatives, in which the side chain was used to link the cytostatic moiety, such as happens in Bamet-UD2, were also transported by this carrier.

Several members of a large family of Na^+-independent carriers, the organic anion transporting polypeptides (OATPs), are able to transport bile acids. Since the nomenclature of these proteins has recently been changed [9], during a transitory period we have decided to use both the previous and new nomenclatures. In the present study, we investigated the presence in healthy and neoplastic intestinal tissue of three isoforms, namely OATP-A/1A2, OATP-C/1B1 and OATP8/1B3. These transporters, expressed in different tissues, are able to carry out the uptake of a broad spectrum of structurally unrelated compounds, such as bile acids and anionic conjugated and neutral steroids, such as ouabain [10]. However, some of them, such as the human OATP-A/1A2, also seem to be able to transport type II organic cations [11]. Such transport is also accomplished mainly by carrier systems known as organic cation transporters (OCTs), whose isoform 1 was included in the present study. These carriers have been found to be involved in the hepatic clearance of many organic cations, including several chemotherapeutic drugs [12].

An important aspect regarding the handling of cytostatic drugs by tumor cells is the existence of mechanisms of extrusion. Whether OATPs and OCTs might also mediate drug efflux from intestinal cells is not known. However, when expressed, the heterodimeric protein OSTα/OSTβ has been suggested to likely play a role as an efflux system in cells handling bile acids [13]. In addition, the extrusion of compounds may partly be due to ATP-binding cassette (ABC) proteins belonging, for instance, to the families of multidrug resistance proteins (MDR), such as MDR1, and multidrug resistance-associated proteins, such as MRP2 and MRP3. These proteins are already expressed in normal gastrointestinal epithelial cells [14] and are often overexpressed in several tumors derived from these cells [15]. When this occurs, these ABC proteins may play an important role contributing to the failure of chemotherapy by efficiently pumping the cytostatic agents, including cisplatin, out of these cells [16]. Among the aims of the present study, we also investigated the expression of these efflux transporters that may mediate the extrusion of bile acid derivatives from healthy and tumor intestinal cells.

2. Materials and methods

2.1. Chemicals and samples

Cisplatin, sodium taurocholate (TC) and ursodeoxycholic acid were obtained from Sigma–Aldrich (Madrid, Spain). [^{3}H]-TC

Table 1 – Gene-specific primers used for quantitative real-time RT-PCR analysis

Name	Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)	Product size (bp)	Accession number
ASBT	*SLC10A2*	TGAGCGTCAGCATGACCACA	CCCAGAGTCGACCCACATTTT	95	NM_000452
OSTα	*MGC39807*	TGTCCACACTGCTGGCTCTCT	GGCTCCCATGTTCTGCTCAC	83	NM_152672
OSTβ	*MGC118959*	TGGCAGCTGTGGTGGTCATT	TGGTGGCTGCATCGTTTCTT	83	NM_178859
OCT1	*SLC22A1*	AAAGCCCAAAGAAAACACGATTTA	CGCCGCAAAACATCTCTCTC	79	NM_003057
OATP-A/1A2	*SLCO1A2*	AAGACCAACGCAGGATCCAT	GAGTTTCACCCATTCCACGTACA	101	NM_134431
OATP-C/1B1	*SLCO1B1*	GAATGCCCAAGAGATGATGCTT	AACCCAGTGCAAGTGATTTCAAT	154	NM_006446
OATP8/1B3	*SLCO1B3*	GTCCAGTCATTGGCTTTGCA	CAACCCAACGAGAGTCCTTAGG	111	NM_019844
MDR1	*ABCB1*	GCCTACTTGGTGGCACATAAAC	GCACCAAAGACAACAGCTGAAA	74	NM_000927
MRP2	*ABCC2*	GGCAGTGAAGAAGAAGACGATGA	ATTGGACCTAGAACTGCGGCT	132	NM_000392
MRP3	*ABCC3*	TCTGTCCTGGCTGGAGTCG	TCAGCTTGATGCGCGAGTC	121	AF085690

(1.85 TBq/mmol) was purchased from American Radiolabeled Chemicals (Itisa Biomedica, Madrid). Bamet-UD2 [*cis*-diammine-bisursodeoxycholate-platinum(II)] was synthesized by binding two ursodeoxycholic acid molecules to cisplatin [17]. All other reagents were of analytical grade and were readily available from commercial sources.

Human tissue samples were the remains of biopsies collected for diagnostic purposes at the Department of Gastroenterology, University Hospital of Salamanca, in accordance with the protocols and consent forms approved by the Human Subjects Committee of this Hospital.

2.2. *Quantitation of gene expression by real-time RT-PCR*

Freshly obtained human tissue was immersed in the RNA-stabilization reagent RNAlater (Ambion, bioNova Cientifica, Madrid) and stored at −80 °C until use. Total RNA was isolated from these samples using RNeasy spin columns from Qiagen (Izasa, Barcelona, Spain). After treatment with RNase-free DNase I (Roche Diagnostics, Barcelona), RNA

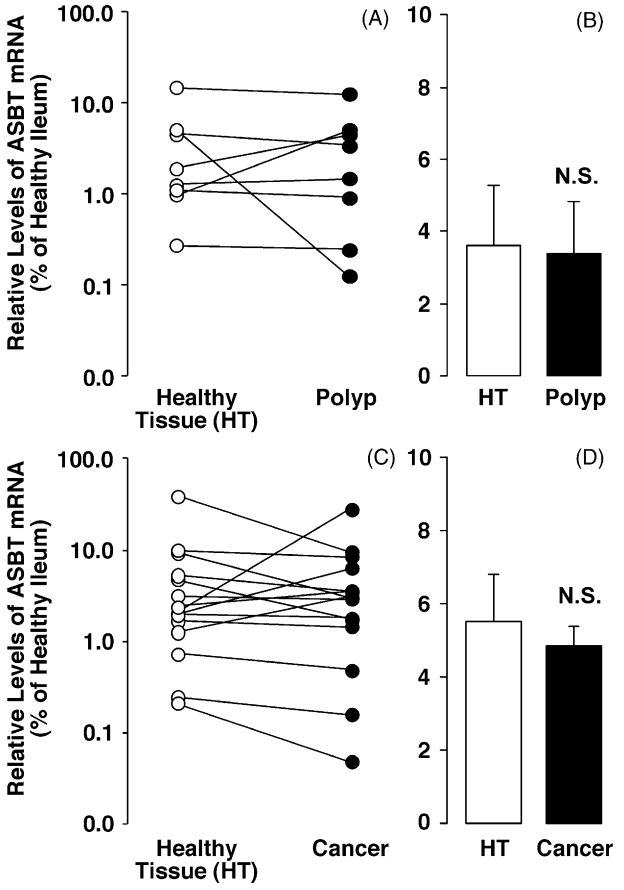

Fig. 1 – Relative abundance of ASBT mRNA in paired samples of colon polyps (A and B; $n = 8$) and colon cancer (C and D; $n = 15$) and the surrounding healthy tissue (HT) as compared to that of healthy ileum. Values are shown as individual pairs (A and C) and as means ± S.E.M. (B and D). N.S., $p > 0.05$, on comparing healthy and neoplastic tissue using the paired *t*-test.

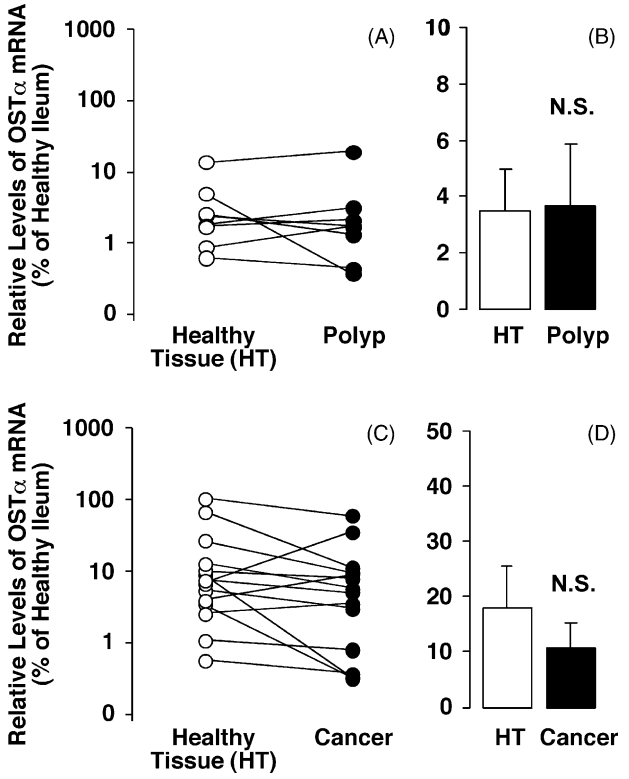

Fig. 2 – Relative abundance of OSTα mRNA in paired samples of colon polyps (A and B; $n = 8$) and colon cancer (C and D; $n = 15$) and the surrounding healthy tissue (HT) as compared to that of healthy ileum. Values are shown as individual pairs (A and C) and as means ± S.E.M. (B and D). N.S., $p > 0.05$, on comparing healthy and neoplastic tissue using the paired *t*-test.

was quantified fluorimetrically with the RiboGreen RNA-Quantitation kit (Molecular Probes, Leiden, The Netherlands). DNA was synthesized from 2 μg total RNA using random nonamers and avian myeloblastosis virus reverse transcriptase (Enhanced Avian RT-PCR kit; Sigma-Genosys, Cambridge, UK).

Primer oligonucleotides obtained from Sigma-Genosys (Table 1) were designed with the assistance of Primer Express software (Perkin-Elmer Applied Biosystems, Madrid) for DNA fragments in described sequences, and their specificity was checked using BLAST. Real-time quantitative PCR was then performed using AmpliTaq Gold polymerase (Perkin-Elmer Applied Biosystems) in an ABI Prism 5700 Sequence Detection System (Perkin-Elmer Applied Biosystems). The thermal cycling conditions were as follows: a single cycle at 95 °C for 10 min followed by 45 cycles at 95 °C for 15 s and at 60 °C for 60 s.

Detection of amplification products was carried out using SYBR Green I (Perkin-Elmer Applied Biosystems). The absence of non-specific products of PCR was investigated with 2.5% agarose gel electrophoresis and melting temperature curves. No artifacts were found, which permitted the use of SYBR Green I detection in all cases, except for MRP3, for which a

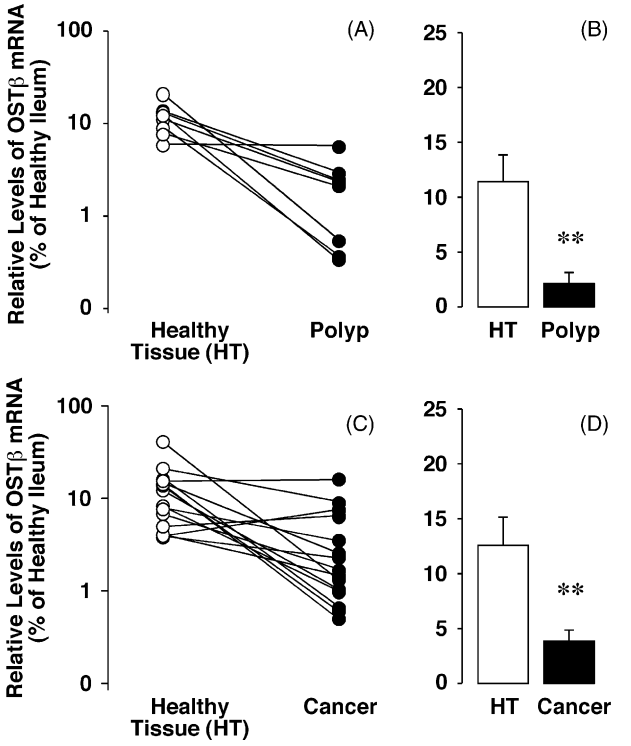

Fig. 3 – Relative abundance of OSTβ mRNA in paired samples of colon polyps (A and B; $n = 8$) and colon cancer (C and D; $n = 15$) and the surrounding healthy tissue (HT) as compared to that of healthy ileum. Values are shown as individual pairs (A and C) and as means ± S.E.M. (B and D). $^{**}p < 0.01$, on comparing healthy and neoplastic tissue using the paired t-test.

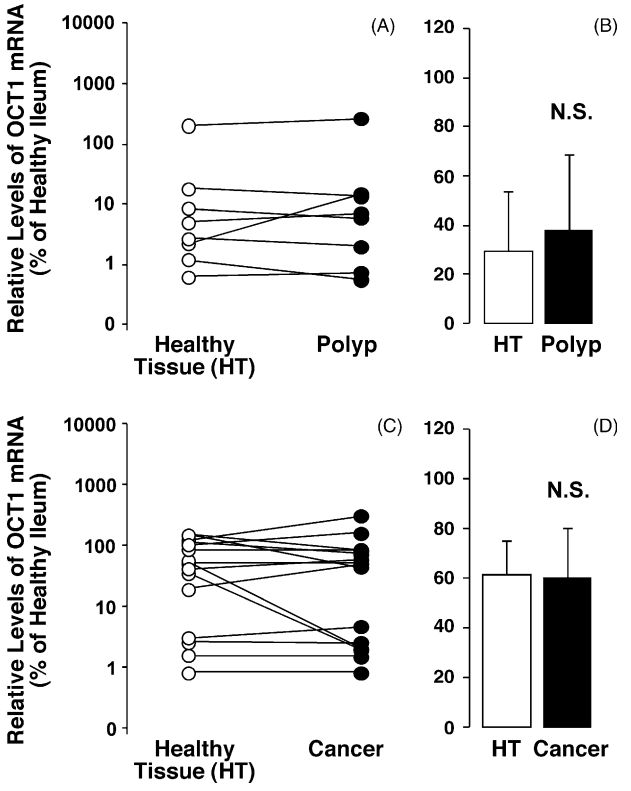

Fig. 4 – Relative abundance of OCT1 mRNA in paired samples of colon polyps (A and B; $n = 8$) and colon cancer (C and D; $n = 15$) and the surrounding healthy tissue (HT) as compared to that of healthy ileum. Values are shown as individual pairs (A and C) and as means ± S.E.M. (B and D). N.S., $p > 0.05$, on comparing healthy and neoplastic tissue using the paired t-test.

TaqMan® probe (5′-TGGCCGTGAAGATGCGC-3′) was used. The results of mRNA abundance for each target gene in each sample were normalized on the basis of its 18S rRNA content, which was measured with the TaqMan® Ribosomal RNA Control Reagents kit (Perkin-Elmer Applied Biosystems).

2.3. *Uptake studies in Xenopus laevis oocytes*

Mature female frogs (*X. laevis*), purchased from Regine Olig (Hamburg, Germany), were used. The amphibians received humane care as outlined in "Guide for the Care and Use of Laboratory Animals" (NIH Publication 80–23, revised 1985). Experimental protocols were approved by the Ethical Committee for Laboratory Animals of the University of Salamanca. Oocyte harvesting and preparation were carried out as described elsewhere [18].

X. laevis oocytes were microinjected with TE buffer (1 mM EDTA, 10 mM Tris, pH 8.0) alone (control); with 20 ng RNA obtained from human tissue samples as described above, or with 9 ng of the cRNA of the rat orthologue of ASBT synthesized using the T7 mMessage mMachine kit (Ambion) and a recombinant plasmid obtained by subclonning between the *Eco*RI and *Hin*dIII sites of the pSPORT 1 plasmid the ORF of this transporter, kindly supplied by Dr. Paul Dawson (Wake Forest University School of Medicine, Winston-Salem, North Carolina) cloned in the pCMV5/rIbat plasmid. Oocytes were used 2 days after RNA injection, when – on the basis of preliminary experiments on the time-course of functional expression for this carrier – the uptake rate was highest (data not shown).

Uptake studies were carried out using groups of 10–12 oocytes per data-point. Experiments were repeated using three different frogs. Oocytes were washed with substrate-free uptake medium and incubated with 100 μL of uptake medium (100 mM sodium chloride or 100 mM choline chloride, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 10 mM Hepes/Tris, pH 7.0) containing the desired amount of the substrate and inhibitor to be tested at 25 °C for 60 min. Uptake was stopped by the addition of 4 mL ice-cold uptake medium. The oocytes were washed a further three times before being collected and placed in vials for dissolution in 200 μL of 10% (w/v) SDS in groups of two to increase the signal of radioactivity (when using radiolabeled TC as substrate) or the platinum signal (when using Bamet-UD2 as substrate). Platinum contents were determined by flameless atomic absorption spectrophotometry [18].

2.4. Statistical methods

Results are expressed as individual values or as means ± S.E.M. To calculate the statistical significance of the differences between groups, the paired *t*-test or the Bonferroni method for multiple range testing were used, as appropriate.

3. Results

To obtain indirect evidence of the ability of neoplastic colon tissue to take up cytostatic drugs synthesized by using bile acids as targeting elements, the expression of ASBT, the major carrier involved in active sodium-dependent intestinal absorption of bile acids, was investigated (Fig. 1). The mRNA of this transporter was detected in healthy colon at levels of less than 10% of those found in normal ileum. No significant decrease in ASBT expression in colon cancer and polyps was detected (Fig. 1).

Similarly, the mRNA of both components of the heterodimeric transporter OSTα/OSTβ, which is involved in bile acid efflux across the basal plasma membrane of ileal mucosa cells, was also found in healthy colon tissue at levels approximately one-tenth of those seen in normal ileum (Figs. 2 and 3).

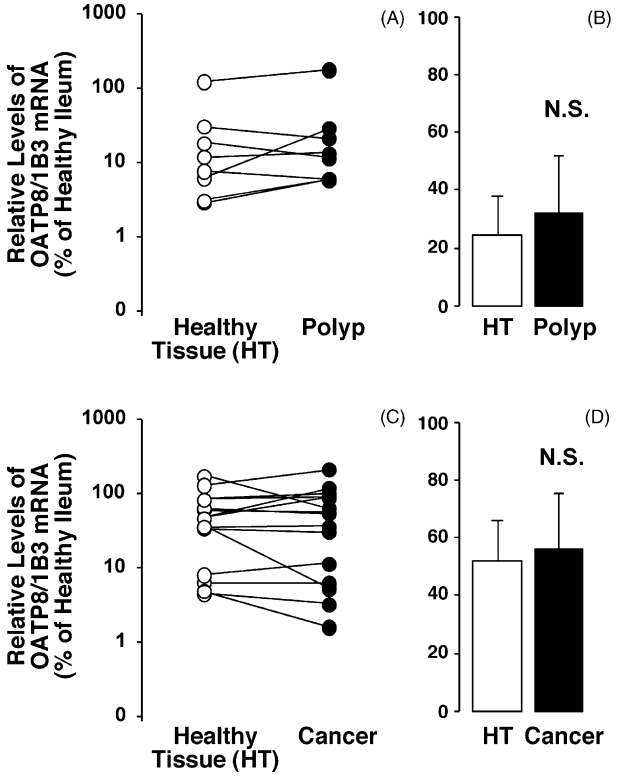

Fig. 5 – Relative abundance of OATP8/1B3 mRNA in paired samples of colon polyps (A and B; *n* = 8) and colon cancer (C and D; *n* = 15) and the surrounding healthy tissue (HT) as compared to that of healthy ileum. Values are shown as individual pairs (A and C) and as means ± S.E.M. (B and D). N.S., $p > 0.05$, on comparing healthy and neoplastic tissue using the paired *t*-test.

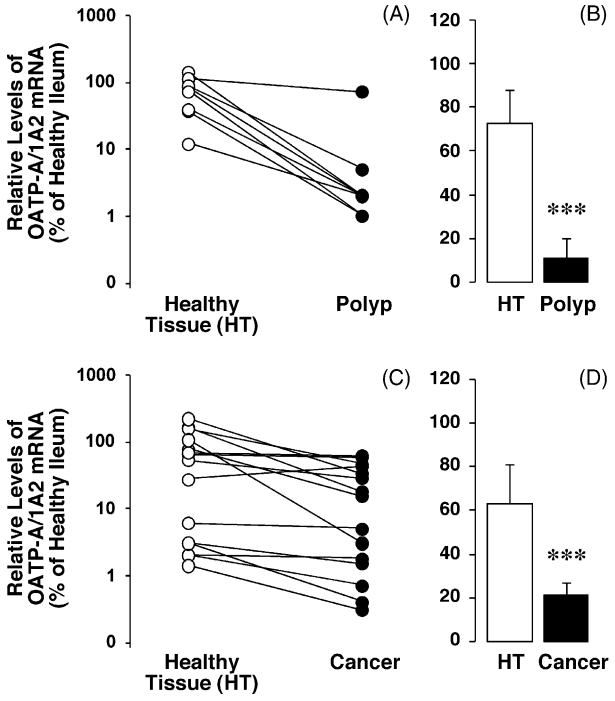

Fig. 6 – Relative abundance of OATP-A/1A2 mRNA in paired samples of colon polyps (A and B; *n* = 8) and colon cancer (C and D; *n* = 15) and the surrounding healthy tissue (HT) as compared to that of healthy ileum. Values are shown as individual pairs (A and C) and as means ± S.E.M. (B and D). *** $p < 0.001$, on comparing healthy and neoplastic tissue using the paired *t*-test.

Moreover, whereas the expression of OSTα was not markedly impaired in colon cancer and polyps (Fig. 2), that of OSTβ was significantly decreased (Fig. 3).

Next, the expression of other carriers previously reported to be able to transport bile acid derivatives [18], such as Bamet-UD2, namely OCT1 and several members of the OATP family, was investigated. As compared with healthy ileum, the expression of OCT1 (Fig. 4) and OATP8/1B3 (Fig. 5) in healthy colon tissue was approximately 50% and was not significantly modified in colon cancer and polyps. In contrast, the expression of OATP-A/1A2, which was closer to that found in normal ileum, was markedly impaired in colon cancer and polyps (Fig. 6). Finally, OATP-C/1B1 mRNA was less abundant in healthy colon tissue but this increased in colon cancer and polyps above values found in healthy ileum (Fig. 7).

Regarding the export ABC proteins investigated here, the expression of MDR1 (Fig. 8) and MRP2 (Fig. 9) in healthy colon tissue was very low as compared to that observed in healthy ileum. In contrast, the abundance of MRP3 mRNA was higher in colon than in ileum (Fig. 10). Nevertheless, the expression of these proteins was not significantly changed in colon cancer and polyps.

To investigate whether the expression of the mRNAs detected in healthy and neoplastic tissue does in fact confer the ability to transport bile acids and their derivatives, the RNA obtained from normal ileum or paired samples of

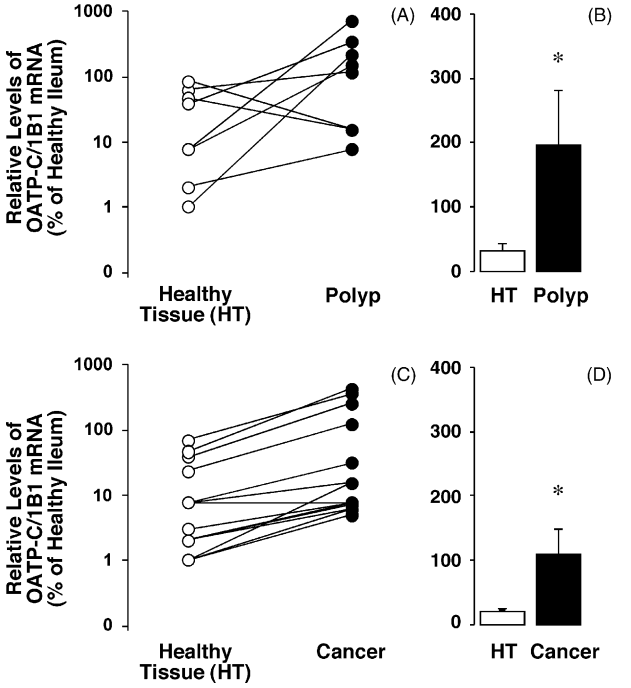

Fig. 7 – Relative abundance of OATP-C/1B1 mRNA in paired samples of colon polyps (A and B; $n = 8$) and colon cancer (C and D; $n = 15$) and the surrounding healthy tissue (HT) as compared to that of healthy ileum. Values are shown as individual pairs (A and C) and as means ± S.E.M. (B and D). $^{*}p < 0.05$, on comparing healthy and neoplastic tissue using the paired t-test.

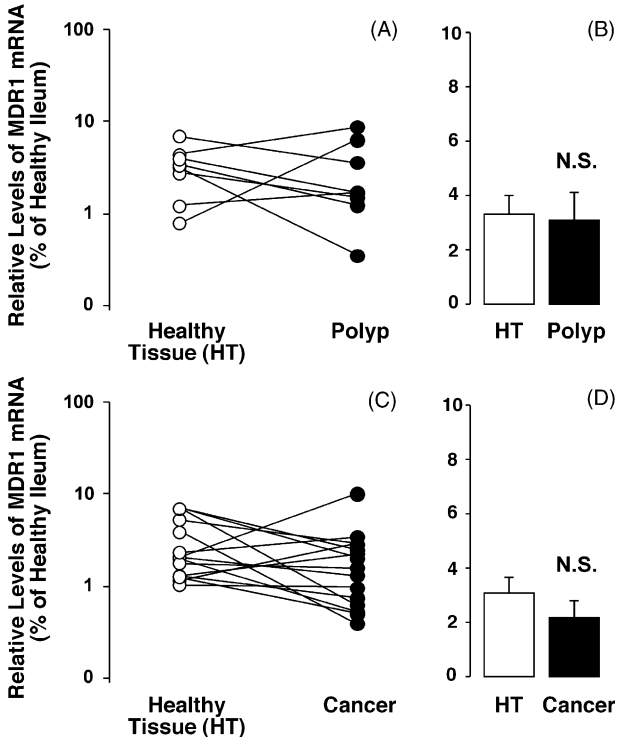

Fig. 8 – Relative abundance of MDR1 mRNA in paired samples of colon polyps (A and B; $n = 8$) and colon cancer (C and D; $n = 15$) and the surrounding healthy tissue (HT) as compared to that of healthy ileum. Values are shown as individual pairs (A and C) and as means ± S.E.M. (B and D). N.S., $p > 0.05$, on comparing healthy and neoplastic tissue using the paired t-test.

healthy colon and colon cancer and polyps was injected into *X. laevis* oocytes; after 2 days these were used to evaluate their ability to take up either radiolabeled taurocholate (Fig. 11) or Bamet-UD2 (Fig. 12). In oocytes injected with RNA from colon cancer and polyps, no impaired ability to take up these compounds was found as compared to that found in oocytes injected with RNA from healthy colon. For both radiolabeled taurocholate (Fig. 11) and Bamet-UD2 (Fig. 12), this ability was more than two-fold higher in oocytes injected with RNA from healthy ileum. Moreover, in all cases the ability to take up radiolabeled taurocholate was markedly lower than that observed for Bamet-UD2, probably due to the fact that ASBT is able to transport both substrates whereas Bamet-UD2 can be also efficiently transported by several different carriers [18].

To elucidate whether ASBT was indeed involved in the uptake of Bamet-UD2, the rat isoform of this transporter was expressed in oocytes. This conferred these cells the ability to efficiently take up radiolabeled taurocholate in a sodium-dependent manner (Fig. 13A). Moreover, this uptake was significantly inhibited by unlabeled taurocholate. The oocytes expressing rat ASBT also exhibited the ability to carry out sodium-dependent and taurocholate-inhibitable Bamet-UD2 uptake (Fig. 13B), although less efficiently than for taurocholate.

4. Discussion

The expression in certain tissues of transporters able to carry out an efficient and relatively specific uptake of structurally related compounds has been the basis of a promising strategy for tissue-selective drug delivery. This consists of coupling pharmacological active agents, such as cytostatic drugs, to shuttle molecules that can be recognized as substrates by these carrier proteins [19]. In this respect, the restricted expression of bile acid carriers able to mediate the uptake of bile acid derivatives to only certain cell types offers an excellent example of where this strategy could be employed [4].

Since first proposed for delivering different kinds of drugs to the liver [20], many bile acid-drug conjugates have been synthesized [21–23]. In previous studies we have reported the interest of several members of a family of anti-tumor compounds obtained by binding cisplatin to the side chain of bile acids [17,24,25]. Some of these compounds, such as Bamet-UD2, have been suggested to be potentially useful in the chemotherapy of liver tumors, owing to their beneficial characteristics, which include: liver organotropism [23,26], strong cytostatic activity ''in vitro'', and anti-tumor effect against tumors implanted in the liver of nude mice [5,27,28]. Moreover, using laboratory animals and

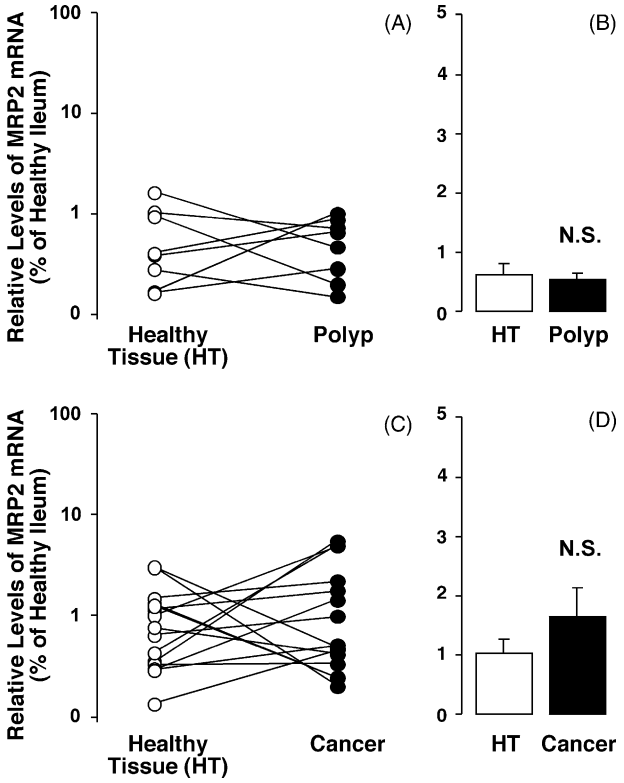

Fig. 9 – Relative abundance of MRP2 mRNA in paired samples of colon polyps (A and B; $n = 8$) and colon cancer (C and D; $n = 15$) and the surrounding healthy tissue (HT) as compared to that of healthy ileum. Values are shown as individual pairs (A and C) and as means ± S.E.M. (B and D). N.S., $p > 0.05$, on comparing healthy and neoplastic tissue using the paired *t*-test.

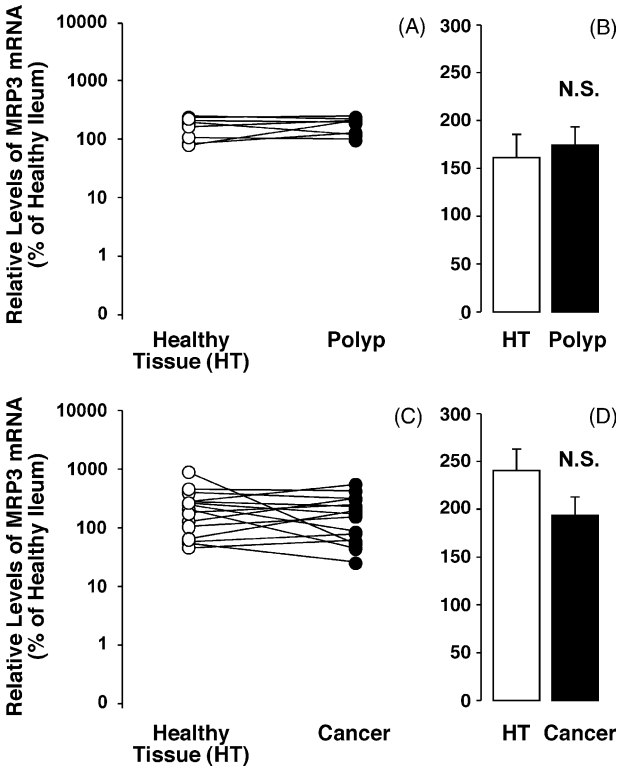

Fig. 10 – Relative abundance of MRP3 mRNA in paired samples of colon polyps (A and B; $n = 8$) and colon cancer (C and D; $n = 15$) and the surrounding healthy tissue (HT) as compared to that of healthy ileum. Values are shown as individual pairs (A and C) and as means ± S.E.M. (B and D). N.S., $p > 0.05$, on comparing healthy and neoplastic tissue using the paired *t*-test.

cultured cells, recent studies have indicated that Bamet-UD2 is efficiently taken up and overcome the resistance to cisplatin when this is in part due to an enhanced ability of intestinal tumor cells to reduce intracellular cisplatin contents [6].

Bamet-UD2 has a strong pro-apoptotic activity but a weak pro-necrotic effect on intestinal tumor cells, which together with its mild toxicity to normal tissues, including the intestinal mucosa, may account for the high anti-tumor activity of Bamet-UD2, together with its very few side effects [5,6]. In this context, an important question that remained unanswered, which has been addressed in the present study, was whether proteins able to transport Bamet-UD2 are indeed expressed in human colon tumors. Although owing to the large phenotypic variability among human tumors it is not possible to propose a general rule, the results obtained here suggest that Bamet-UD2 is probably efficiently taken up by most colon tumors: both colon cancer and polyps. The ability of ASBT to transport Bamet-UD2 may contribute, together with other transporters such as OATPs and OCTs, to mediating the uptake of this drug by tumor cells. The ability of OCTs to take up Bamet-UD2 has been previously demonstrated [18] and is not surprising, since in aqueous solution this compound is partly present in cationic form.

Additionally, among the large list of known substrates for members of the OATP family, most of which are organic anions, both organic cations [11,29] and neutral compounds [10] are also included. This means that these transporters might represent a route for the uptake of different ionization states of the same compound, such as Bamet-UD2, which in aqueous solution is in equilibrium between neutral and cationic forms. This is consistent with previous studies reporting that the uptake of Bamet-UD2 by OATP-A/1A2 is enhanced in the absence of chloride, which leads to a displacement of the equilibrium towards the formation of cationic DNA-reactive ''aquo'' groups. However, this maneuver decreases Bamet-UD2 uptake by OATP-C/1B1, suggesting that this carrier does not prefer the cationic form of this drug as a substrate [18].

An important aspect regarding drug targeting that should be taking into consideration when evaluating the usefulness of this strategy is the fact that the intracellular, pharmacologically active concentration of any drug is the balance between uptake and neutralizing pathways, either by biotransformation or extrusion from the targeted cells. In this regard, it is noteworthy that the major pathway for bile acid efflux from intestinal mucosa cells is the heterodimeric transporter OSTα/OSTβ [13], and that one of its components,

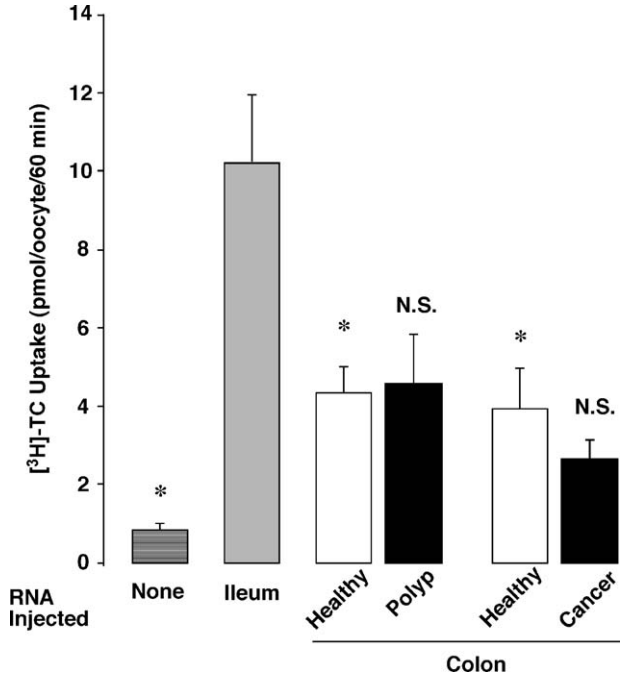

Fig. 11 – Uptake of radiolabeled taurocholate ([^{3}H]-TC) by *Xenopus laevis* oocytes injected with TE buffer alone or containing RNA obtained from healthy ileum (n = 5) and paired samples of colon polyps (n = 5) and colon cancer (n = 7) and the surrounding healthy colon tissue 2 days before being incubated with 100 μM substrate in presence of sodium for 60 min. Values are means ± S.E.M. from between 10 and 20 oocytes from three different frogs per data-point. $^*p < 0.05$ as compared TE-injected control oocytes and healthy colon to ileum by Bonferroni method of multiple range testing. N.S., $p > 0.05$, on comparing healthy and neoplastic tissue using the paired t-test.

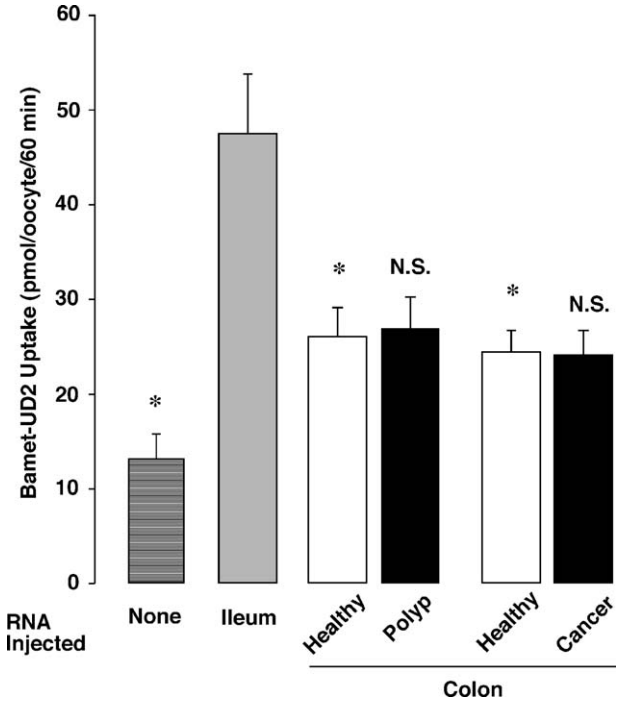

Fig. 12 – Uptake of Bamet-UD2 by *Xenopus laevis* oocytes injected with TE buffer alone or containing RNA obtained from healthy ileum (n = 5) and paired samples of colon polyps (n = 5) and colon cancer (n = 7) and the surrounding healthy colon tissue 2 days before being incubated with 100 μM substrate in presence of sodium for 60 min. Values are means ± S.E.M. from between 10 and 20 oocytes from three different frogs per data-point. $^*p < 0.05$ as compared TE-injected control oocytes and healthy colon to ileum by Bonferroni method of multiple range testing. N.S., $p > 0.05$, on comparing healthy and neoplastic tissue using the paired t-test.

namely OSTβ, was markedly down-regulated in neoplastic colon tissue, probably leading to a reduction in the overall ability of tumor cells to extrude bile acid derivatives through this pathway. Thus, the normal levels of ASBT expression combined with reduced OSTβ in neoplastic tissue might determine a preferential accumulation of conjugated drug in tumors. As tumor cells are not polarized this might be true for both orally and systemically administered drug.

Regarding ABC transporters, MRP3, which is expressed in healthy colon tissue as well as in colon cancer and polyps, is known to transport bile acids [30,31] but whether it is involved in the extrusion of bile acid derivatives is not known. Even in this latter case, it is interesting to note that this transporter was not up-regulated in neoplastic colon tissue. Although MRP2, and perhaps to a lower extent also MDR1, which primarily accepts amphipathic cations and neutral compounds as substrates [32], seem to be involved in the resistance to cisplatin [16,33], here they were poorly expressed in colon cancer and polyps. Our results are consistent with those reported in a previous study concerning the expression of these ABC proteins in colorectal cancer and non-cancerous tissue, in which it was reported that, although a large variability among tumors was found, there was a tendency for the expression of MRP2 to increase and that of MDR1 and MRP3 to decrease [34]. Nevertheless, even though these proteins may be over-expressed in certain individuals, Bamet-UD2 has been shown to overcome resistance due to these export pumps [6].

In sum, our findings indicate that ASBT is able to transport Bamet-UD2, and that this and other transporters, such as OATPs and OCTs, which are able to mediate Bamet-UD2 uptake, are expressed in colon cancer and polyps, whereas the efflux through OSTα/OSTβ might be impaired. This suggest that the use of bile acids as shuttles to deliver cytostatic drugs might be a useful pharmacological strategy for drug targeting to colon tumors.

Conflict of interest statement

In the period of research leading up to this publication we have not received any financial support that may affect in any way

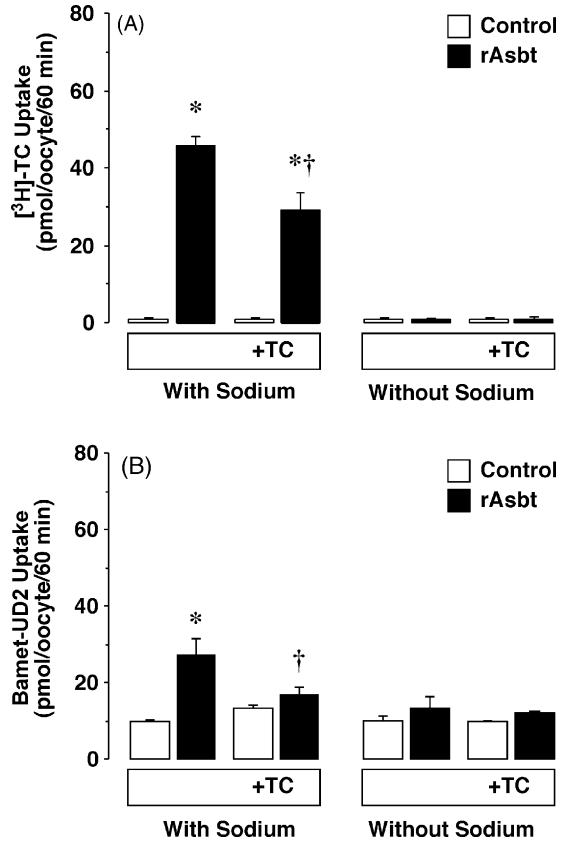

Fig. 13 – Uptake of radiolabeled taurocholate ([^{3}H]-TC) (A) or Bamet-UD2 (B) by *Xenopus laevis* oocytes injected with the cRNA of rat ASBT (rAsbt) or TE buffer (control) 2 days before being incubated with 10 μM [^{3}H]-TC or 100 μM Bamet-UD2 in the absence or the presence of sodium and in the absence or the presence of 50 or 500 μM unlabeled taurocholate (+TC), respectively, for 60 min. Uptake values are means ± S.E.M. from measurements carried out in 30 oocytes from three different frogs per data-point.

the conclusions of our article. Moreover, the authors have no direct or indirect commercial interest in any company that might be financially affected by the conclusions of the present article.

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