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Efficacy of ST1968 (namitecan) on a topotecan-resistant squamous cell carcinoma

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ABSTRACT

ST1968 (namitecan), a novel 7-modified hydrophilic camptothecin, was found to be effective against tumor models relatively resistant to topotecan and irinotecan. Based on this observation, this study was designed to investigate the cellular and antitumor effects of ST1968 in a subline of A431, squamous cell carcinoma, selected for resistance to topotecan (A431/TPT). This model was characterized by a slow growth rate, associated with downregulation of EGFR and topoisomerase I. In contrast to other camptothecins (SN38 and gimatecan), ST1968 was able to overcome almost completely the resistance at cellular level. The cellular pharmacokinetics indicated a comparable accumulation and retention of ST1968 in sensitive and resistant cells, in spite of expression of the efflux transporter, P-glycoprotein, in resistant cells. The uptake and retention of topotecan were dramatically reduced in both tumor cell lines, but more evident in the resistant one. In contrast to topotecan, ST1968 retained an outstanding efficacy in vivo against the resistant tumor (A431/TPT). The results are consistent with the interpretation that ST1968 was able to overcome the most relevant mechanisms associated with the development of topotecan resistance (i.e., slow proliferation and target downregulation) owing to its peculiar pharmacokinetic behaviour.

1. Introduction

Camptothecins are among the most effective antitumor agents that stabilize the covalent binding of topoisomerase I to DNA during the enzyme catalytic process forming a reversible ternary complex [1–3]. During DNA synthesis the collision of the replication forks with the cleavable complex (topo I-DNA) converts the single-strand breaks into irreversible lethal double-strand breaks [1,4]. On the basis of this mechanism and of the reversibility of the cleavable complex, prolonged intracellular accumulation and stability of the ternary complex are required to exploit the S-phase-specific activity and therapeutic efficacy of camptothecins [4]. We have reported that the presence of highly lipophilic substituents at position 7 provides favourable features, in terms of cellular pharmacokinetics, lactone stability and ternary complex stabilization [5–7]. However, the ability to stabilize the cleavable complex is a favourable feature of several derivatives of the 7-oxyiminomethyl series [8]. In particular, the hydrophilic derivative namitecan (Fig. 1) exhibit an excellent activity, superior to that of irinotecan and topotecan (TPT), against several tumor models, including slowly growing tumors [9,10]. To better understand the basis of the peculiar profile of ST1968, we have performed a comparative study of ST1968 and topotecan in a squamous cell carcinoma model selected for resistance to topotecan (A431/TPT). The results support the ability of ST1968 to partially overcome the acquired resistance of A431/TPT cells, likely reflecting an improved uptake and persistent intracellular accumulation which allow efficient target inhibition and antitumor efficacy in spite of reduced proliferation rate and reduced topoisomerase I expression.

2. Materials and methods

2.1. Cell lines and culture conditions

The human squamous cell carcinoma A431 and its subline selected for resistance to topotecan (A431/TPT), were maintained in RPMI 1640 (Lonza Group Ltd., Verviers, Belgium) supplemented with 10% FBS (Gibco® Invitrogen, Segrate, Italy). The A431/TPT cell line was selected by growing A431 cells for 4 months in culture medium containing 0.05 μM topotecan (Hycamtin, GlaxoSmithK-line). A431/TPT cells were subcultured in the presence of the drug every two split.

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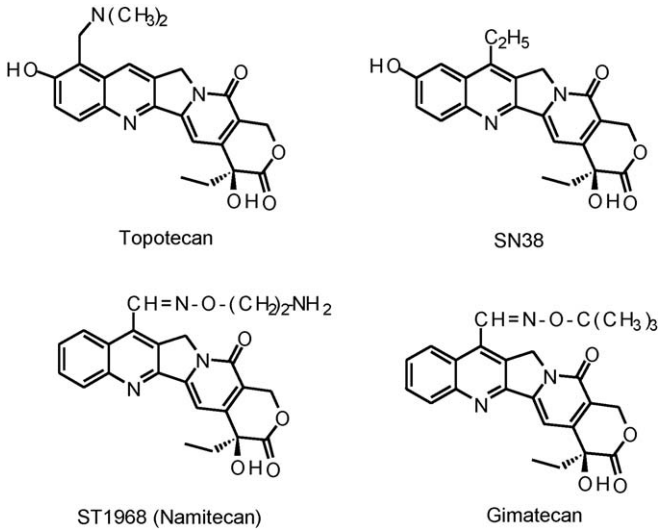

Fig. 1. Chemical structure of ST1968.

2.2. Drugs and antibodies

ST1968 was synthesized as previously described [5]. For in vitro studies, ST1968, gimatecan and SN38 were dissolved in dimethylsulfoxide. Topotecan was dissolved in sterile distilled water. For in vivo studies, ST1968 was dissolved in sodium lactate buffer (50 mM) adjusted to pH 4.0 with the addition of hydrochloric acid. Topotecan was dissolved in sterile distilled water. The drugs were administered by a slow i.v. injection in a volume of 10 ml/kg body weight.

The following antibodies were used: anti-EGFR (Upstate Biotechnologies, Lake Placid, NY); anti-p-glicoprotein (MDR1), β-Tubulin and -actin (Sigma, St. Louis, MO), anti-BCRP (Alexis Biochemicals, Lausen, Switzerland), anti-MRP-1 and anti-V-ATPase-D (Santa Cruz Biotechnology, Santa Cruz, CA); anti-DNA topoisomerase I (BD Bioscience, San Josè, CA).

2.3. Cell sensitivity studies

Cell sensitivity to the drugs was determined by growth inhibition assay. Cells were seeded in duplicate into 12-well plates and exposed to the drugs for 1 h. Adherent cells were trypsinized 72 h after treatment and counted by a cell counter (Beckam Coulter Inc., Fullerton, CA). IC_{50} values, determined from dose–response curves, were defined as the drug concentrations producing 50% inhibition of cell growth. The reported values represent the mean ± standard deviation (SD) derived from at least three independent experiments.

2.4. Determination of apoptosis

Apoptosis was determined by TUNEL assay 72 h after treatment. Cells were fixed in 4% paraformaldehyde (45 min), washed and resuspended in ice-cold PBS. The "in situ cell death detection" kit (Roche, Germany) was used according to the manufacturer's instructions, and samples were analyzed by flow cytometry (FACScan, Becton Dickinson, Franklin Lakes, NJ).

2.5. Cellular pharmacokinetics

Cells were seeded in six-well plates at a subclonfluent density (around 100,000 cells/cm^2). After 24 h, samples, in triplicate, were washed with serum-free medium and exposed for 30 min, 1 or 2 h to the drug in serum-free medium. Treated cells were washed three times with PBS to remove the extracellular drug and, for retention studies, maintained in a drug-free medium for further 5 h. At different times, washed cells were resuspended in 150 μl of PBS and subjected to 5 cycles of freezing in liquid nitrogen and thawing in thermostated bath at 37 °C. Each cycle lasted 5 min. Cell lysates were frozen at −20 °C. Samples were processed by adding cold 0.1% acetic acid/methanol (1:5, v:v). Analysis was performed using the 32Karat Software (Beckam Coulter) with a fluorescence detector (Jasco or Shimadzu) fixed at 370 nm as excitation wavelength and 510 nm as emission wavelength. ST1968 and topotecan were eluted on a Discovery HS F5 column (5 mm, 100 mm × 4.6 mm, Supelco) kept at room temperature in isocratic condition (0.1 M acetic acid, 0.1%TEA:CAN, 75:25, v:v) with a flow rate of 1 ml/min.

2.6. Intracellular drug distribution

Subconfluent cells, grown on coverslips, were treated with ST1968 or topotecan 10 μg/ml for up to 2 h. Fluorescence images were acquired at different times by means of an Argus VIM 100 processor digital system (Hamamatsu Photonics Deutschland GmbH, Herrsching am Ammersee, Germany), using an high sensitive ISIT camera (Hamamatsu C2400-09) coupled to a Leica (Wetzlar, Germany) fluorescence microscope. The images were digitally stored on a magnetic mass memory support and processed by means of the Hamamatsu Argus 100 control program. For drug localization study, images under excitation at 366 nm (366 nm interference filter; *T*% = 40) were acquired at wavelengths >440 nm by means of a 11000v3 filter combination set (Chroma Technology Corp, Rockingham, VT). The emission spectrum of topotecan was in the range of 450–600 nm with a maximum at 520 nm. The emission spectrum of ST1968 (430–550 nm) exhibited a maximum at 480 nm.

LysoTracker Red DND-99 (LTR) (Invitrogen) was used for lysosomal staining. Sequential images were recorded from the same field at wavelengths >440 nm (covering the emission region of ST1968, in the 440–540 nm range, and of LTR in the 580–630 nm range), and >570 nm (selective for LTR signal). Images were also recorded from cells incubated with LTR alone, to determine the contribution of the dye signal to the images recorded at wavelengths >440 nm. An image analysis was performed on each couple of images, and ratio values ($F.I._{>440}/F.I._{>570\ nm}$) were calculated pixel by pixel on the lysosomal areas. The comparison of the ratio values from the cells incubated with LTR alone and from those incubated with both ST1968 and LTR allowed the determination of the contribution of ST1968 signal to that of LTR in the lysosomes.

2.7. Western blot analysis

Cells were rinsed twice with PBS, added with 0.1 mM sodium orthovanadate and lysed in hot sample buffer [11]. After determination of protein concentration, whole-cell extracts were separated by SDS polyacrylamide gel electrophoresis (PAGE) and transferred onto nitrocellulose filters. The filters were incubated with primary antibodies overnight and with peroxidase-conjugated secondary antibodies at room temperature for 1 h. Immunoreactive bands were revealed by using the enhanced chemiluminescence detection system from Amersham Biosciences (Rockford, IL).

2.8. Assessment of lysosomal membrane permeability

The lysosomal acidification was determined by incubating cells with 5 μM acridine orange (Sigma–Aldrich, St. Louis, MO) for 17 min at 37 °C. FL-3 fluorescence was measured by flow cytometry (FACScan). The same samples were examined by a fluorescence microscope (Leica, Wetzlar, Germany).

2.9. Antitumor activity in vivo

All experiments were carried out using female athymic Swiss nude mice, 6–8 weeks-old (Charles River, Calco, Italy). Mice were maintained in laminar flow rooms keeping temperature and humidity constant. Mice had free access to food and water. Experiments were approved by the Ethics Committee for Animal Experimentation of the Fondazione IRCCS Istituto Nazionale dei Tumori of Milan according to institutional guidelines and to the UK Coordinating Committee on Cancer Research Guidelines [12].

Exponentially growing tumor cells (10^7 cells/mouse) were s.c. injected into mice flank. Tumor lines were achieved by serial s.c. passages of fragments (about 2 mm × 2 mm × 6 mm) from growing tumors into healthy mice, as previously described [13]. Groups of 5–8 mice bearing s.c. tumors implanted in one or both flanks were employed. Tumor fragments were implanted on day 0 and tumor growth was followed by biweekly measurements of tumor diameters with a Vernier caliper. Tumor volume (TV) was calculated according to the formula: TV (mm^3) = $d^2 \times D/2$ where d and D are the shortest and the longest diameter, respectively. Drugs were delivered i.v. every fourth day for four times (q4d×4) starting when tumors were just palpable.

The efficacy of the drug treatment was assessed as:

(i) Tumor volume inhibition percentage (TVI%) in treated versus control mice, calculated as: TVI% = 100 − (mean TV treated/mean TV control × 100).
(ii) Complete responses (CR), i.e., tumors not grown until the end of experiment.

The toxicity of the drug treatment was determined as body weight loss and lethal toxicity.

3. Results

3.1. Pattern of cellular response to selected camptothecins

The A431/TPT cell subline, selected for resistance to topotecan, exhibited an increased doubling time as compared with the parental line (28 h vs. 23 h in resistant and parental cells, respectively). The slow proliferation of resistant cells was reflected in a reduced in vivo growth rate, because the doubling time of resistant subline was substantially increased as compared with the parental line (5.8 days vs. 3.5 days). Table 1 shows the pattern of cross-resistance to topotecan, SN38 (the active metabolite of irinotecan), gimatecan and ST1968, a novel hydrophilic camptothecin. The comparison of antiproliferative effects of camptothecins following 1-h exposure indicated a significant degree of resistance of A431/TPT to the selecting agent (TPT), SN38 and gimatecan, but only a marginal resistance to ST1968.

Table 1
Pattern of cross-resistance of A431/TPT cells[a].

Drug	IC_{50} (μM)[b]		RI[c]
	A431	A431/TPT	
Topotecan	1.00 ± 0.13	3.13 ± 0.97	3.1
ST1968	0.21 ± 0.10	0.29 ± 0.11	1.4
SN38	0.11 ± 0.04	0.34 ± 0.13	3.1
Gimatecan	0.022 ± 0.012	0.083 ± 0.023	3.8

[a] Drug sensitivity was measured by growth inhibition assay after 1-h exposure.
[b] IC_{50}, drug concentration causing a 50% decrease in cell growth.
[c] RI, resistance index, ratio between the IC_{50} of topotecan-resistant and -sensitive cells.

To investigate the susceptibility to induction of apoptosis in response to topotecan and ST1968, we have determined the extent of apoptotic cell death at 72 h after 1-h exposure to a concentration required for about 80% growth inhibition (Fig. 2). In contrast to topotecan which exhibited a low proapoptotic effect in resistant cells (21 ± 6 and 56 ± 5, in resistant and parental cells, respectively), ST1968 induced a comparable level of apoptosis in A431 and A431/TPT (78 ± 5 and 87 ± 3%, respectively). The moderate apoptotic response of A431/TPT cells to topotecan at a concentration which produced a marked inhibition of cell proliferation (around IC_{80}) suggested a cytostatic rather than cytotoxic effect of the drug.

3.2. Biochemical features of the resistant subline

Since the squamous cell carcinoma cells A431 are known to be characterized by overexpression of EGF receptor (EGFR), we determined the expression level of the protein in topotecan-resistant cells. As shown in Fig. 3, the EGFR expression was

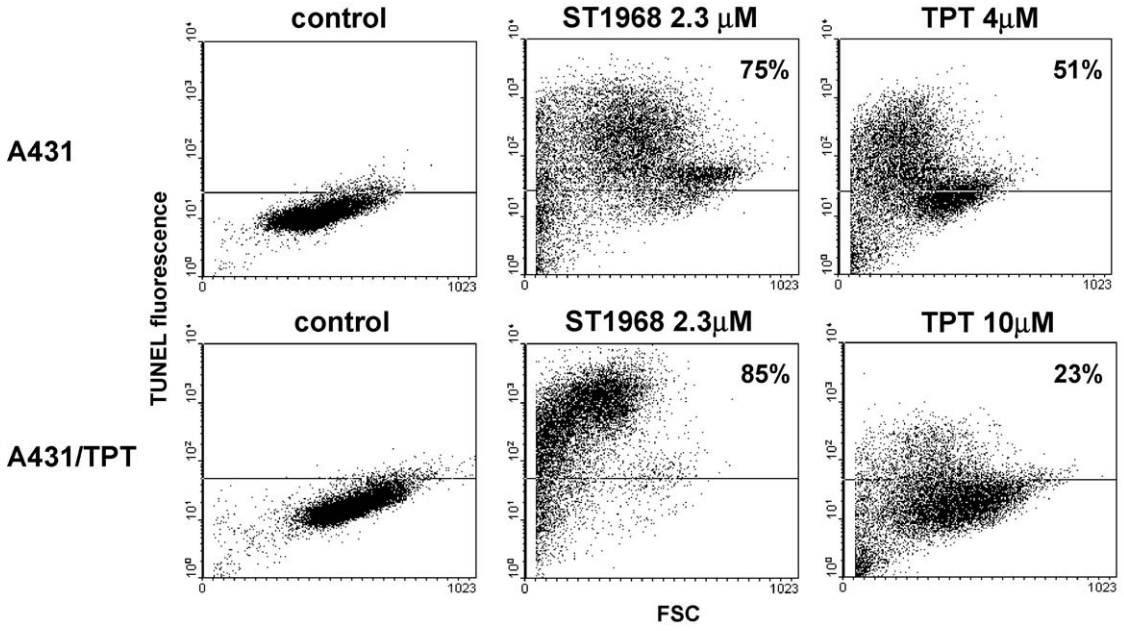

Fig. 2. Apoptosis induced by equitoxic concentrations of ST1968 and topotecan (TPT). Cells were exposed 1 h to 2.3 μM ST1968 corresponding to the IC_{80} value in both cell lines or to TPT 4 or 10 μM corresponding to the IC_{80} value in A431 and A431/TPT, respectively. Apoptosis was detected by TUNEL assay and determined by FACS analysis.

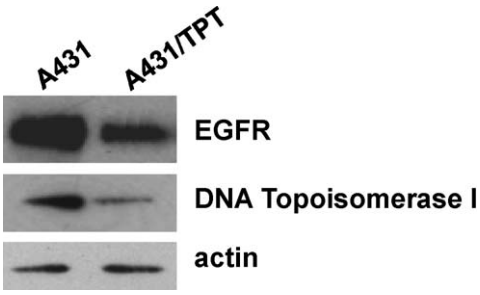

Fig. 3. Expression of EGFR and DNA topoisomerase I in A431 and A431/TPT cells. Protein expression was analyzed on whole-cell lysates by Western blotting with the specific antibodies. Actin is shown as control for protein loading.

markedly reduced in A431/TPT cells as compared to the parental cells. This change could be consistent with the reduced rate of proliferation. In an attempt to explore the molecular basis of the topotecan resistance, we examined the expression of the intracellular target, topoisomerase I, and of ABC transporters which potentially could contribute to the resistant phenotype. A431/TPT cells revealed a marked reduction of topoisomerase I expression (Fig. 3). An analysis of the expression of proteins usually associated with multidrug resistance (Pgp, BCRP, MRP4 and V-ATPase) indicated upregulation of only Pgp (MDR1) and V-ATPase (Fig. 4A) in topotecan-resistant cells. The concomitant over-expression of the vacuolar ATPase has been already reported in multidrug resistant cells [14,15]. Since the vacuolar-type H^+-ATPase (V-ATPase) is responsible for acidification of eukaryotic intracellular organelles, the lysosomal acidification was investigated by staining with acridine orange, a lysosomotropic agent which accumulates in acidic organelles [15,16]. In agreement with the upregulation of V-ATPase D, the resistant subline was characterized by increased lysosomal acidification (as shown by the increase of acridine orange fluorescence, Fig. 4B) and of granules in the cytosol (Fig. 4C).

3.3. Cellular pharmacokinetics

Drug uptake was studied after 30 min, 1 and 2 h of drug exposure, and drug retention was determined following 5-h incubation in a drug-free medium (Fig. 5). In A431 cells treated with 1 μM, the intracellular accumulation of ST1968 was time-dependent and reached a plateau at around 2-h exposure [10]. The pattern of intracellular accumulation in the resistant subline was similar, but the intracellular content of ST1968 was appreciably higher than that of the parental line. The analysis of the intracellular content following 5-h incubation in drug-free medium indicated a substantial retention of the camptothecin in both cell lines (about 15–25% of the drug content at the end of the exposure). This behaviour, in spite of upregulation of the Pgp in the resistant cells, suggested that ST1968 was a poor substrate for this efflux transporter. At the same concentration (1 μM) the intracellular accumulation of topotecan was markedly lower in either A431 and A431/TPT cells. An intracellular topotecan content comparable to that of ST1968 required exposure to 10 μM. Under these conditions, the plateau of drug uptake was reached at 30-min exposure. In contrast to A431 cells, a consistent reduction of intracellular accumulation was observed in A431/TPT by increasing the exposure time. In both cell lines, the topotecan retention following removal of extracellular drug was negligible, thus suggesting a fast drug efflux.

A study of intracellular drug distribution was performed by fluorescence imaging analysis at the single cell level (Fig. 6). Both ST1968 and topotecan showed a prevailing cytoplasmic

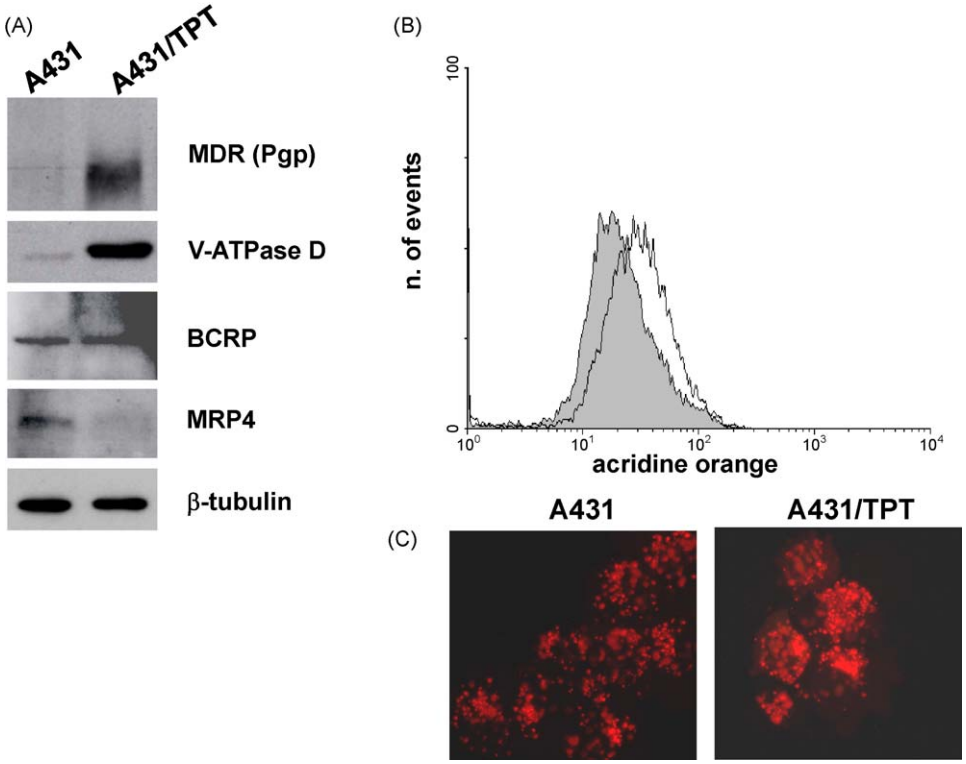

Fig. 4. Expression of ABC transporters. (A) MDR, V-ATPase D, BCRP and MRP-4 expression was analyzed on whole-cell lysates by Western blotting with the specific antibodies. β-Tubulin is shown as control for protein loading. (B) Acridine orange staining used as marker of lysosomal acidification. Cells were incubated with 5 μM acridine orange for 17 min at 37 °C and intracellular red fluorescence was measured by flow cytometry. Grey profile: A431; white profile: A431/TPT. (C) Acridine orange staining as by fluorescence microscopy.

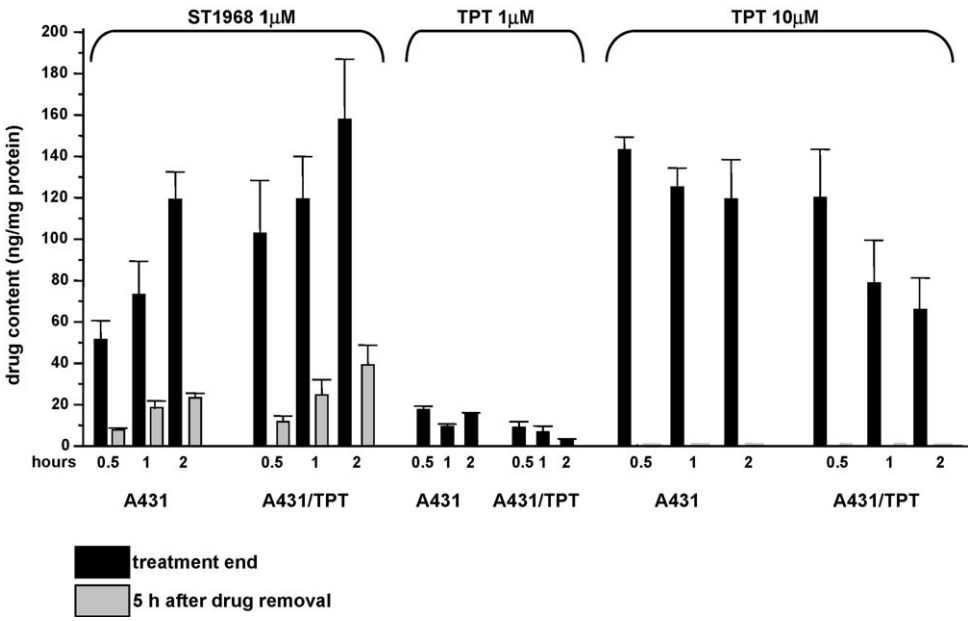

Fig. 5. Cellular pharmacokinetics of ST1968 and topotecan (TPT). Drug uptake was determined after 0.5-, 1- or 2-h exposure to 1 μM. In the case of topotecan, intracellular drug content was measured also after exposure to 10 μM. Drug retention was determined after drug removal and 5-h incubation in drug-free medium.

localization of the fluorescence signal, with darker areas corresponding to the nuclei, in the two cell lines. As shown in Fig. 6A, A431 cells treated with ST1968 exhibited a perinuclear and polar fluorescence signal till 30 min, followed by a prevailing localization in few organelles at around 60 min in comparison with A431 cells. The subcellular distribution of ST1968 in A431/TPT cells was more marked in the perinuclear areas at 15 min, with a localization in the organelles already at 30 min. This pattern was even more marked at longer times, with many organelles being well evident because of a high fluorescence intensity. The pattern of subcellular distribution of ST1968 was reminiscent of the behaviour of gimatecan [17]. Topotecan (Fig. 6B) showed a diffuse distribution pattern of fluorescence signal, with a prevailing perinuclear localization in both cell lines at all incubation times, and only an occasional presence in a few organelles. On the basis of their shape and topological distribution inside the cells, the granular structures engaged in drug intracellular accumulation and retention were likely lysosomes, as supported by similar fluorescence patterns obtained with acridine orange staining (not shown). This interpretation was confirmed by colocalization of a lysosome-specific dye, LysoTracker Red and ST1968 (Fig. 6C).

3.4. Antitumor activity studies

The study of antitumor activity of topotecan and ST1968 was performed in tumor xenografts obtained by s.c. inoculation of A431 and topotecan-resistant cells in athymic nude mice. For this comparative study, we used the same treatment schedule (q4d×4, i.v. administration and optimal doses of each drug, 10 and 25 mg/kg, for topotecan and ST1968, respectively), i.e., well-tolerated doses which caused acceptable body weight loss (≤10%) and no toxic deaths. Under these conditions, the activity of ST1968 was substantially higher than that of topotecan in both models (Fig. 7). Topotecan caused a partial inhibition of tumor growth in A431 tumor (maximum tumor growth inhibition, around 75%), but a marginal effect in A431/TPT, thus reflecting the cellular drug resistance. In contrast, ST1968 produced a 100% complete response rate in the mice bearing the A431 tumor, and retained a relevant activity in the topotecan-resistant tumor (TVI, 96%).

4. Discussion

A number of problems limit the therapeutic efficacy of camptothecins and could account for the clinical resistance and low efficacy in several tumor types. As with other clinically effective agents, the clinical resistance to camptothecins may be a multifactorial phenomenon likely involving cellular, pharmacological and tumor-specific factors [18,19]. On the basis of the peculiar features of the camptothecin structure (i.e., opening of the lactone ring) and of the mechanism of action (i.e., conversion of the single-strand breaks in irreversible double-strand breaks during S-phase), prolonged drug exposure is a critical requisite to overcome the shortcoming related to reversibility of the cleavable complex. This is a critical aspect of the clinical use of camptothecins considering the slow growth of tumors in the clinical setting as compared to the preclinical tumor models.

The role of intracellular drug accumulation and retention as a determinant of cellular sensitivity and tumor responsivity is supported by the observations reported in our study performed on a topotecan-resistant subline of the A431 tumor. This variant selected following continuous exposure to topotecan was characterized by (i) a reduced proliferation rate both in vitro and in vivo, (ii) a decreased expression of topoisomerase I and (iii) upregulation of MDR1. Although several factors have been implicated in the mechanisms of cellular resistance to camptothecins [18], the alterations associated with the development of resistance to topotecan and the pattern of cross-resistance to other camptothecins in our model may be relevant as determinants of clinical resistance, because the features of A431/TPT cells are expected to influence cellular sensitivity on the basis of the recognized mechanism of action of camptothecins.

The novel hydrophilic camptothecin of the 7-oxyiminomethyl series ST1968 exhibited ability to overcome, at least in part, the mechanisms implicated in the development of resistance in A431/TPT. In contrast to a substantial resistance to topotecan, ST1968 retained a good antitumor efficacy in vivo, in spite of the slow growth of the tumor. The cytotoxic and proapoptotic effects of ST1968 in topotecan-resistant cells were comparable to those observed in parental cells in spite of the marked reduction of topoisomerase I protein levels. A plausible explanation for the efficacy of ST1968 against this model could be its favourable

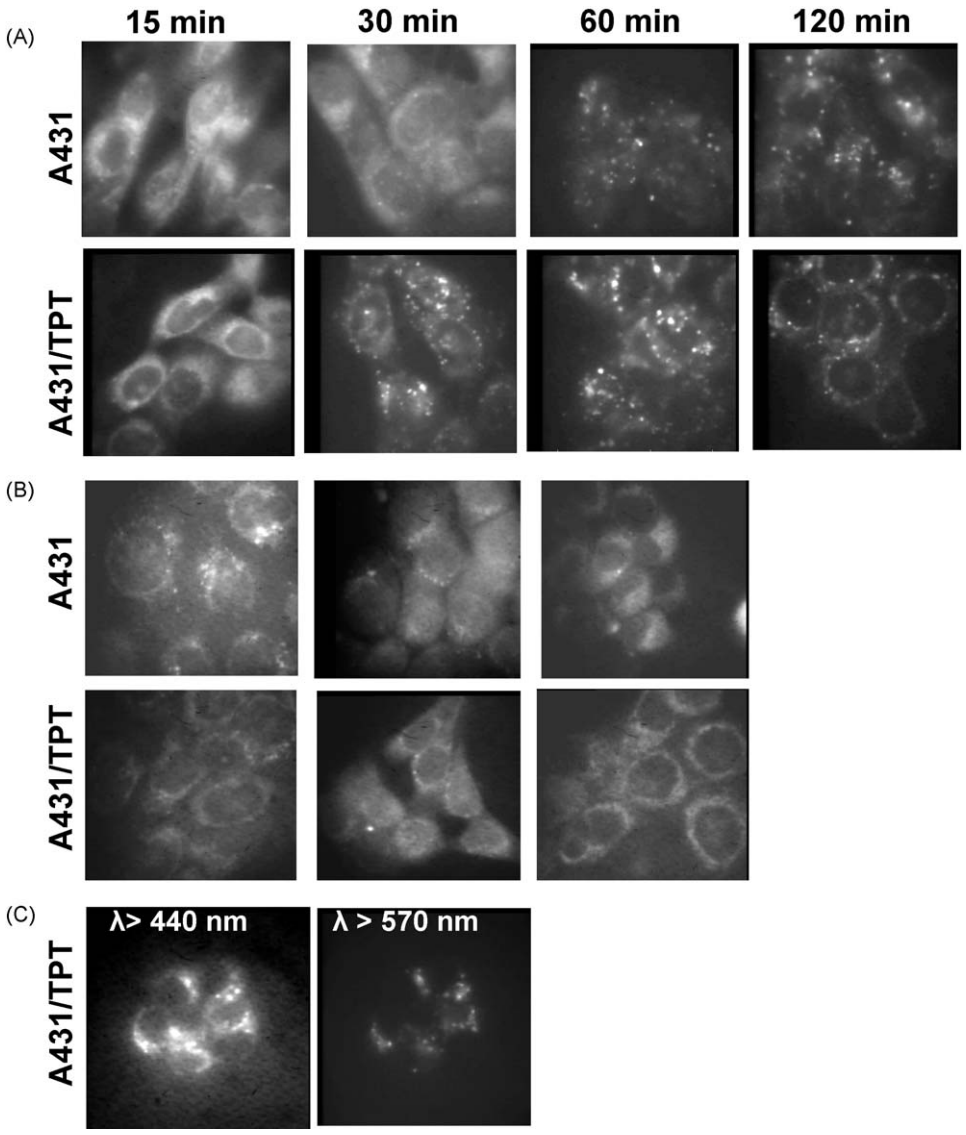

Fig. 6. Intracellular drug distribution. Fluorescence images of A431 and A431/TPT cells incubated with 10 μM ST1968 (A) or topotecan (B) for the indicated times were acquired by means of a processor digital system using a high-sensitivity ISIT camera coupled to a fluorescence microscope under excitation at 366 nm and emission >440 nm. Pictures were adjusted to comparable gray levels to allow an easier comparison of the fluorescence patterns. (C) Lysosomal localization of ST1968. A431/TPT cells were exposed to ST1968 (10 μM) and to LysoTracker Red (LTR) (1 μM) for 1 h. Fluorescence images were acquired at wavelength >440 nm (ST1968 + LTR) and at wavelength >570 nm (LTR) under excitation at 366 nm (366 nm interference filter, T% = 40, mounted in a 110000v3 filter set cube provided by Chroma Technology Corp., Rockingham, VT). The imaging analysis resulted in ratio values ($F.I._{>440}/F.I._{>570}$) of 3.43 ± 0.42 for LTR alone and 5.20 ± 0.6 for the lysosomal areas observed in cells incubated with both ST1968 and LTR.

cellular pharmacokinetics characterized by a marked intracellular accumulation and retention and a peculiar subcellular distribution. This behaviour, already observed in other squamous cell carcinoma systems, is expected to ensure prolonged drug effects as suggested by the persistence of DNA damage [10]. It is conceivable that the persistence of intracellular drug may compensate the effects of the alterations which were likely responsible for the development of resistance to topotecan. Indeed, an unexpected finding of this study was a similar cellular pharmacokinetics of ST1968 in resistant and parental cells, in spite of overexpression of the transport system, Pgp, in the resistant subline. This finding suggested that ST1968 was a poor substrate for Pgp (MDR1), as already found for other derivatives of the 7-oxyiminomethyl series [20]. However, taking into account the concomitant expression of the vacuolar-ATPase in resistant cells, it is conceivable that both v-ATPase and Pgp are implicated in drug subcellular distribution, because the drug localization in cytoplasmic organelles (presumably lysosomes) was more fast and marked in the resistant subline. The acidic environment of the lysosomes may favour the stabilization of the lactone form of the drug and lysosomal localization could represent a store allowing intracellular release of the active drug [17]. Topotecan exhibited a different subcellular localization. However the implications of the different behaviour of the two camptothecin at subcellular level remain uncertain.

The dramatic reduction of uptake and retention of topotecan by A431 was consistent with reduced cytotoxic potency in vitro and limited antitumor efficacy. The defects in cellular accumulation of topotecan were enhanced in the resistant subline which also exhibited a reduced responsiveness in vivo (maximum TV inhibition, 30 and 75% in A431/TPT and A431 tumor, respectively). In a cellular context characterized by low levels of topoisomerase expression and a slow proliferation, it is conceivable that the intracellular concentration of topotecan was inadequate to cause sufficient lethal lesions. In contrast the favourable pharmacokinetic behaviour of ST1968 might overcome the resistance mediated by these changes, as a consequence of drug persistence inside the cell. If

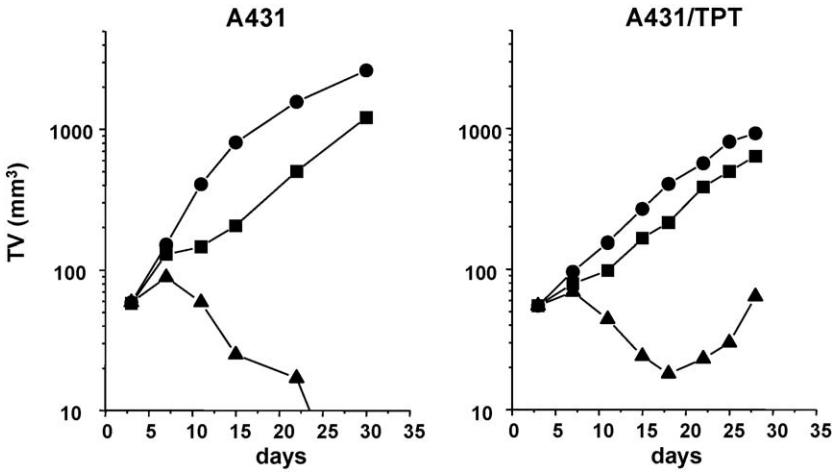

Fig. 7. Comparison of the antitumor activity of ST1968 and topotecan against A431 and A431/TPT tumor xenografts in nude mice. Drugs were delivered i.v. every fourth day for four times (q4d×4) starting when tumors were just palpable. Circle: control; square: 10 mg/kg TPT; triangle: 25 mg/kg ST1968.

this interpretation is correct, the peculiar features of ST1968 may have relevant therapeutic implications in clinical development of the novel camptothecin, because the slow growth of human tumor in patients and the reversibility of drug effects are critical limitations of the efficacy of conventional camptothecins [4].

Our study indicating the efficacy of ST1968 against topotecan-resistant tumors provide further support to the therapeutic interest of the novel camptothecin. Additional advantages of ST1968 over conventional camptothecins are the improvement of the therapeutic index and efficacy in a wide range of well-tolerated doses [9]. In spite of its cytotoxic potency (higher than that of topotecan, Table 1), ST1968 was well-tolerated in vivo at doses substantially higher than those of topotecan (25–30 mg/kg vs. 10–12 mg/kg). A favourable pharmacokinetics characterized by a prolonged retention in tumor tissue as compared with normal tissue may account for the improvement of the therapeutic index reflecting an increased tumor selectivity of the drug (unpublished data). The molecular basis of the improved pharmacological profile of ST1968 remains to be defined. We have reported that ST1968, characterized by the presence of a free amino group, induces a markedly stable ternary complex [8]. Thus the molecular and pharmacokinetic features could contribute to improve antitumor efficacy and selectivity of the novel camptothecin.

Conflict of interest

None.

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