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Preclinical efficacy of ST1976, a novel camptothecin analog of the 7-oxyiminomethyl series

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ABSTRACT

In previous studies, we have documented the potential therapeutic advantages of camptothecin analogs modified at the 7-position, i.e., 7-oxyiminomethyl derivatives. The present study was performed to explore the therapeutic potential of novel hydrophilic derivatives of this series. With one exception (ST1976), the tested camptothecins exhibited a reduced antiproliferative activity and all compounds retained ability to stabilize the topoisomerase I-mediated cleavable complex. The two analogs (ST1976 and ST1968) characterized by the presence of a free amino group in the side chain also exhibited the formation of persistent cleavable complexes. The most potent compound, ST1976 (7-(4-aminobenzyl)oxyiminomethylcamptothecin), was selected for evaluation of its preclinical profile of antitumor activity in a large panel of human tumor xenografts. As expected on the basis of the introduction of a hydrophilic substituent, the novel camptothecin was a substrate for BCRP. However, in spite of an apparent recognition by BCRP, ST1976 was effective following oral administration. The antitumor activity was evaluated using various schedules and routes of administration (i.v. and p.o.). ST1976 exhibited a remarkable activity in all tested tumors and was effective in a number of tumors which are resistant to irinotecan. The biological and pharmacological profile of ST1976 supports the therapeutic potential of camptothecins containing hydrophilic substituents at the 7-position. On the basis of its excellent activity in preclinical models, ST1976 is a promising candidate for clinical development.

1. Introduction

Camptothecins continue to be the subject of intense investigation [1]. The preclinical and clinical efficacy of topoisomerase I inhibitors has generated high expectations in the development of the novel generation of camptothecins. A major limitation of camptothecins is related to their peculiar chemical structure [2,3]. The chemical instability of the lactone form at physiological pH generates the carboxylate form which exhibits high affinity for human serum albumin [4]. This interaction shifts the equilibrium toward the open form, thus favoring the hydrolysis of the lactone and limiting the therapeutic level of the active form [2,4]. The drug-stabilized topoisomerase I-DNA cleavable complex, containing single-strand break, is reversible [5]. This lesion is converted in the more lethal double-stranded breaks during DNA synthesis [6]. Therefore, as a result of this mechanism of

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action, stability of the cleavable complex (i.e., drug–enzyme–DNA ternary complex) or prolonged exposure to the active drug form are critical requisites for therapeutic efficacy of camptothecins [2,3].

We have reported that 7-substituted lipophilic camptothecins exhibit favorable molecular and pharmacological features resulting in potential therapeutic advantages [7–13]. The promising pharmacological profile of analogs of this series likely reflects a rapid intracellular accumulation (a favorable event to minimize drug–plasma protein interaction) and a persistent stabilization of the cleavable complex. However, recent studies on the molecular structure of the cleavable complex stabilized by topotecan have indicated the participation of water in the enzyme-mediated cleavable complex [14], which likely provides stabilization of the ternary complex. Based on this model, hydrophilic analogs modified at the 7-position able to form extensive hydrogen bond networks have been prepared [15]. The reported hydrophilic camptothecin analogs form stable cleavable complex with DNA and topoisomerase I and the *in vivo* activity of the tested 7-modified analogs was found promising.

The present study was performed to explore the influence of hydrophilic substituents at the 7-position of novel camptothecins of the 7-oxyiminomethyl series [9]. Among the tested camptothecins we selected a potent analog, ST1976, for detailed antitumor activity studies.

2. Materials and methods

2.1. Drugs

The procedure for the synthesis of 7 modified camptothecins has been previously described [7,9] and the chemical structures are presented in Fig. 1. The clinical preparations of topotecan and irinotecan were used as standard reference. ST1600, ST1587, and ST1968 were dissolved in distilled water; ST1976 was dissolved in DMSO and diluted in sterile distilled water before use with a final DMSO concentration of 10% for oral and 5% for i.v. delivery. Irinotecan was dissolved in sterile, distilled water keeping it under magnetic stirring for about two hours, and administered i.v. very slowly. All drugs were delivered in a volume of 10 ml/kg of body weight.

2.2. Antiproliferative activity

Cell lines used were the non-small cell lung cancer cell line NCI-H460 (ATCC, HTB-177), the colorectal adenocarcinoma cell line HT29 (ATCC, HTB-38) and the corresponding mitoxantrone resistant variant HT29/mit [16].

Cells were cultured in RPMI-1640 containing 10% foetal calf serum. Cytotoxicity was assessed by growth inhibition assay after 1 h drug exposure. Cells in the logarithmic phase of growth were harvested and seeded in duplicates into 6-well plates. Twenty-four hours after seeding, cells were exposed to the drug and harvested 72 h after exposure and counted with a Coulter counter. IC_{50} is defined as the inhibitory drug concentration causing a 50% decrease of cell growth over that of untreated control.

2.3. Topoisomerase I-dependent DNA cleavage assay

A 3′-end labeled gel purified 751-bp *Bam*HI-*Eco*RI fragment of SV40 DNA was used for the cleavage assay. SV40 plasmid was first linearized with *Bam*HI enzyme and then 3′-labeled by using DNA polymerase I large (klenow) fragment (Invitrogen, Paisley, UK) in presence of dGTP and α^{32}P ddATP. The labeled DNA was then restricted with *Eco*RI enzyme and the corresponding 751-bp was purified on agarose gel. Topoisomerase I DNA cleavage reactions (20,000 cpm/sample) were performed in 20 μl of 10 mM Tris–HCl (pH 7.6), 150 mM KCl, 5 mM $MgCl_2$, 15 μg/ml BSA, 0.1 mM dithiotritol, and 640 ng of human recombinant enzyme (full length topoisomerase I) [17] for 30 min at 37 °C. Reactions were stopped by 0.5% SDS and 0.3 mg/ml of proteinase K for 45 min at 42 °C. Persistence of DNA cleavage at different time points was examined by adding 0.6 M NaCl after 30 min of incubation. After precipitation DNA was resuspended in denaturing buffer (80% formamide, 10 mM NaOH, 0.01 M EDTA and 1 mg/ml dyes) before loading on a denaturing 8% polyacrylamide gel in TBE buffer. Overall DNA cleavage levels were measured with a PhosphoImager 425 model (Molecular Dynamics).

2.4. Antitumor activity studies

All experiments were carried out using female athymic Swiss nude mice, 7–10 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 Istituto Nazionale Tumori of Milan according to institutional guidelines [18].

Human tumor lines were maintained by serial s.c. passages of fragments (about 2 mm × 2 mm × 6 mm) of

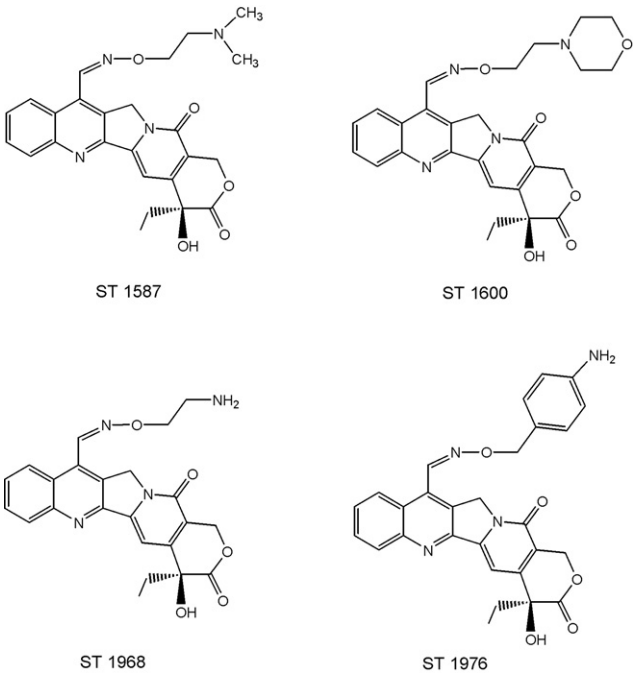

Fig. 1 – Chemical structures of novel hydrophilic camptothecins.