Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical Pharmacology 70 (2005) 1125–1136
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

# The novel lipophilic camptothecin analogue gimatecan is very active in vitro in human neuroblastoma: A comparative study with SN38 and topotecan

Angela Maria Di Francesco [a,*], Anna Shirley Riccardi [a], Giuseppe Barone [a], Sergio Rutella [b], Daniela Meco [a], Roberta Frapolli [c], Massimo Zucchetti [c], Maurizio D'Incalci [c], Claudio Pisano [d], Paolo Carminati [d], Riccardo Riccardi [a]

[a] *Division of Paediatric Oncology, Catholic University, Rome, Italy*
[b] *Division of Haematology, Catholic University, Rome, Italy*
[c] *Department of Oncology, Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy*
[d] *Research & Development, Sigma-Tau, Rome, Italy*

Received 3 May 2005; accepted 18 July 2005

**Abstract**

Neuroblastoma is one of the most common extracranial solid tumours in childhood with a poor prognosis in its advanced stage. Treatment failure is often associated to the occurrence of drug resistance. To date, treatment of paediatric neuroblastoma is still dismal, and therefore novel effective drugs are awaited. In recent years, an increasing interest has concentrated on camptothecin analogues. Topotecan and irinotecan, the only two clinically relevant camptothecin derivatives to date, have entered clinical trials in neuroblastoma but so far the results have been disappointing. Gimatecan (ST1481, LBQ707; 7-*t*-butoxyiminomethylcamptothecin), is a novel lipophilic camptothecin derivative that was selected from a series of lipophilic analogues rationally designed and synthesized in order to overcome some of the main drawbacks of conventional camptothecins, limiting their clinical efficacy. Gimatecan is endowed with potent antitumour activity, strong topoisomerase I inhibition, stable drug–target interactions and a better pharmacological profile. The present study deals with the comparative evaluation of cellular pharmacology features of gimatecan, topotecan and SN38 in neuroblastoma cell lines. We show that, despite the lowest intracellular accumulation, gimatecan was the most active among the camptothecin analogues studied. Our findings suggest that the high activity of gimatecan in neuroblastoma is related to the ability of this novel analogue to cause a very high number of DNA breaks as assessed by the Comet assay in both cellular or sub-cellular systems. We propose that DNA strand breaks efficiency as measured by the Comet assay might provide important information about the stability of the ternary complexes induced by camptothecin compounds.


*Keywords:* Gimatecan; Camptothecins; Topoisomerase I inhibitors; Neuroblastoma; Alkaline comet assay; DNA strand breaks

## 1. Introduction

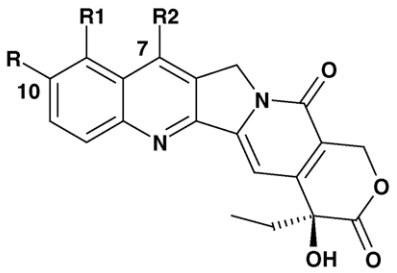
Neuroblastoma is a paediatric tumour with a poor prognosis in its advanced stage. Although some progresses in therapy, neuroblastoma it is still responsible for about 15% of cancer-related deaths in children [1–4]. In recent years, different approaches to the treatment of advanced neuroblastoma have been attempted and new agents such as camptothecin analogues have shown some interesting results [5–8]. Camptothecins are semi-synthetic drugs derived from the alkaloid camptothecin that was first isolated from the Chinese tree Camptotheca acuminata. They represent an important class of anticancer drugs with a wide spectrum of activity in many solid tumours such as lymphoma, gastric cancer, small cell lung cancer, non small cell lung cancer, cervical cancer and colorectal cancer (see ref. [8] and references within). There is firm evidence that the molecular target of camptothecins is the nuclear enzyme topoisomerase I, that plays a key role in DNA replication,

* Corresponding author. Present address: Laboratory of Pharmacology, Division of Paediatric Oncology, Catholic University of Rome, L.go A. Gemelli 8, 00168 Rome, Italy. Tel.: +39 063058203; fax: +39 063052751.
*E-mail address:* labfarm2@rm.unicatt.it (A.M. Di Francesco).

0006-2952/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2005.07.009

transcription and repair. Topoisomerase I acts by relaxing torsionally strained duplex DNA through the insertion of DNA single strand breaks. Camptothecins are able to form a labile cleavable complex drug–enzyme–DNA that inhibits the DNA relegation step. Because of the reversibility of these topoisomerase I–DNA complexes, toxicity occurs only when they are converted to irreversible DNA strand breaks [9]. The collision between the replication-fork and these complexes has been proposed to explain the camptothecin-driven S-phase specific cytotoxicity and the arrest in the G2-M phase of the cell cycle [10]. Furthermore, the blockage of the RNA polymerase elongation systems by topoisomerase I–DNA covalent complexes induces transcription arrest and triggers 26S proteasome-mediated degradation of both topoisomerase I and the large subunit of RNA polymerase II (RNA Pol $II_0$) [11–13]. This effect is considered a repair response to the cytotoxic action of camptothecins, as degradation of topoisomerase I results in the exposure of single strand breaks that can then be repaired through functional transcription-coupled repair (TCR) [13]. For their ability to transform topoisomerase I in a cytotoxin, camptothecins are often referred to as topoisomerase I poisons.

To date, two water-soluble derivatives of camptothecin, topotecan and irinotecan, represent the main clinically relevant drugs of this class for the treatment of many solid tumours. Topotecan is used as standard regimen treatment in ovarian and lung cancers [14,15], whereas irinotecan is used in first- and second-line treatments in advanced colorectal cancer [16,17]. Although topotecan has been rarely used in paediatric malignancies, there is a recent report of a phase II study in which the combination topotecan-cyclophosphamide administered i.v., showed significant activity in children with newly diagnosed neuroblastoma and this treatment was more efficacious than topotecan alone [18]. On the other hand, oral topotecan therapy showed antitumour activity only in a small percentage of patients with relapsed or refractory neuroblastoma where the drug was administered at a dose of 1 mg/($m^2$ day) in two divided doses for 21 consecutive days. However, due the high toxicity reported, a dose adjustment was required in order to limit the side effects of the drug [7]. In fact, pre-clinical studies showed that, in several tumours, protracted schedules of daily administration of low-dose topotecan were more effective than more intense shorter schedules of administration [19]. Consistently, a recent phase I clinical trial using oral topotecan at a low dose (0.8 mg/($m^2$ day)) in combination with oral cyclophosphamide for 10–17 days, showed reduced toxicity but only a partial response in one neuroblastoma patient [20]. So, the low-oral dose schedule of topotecan appears of use mainly as last-line therapy in pre-treated neuroblastoma patients. With irinotecan, phase I studies in paediatric tumours were conducted in Japan, USA and France [21–23] and Phase II studies are planned. Overall, irinotecan has shown some activity in neuroblastoma but a prolonged schedule and the i.v. administration route appeared necessary for better results [8].




| Drug | R | R1 | R2 |
|---|---|---|---|
| Camptothecin | H | H | H |
| Gimatecan | H | H | $CH{=}NOC(CH_3)_3$ |
| SN38 | OH | H | $CH_2$-$CH_3$ |
| Topotecan | OH | $CH_2N(CH_3)_2$ | H |

Scheme 1. Chemical structures of camptothecin analogues considered in this study.

The main drawbacks of camptothecin derivatives is the instability of the α-hydroxylactone ring (the active form of the drug) and the lability of the cleavable complex. In recent years, many efforts have been made in the medicinal chemistry field to overcome these limitations and to maintain antitumour potency. A series of modified lipophilic analogues was synthesized in order to stabilize drug-target interactions. Gimatecan (ST1481, LBQ707; 7-*t*-butoxyiminomethylcamptothecin; Scheme 1), is a novel analogue that was selected from this series as it is endowed with potent antitumour activity, strong topoisomerase I inhibition and a better pharmacological profile than other conventional camptothecins [24–26]. To date, there are already a few encouraging reports showing that gimatecan did not fail the initial expectations. Furthermore, there are evidences that unlike topotecan, gimatecan is able to overcome drug resistance mediated by the MDR phenotype [26] and BCRP [27]. The availability of the lipophilic derivative and orally active gimatecan showing both a better pharmacological profile and a lack of cross-resistance to topotecan and irinotecan has attracted our interest, and has prompted us to compare it to other conventional camptothecins in neuroblastoma. During our in vitro studies we selected topotecan and SN38, the active metabolite of irinotecan, as reference camptothecins.

## 2. Materials and methods

### 2.1. Drugs

Gimatecan and SN38 were kindly provided by Sigma Tau (Pomezia, Rome, Italy). The drugs were dissolved in