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Commentary

Relevance of extracellular and intracellular interactions of camptothecins as determinants of antitumor activity

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ABSTRACT

Camptothecins are potent antitumor agents that stabilize the covalent binding of topoisomerase I to DNA forming a reversible ternary complex which, following collision with the replication forks, converts the single-strand breaks into lethal double-strand breaks. This cytotoxic mechanism has been originally ascribed to the closed lactone form, because opening of the lactone ring resulted in loss of antitumor activity. Since the lipophilic lactone favours passive diffusion into the cancer cells, the stability of the closed form is expected to be predictive for activity. Thus, the *in vivo* pharmacological behavior of camptothecins, which is dependent on the pH-dependent dynamics, is likely a critical determinant of their antitumor efficacy and therapeutic index. The physicochemical properties could influence a number of cellular and *in vivo* interactions, including stability of the ternary DNA–enzyme–drug complex, binding to serum proteins, recognition by transport systems. These interactions are also implicated in the processes responsible of toxic side effects and drug resistance which are major limitations of the efficacy of camptothecin-based therapy. A number of strategies have been developed to overcome the limitations associated with the peculiar *in vivo* reactivity and the reversibility of drug–target interaction. Modifications with hydrophilic or lipophilic substituents at specific positions may have a variable (and somewhat opposite) influence on interaction with the intracellular target and plasma proteins and on recognition by membrane transporters. Here, we highlight the interactions of camptothecins which could be exploited to optimize therapeutic efficacy.

1. Introduction

The cytotoxic and antitumor activity of camptothecins (CPT) is ascribed to their ability to stabilize the covalent binding of topoisomerase I (Top1) to DNA (the cleavable complex) forming a reversible ternary complex. These stabilized single-strand DNA breaks are fully reversible and non-lethal. However, when a DNA replication fork collides with the cleavable complex, single-strand breaks are converted into irreversible double-strand breaks (Fig. 1). On the basis of S-phase-specific activity of camptothecins and of the reversibility of cleavable complex, prolonged drug exposure is a critical requisite for therapeutic efficacy.

CPT has a peculiar pentacyclic structure containing an hydrolyzable ring E (Fig. 2) [1]. The molecule in solution is almost planar with an asymmetric carbon in position 20 of ring

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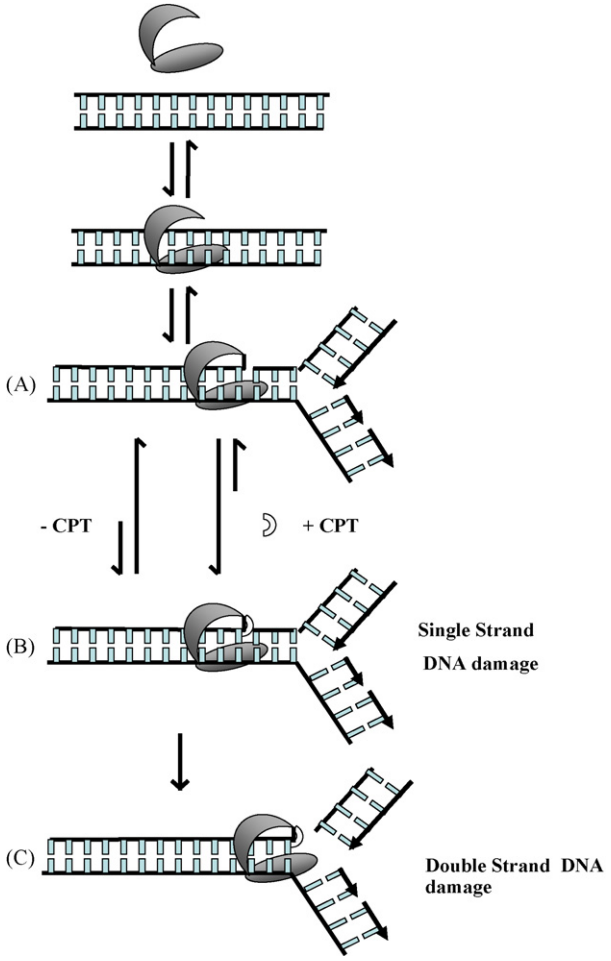

Fig. 1 – Mechanism of DNA damage mediated by topoisomerase I. (A) Cleavage reaction of topoisomerase I. (B) Drug-stabilized clavable complex. (C) Collision with the replication fork.

E, and with a single out-of-plane nitrogen atom of ring C connected with the slightly out-of-plane carbonyl group of ring D [2]. The CPTs can undergo a pH-dependent reversible interconversion between the lactone form and the ring-opened carboxylate. At neutral or physiologic pH, the equilibrium between the two species favours the carboxylate form (Fig. 2). A closed lactone ring strengthens the covalent structure of the CPT chromophore and its hydrolysis leads to relaxation of the system and to inversion of the position of the nitrogen atom in ring C, from up to down, relative to the plane of the chromophore molecule, similar to that induced by 20(*S*)-20(*R*)-CPT isomerisation [2]. The extracellular stability of the lactone form of CPT is critical for its anticancer activity. Indeed, the carboxylate form shows a poorer diffusibility through the lipid bilayer than the lactone form and binds preferentially to human serum albumin (HSA) [3]. Factors influencing the lactone-carboxylate equilibrium are clearly important determinants of CPT activity and the hydrolysis of lactone ring represents one of the major drawbacks of this class of agents. Although the only recognized target of CPTs is Top1, their complex polycyclic structure and the presence of various structural functionalities may confer ability to interact with other proteins which influence critically their pharmacological behavior. In the present commentary, we examine the multiple drug interactions which may influence the pharmacological properties of CPT and could be exploited to optimize therapeutic efficacy.

2. Interaction of camptothecins with plasma proteins

CPTs exist in an equilibrium between closed lactone and open carboxylate forms, which is dependent on the pH of the medium, interaction with blood components and likely other factors. The different lactone/carboxylate ratios in plasma of mice versus humans is the most convincing explanation of the interspecies variability of CPT efficacy. The pharmacological basis of this difference is the increased affinity of CPTs for HSA, which is the most important interaction with plasma proteins affecting the toxicity profile and efficacy of these drugs.

HSA is the most abundant plasma protein (5 g/100 ml) which has a high affinity for a wide range of drugs and metabolites. HSA consists of three homologous domains (I–III) probably derived through gene multiplication. A hydrophobic pocket is formed in domain II and is thought to be a binding site for many drugs. Although crystallographic analysis of CPT/HSA complex is not available, spectroscopy studies indicate that carboxylate CPT binds within the domain II [4]. In particular, interactions of CPT with HSA are supposed to include both hydrophobic contacts and an interaction of the CPT carbonyl group of ring D and the carboxylate function of hydrolysed ring E with charged amino acid residues of HSA [4].

The preferential binding of HSA with the carboxylate form of CPTs results in the more rapid opening of lactone ring (Fig. 3). The role of HSA in influencing the lactone/carboxylate equilibrium of CPT has been confirmed in many *in vitro* studies. Cell-free system experiments have shown that at

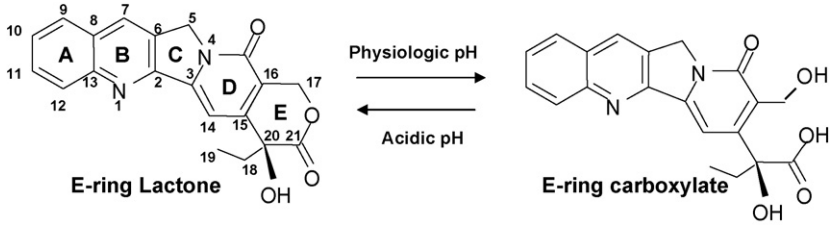

Fig. 2 – Chemical structure of the natural camptothecin and interconversion of the lactone E ring into to the carboxylate form.