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Commentary

Perspectives in the development of hybrid bifunctional antitumour agents

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ABSTRACT

In spite of the development of a large number of novel target-specific antitumour agents, the single-agent therapy is in general not able to provide an effective durable control of the malignant process. The limited efficacy of the available agents (both conventional cytotoxic and novel target-specific) reflects not only the expression of defence mechanisms, but also the complexity of tumour cell alterations and the redundancy of survival pathways, thus resulting in tumour cell ability to survive under stress conditions. A well-established strategy to improve the efficacy of antitumour therapy is the rational design of drug combinations aimed at achieving synergistic effects and overcoming drug resistance. An alternative strategy could be the use of agents designed to inhibit simultaneously multiple cellular targets relevant to tumour growth/survival. Among these novel agents are hybrid bifunctional drugs, *i.e.* compounds resulting by conjugation of different drugs or containing the pharmacophores of different drugs. This strategy has been pursued using various conventional or target-specific agents (with DNA damaging agents and histone deacetylase inhibitors as the most exploited compounds). A critical overview of the most representative compounds is provided with emphasis on the HDAC inhibitor-based hybrid agents. In spite of some promising results, the actual pharmacological advantages of the hybrid agents remain to be defined. This commentary summarizes the recent advances in this field and highlights the pharmacological basis for a rational design of hybrid bifunctional agents.

1. Introduction

Drug resistance of tumour cells is recognized as being a major obstacle to effective cancer treatment [1]. This phenomenon has been ascribed to many mechanisms operating at various cell levels, specifically defence mechanisms, activation of DNA repair mechanisms and modulation of survival/cell death pathways [2]. The heterogeneity of cancer cells and their ability to activate compensatory pathways in response to drug treatment likely account for the intrinsic insensitivity and the frequent development of drug resistance [3]. To overcome these limitations, a well-established approach in cancer therapy for advanced disease is the use of combination of agents with different mechanism of action and non-overlapping toxicity profile [4]. This strategy is based on the compelling evidence that single-agent therapy is not able to provide an effective and durable control of the malignant process. The complex alterations of tumour cells and the redundancy of survival-related pathways contribute to tumour cell survival under stress conditions. For these reasons, the outcome of treatment with both conventional cytotoxic drugs or novel targeted agents is often a cytostatic cellular response rather than induction of cell death.

The *in vivo* combination of agents that exhibit synergistic interaction in cell culture can be less effective than expected due to differential pharmacokinetic behaviour of each drug and to the difficulty to afford optimal concentrations for the required time. Other shortcomings of drug combinations are unpredictable drug–drug interactions and possible enhancement of adverse effects. To avoid problems related to the pharmacokinetic behaviour of the combined agents and to better exploit the expected synergistic interactions, an alternative strategy is the development of hybrid bifunctional agents that may be able to inhibit simultaneously multiple targets involved in tumour cell defence and survival.

The interest in the strategy of multiple target inhibition is also supported by evidence of improved efficacy of dual (or multiple)-action inhibitors, as highlighted by novel kinase inhibitors active against various targets or receptors [5,6]. The multiple inhibitions

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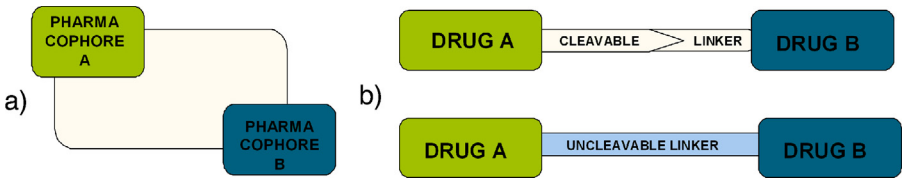

Fig. 1. General structure of hybrid drugs. (a) Molecules containing the pharmacophores of different drugs; (b) molecules resulting by combination of entire drugs connected by a linking arm.

of related pathways may have convergent effects on these pathways, thus enhancing the potency of compounds. Given the structural homology of related kinases, some receptor kinase inhibitors are effective against multiple targets, other (irreversible inhibitors) are active against different kinases by interacting with cysteine residues [6]. The inhibitors of closely related targets containing a single pharmacophore have been extensively studied and the results have been already discussed in recent reviews [6,7].

The development of hybrid molecules represents a different strategy, because these novel agents are conceived as: (a) molecules containing the pharmacophores of different drugs or (b) molecules resulting by combination of entire drugs, usually connected through a linking arm (Fig. 1). The rationale of this approach is based on the expected synergistic interaction of the two pharmacologically active components that could be favoured by optimal pharmacodynamic conditions.

The focus of this commentary is to summarize the most recent advances in the development of hybrid bifunctional compounds as antitumour agents, with particular emphasis on the critical aspects of drug design, the potential drawbacks and future directions of this approach.

The commentary is divided into sections according to the mechanism of action of the hybrid constituting drugs.

2. Hybrid compounds incorporating cytotoxic agents

Hybrid molecules of cytotoxic agents have been designed by overlapping structural motifs of compounds known to interact with the same target or by merging pharmacophore moieties to optimize or enhance interaction with the putative target. A number of molecules of this type have been described in previous reviews [8,9]. Since DNA topoisomerases I and II (topo I and II) are recognized as the primary targets of well-established antitumour drugs, hybrid compounds containing topo I/topo II inhibitors have been explored. Hybrid agents, designed as topo II inhibitors, have been obtained by combining the chemical features of known inhibitors (*e.g.*, DNA intercalating agents such as ametantrone and amsacrine) in the attempt to optimize the interaction of the drug with the DNA-enzyme complex (**1**, Fig. 2) [10]. Although these studies may provide valuable information concerning the drug interaction in the enzyme-DNA ternary complex, the pharmacological activity of these hybrids was not investigated.

Other efforts in this field have been directed to increase the therapeutic potential of multifunctional DNA damaging agents, *i.e.* agents capable to induce genotoxic damage through distinct mechanisms. The compound Alchemix (**2**) is an anthraquinone inducing topo II-mediated DNA damage. Due to the incorporation of an alkylating function, the genotoxic stress is expected to be more persistent and less susceptible to repair [11]. The induction of irreparable damage is consistent with the activity of Alchemix against anthracycline-resistant and platinum-resistant ovarian carcinoma models and the induction of cell death *via* activation of a p53-independent apoptotic pathway [12]. This compound, characterized by intercalating and alkylating properties, preferentially induces the formation of a covalently bound topo IIalpha-drug-DNA ternary complex, but does not stabilize a topo IIbeta-DNA complex. The formation of an irreversible topo II-DNA-drug complex may result in a persistent inhibition of the target enzyme function. In spite of some promising features, including the ability to overcome resistance mechanisms related to recognition by efflux transport systems and to DNA damage response, the therapeutic value of the hybrid compound and the actual advantages over conventional cytotoxic agents was not demonstrated at least in terms of efficacy and tolerability.

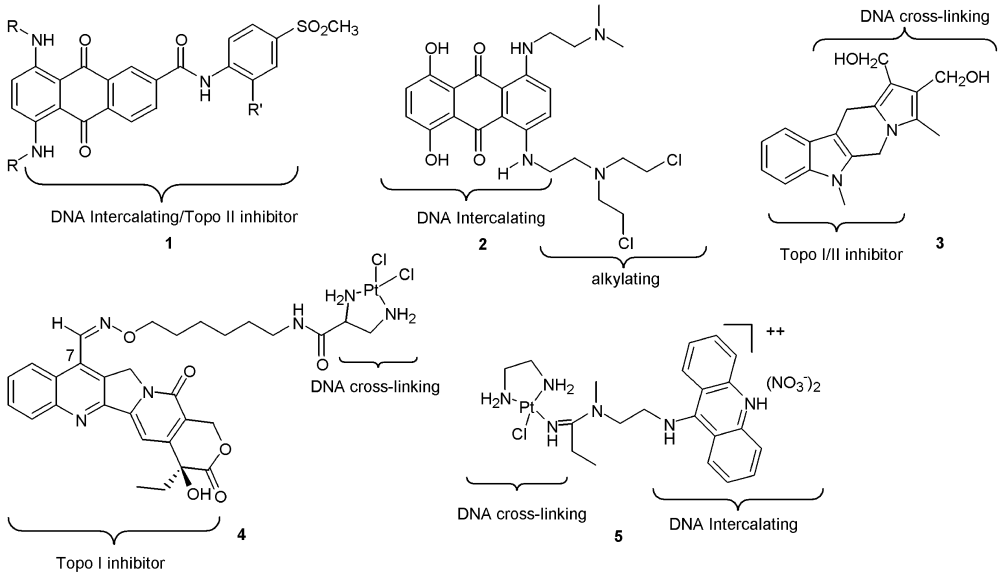

Fig. 2. Hybrid compounds incorporating cytotoxic agents.