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Commentary

Role of the sugar moiety in the pharmacological activity of anthracyclines: development of a novel series of disaccharide analogs

Franco Zunino*, Graziella Pratesi, Paola Perego

Istituto Nazionale per lo Studio e la Cura dei Tumori, via Venezian 1, 20133 Milan, Italy

Abstract

The sugar moiety is an essential component of anthracycline antibiotics for their topoisomerase poisoning activity and antitumor efficacy. Since the sugar interacts with the minor groove, modifications in this moiety could enhance the recognition potential of the drug at the target level. Based on this hypothesis, novel anthracyclines, disaccharides lacking the amino group in the first (aglycone-linked) sugar, were designed. The 3′-amino group in the first sugar was replaced by an hydroxyl group, and the second sugar residue was bound to the first sugar via an α (1–4) linkage. The cytotoxic and antitumor activities of disaccharide analogs of idarubicin were critically dependent on the optimal (axial) orientation of the second sugar residue. Although configurational requirements of the sugar moiety for optimal drug activity support a critical role of the external (non-intercalating) drug domains in the interaction of anthracyclines with the DNA-topoisomerase (ternary complex), the antitumor efficacy of disaccharide analogs is not fully explained by effects mediated by the nuclear enzyme target. The development of this novel disaccharide series may provide insights for a rational synthesis of anthracycline analogs with improved pharmacological profile.

Keywords: Anthracyclines; Disaccharides; Antitumor therapy

1. Introduction

Anthracycline antibiotics represent a major class of antitumor agents, with a wide spectrum of activity in human cancer [1]. In spite of intense efforts in analog synthesis, the most effective agent of this class remains doxorubicin. Like other DNA-intercalating agents effective as antitumor compounds, anthracyclines exert their cytotoxic activity by interfering with the function of DNA topoisomerase II, an essential nuclear enzyme that regulates DNA topology during multiple DNA processes (replication, transcription, recombination) [2]. The inhibitory properties of anthracyclines are not simply related to the DNA–drug intercalation. The recognition that DNA topoisomerase II is the primary target of anthracyclines provides a more rational basis for the design of effective analogs. The topoisomerase II inhibitors form a DNA–drug–enzyme complex (ternary complex) by stabilizing an intermediate of the enzyme reaction (the so-called cleavable complex) in which DNA strands are broken and the enzyme subunits are covalently linked to the broken DNA strands. DNA binding and intercalation are necessary but not sufficient conditions for optimal activity of anthracyclines [3]. Indeed, the external (non-intercalating) moieties of the anthracyclines (Fig. 1) appear crucial for poisoning topoisomerase activity and therapeutic efficacy [4,5]. For example, in spite of a DNA-binding affinity comparable to that of doxorubicin, 9-deoxydoxorubicin is characterized by a substantially reduced ability to stimulate topoisomerase-mediated DNA cleavage and a markedly reduced cytotoxic potency [6,7]. Thus, although DNA intercalation may have a relevant role in the mechanism of drug interaction in the ternary complex, external interactions involving the sugar moiety and cyclohexene ring A appear to be more critical than the strength of the DNA–drug interaction. In particular, the sugar moiety is recognized to be critical for the activity of anthracyclines as topoisomerase poisons. The recent development of a novel series of anthracycline disaccharides provides further insights into the role of the sugar moiety [8,9]. A doxorubicin analog of this series exhibits an improved profile of antitumor activity as compared with doxorubicin and could represent the progenitor of a new generation of promising anthracyclines [10,11].

2. Role of the sugar moiety

Original studies on structure–activity relationship have shown an important role for the structure and stereochem-

* Corresponding author. Tel.: +39-02-2392267; fax: +39-02-2392692.
E-mail address: zunino@istitutotumori.mi.it (F. Zunino).

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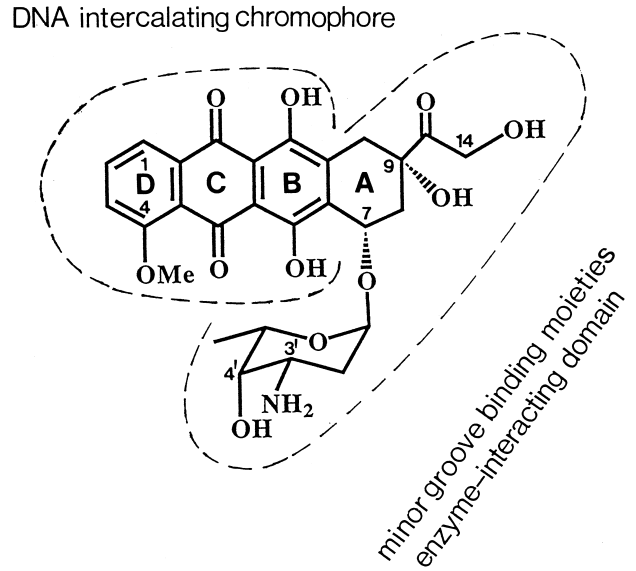

Fig. 1. Structure of doxorubicin showing the drug domains likely relevant for pharmacological activity.

istry of the aminosugar (daunosamine) on the pharmacological activity of anthracyclines related to doxorubicin [12–15]. These studies implicated the basic amino group at C-3′ as a determinant of the stabilization of drug intercalation into DNA [16]. This interpretation was based on the evidence that the blocking of the amino function, as in the amide derivatives, results in a substantial loss of cytotoxic activity and in reduced DNA-binding affinity [15,16]. In addition, the binding of the β anomer of doxorubicin to DNA is markedly different from that of the natural α anomer [14], thus supporting the view that the external binding moieties dictate the mode of binding. However, the presence of a basic amino group at C-3′ is not an essential requirement for activity of doxorubicin-related anthracyclines [5,17]. In spite of a reduced DNA-binding affinity of 3′-substituted analogs, a proper substituent at the 3′-position (e.g. hydoxyl group) confers a comparable cytotoxic and antitumor activity [5]. Relevant to this point is the observation that the substitution of the charged amino group for a more hydrophobic substituent results in compounds able to overcome typical (P-glycoprotein-mediated) multidrug resistance [5].

On the basis of such observations indicating that (a) specific interactions of the sugar moiety are essential for drug activity, and (b) the amino group can be replaced by a proper (small) substituent, a large number of 3′-substituted anthracyclines have been synthesized [15]. The activity of analogs with bulky substituents at the 3′-position is related to a mechanism of action different from topoisomerase II inhibition [5]. Indeed, the presence of a bulky substituent at the 3′-position (as in the 3′-morpholinyl or 3′-methoxymorpholinyl derivatives) completely inhibited the stimulation of the topoisomerase II-mediated DNA cleavage [5]. The 3′-position also has been exploited for the synthesis of alkylating anthracyclines. Alkylcyclines are very potent cytotoxic agents and are characterized by lack of cross-resistance with conventional anthracyclines. The presence of alkylating moieties in the 3′-position confers a distinct mechanism of action for this novel class of anthracyclines [18].

Since the presence of the sugar is an important structural requirement for bioactivity of anthracycline antibiotics, it appears evident that modifications in the amino sugar are of potential interest in modulating drug efficacy. A rational modification of the sugar is still limited by lack of precise information on sugar interactions with DNA and topoisomerase in the ternary complex [19]. The amino sugar is located in the minor groove with a sequence preference [20], which is consistent with the sequence specificity of DNA topoisomerase II-stimulated DNA cleavage [19,21]. Modifications of substituents in the fused ring system (rings B, C, and D; see Fig. 1) could change the orientation of the intercalated chromophore, thus influencing the external interactions of the sugar. Indeed, removal of the 4-methoxy group improved the drug efficacy to stabilize the cleavable complex [3]. Similarly, substitutions on the non-planar cyclohexane ring A could influence the position of the amino sugar in the minor groove [4]. Taken together, all this information supports the hypothesis that bioactivity of anthracyclines requires optimal fit of the sugar at the interface between DNA and topoisomerase, which is determined by its structure and conformation and by DNA interactions with other drug domains [22].

3. Rationale for the development of disaccharide analogs

The interest in modifying the sugar moiety is spawned by evidence that minor groove binders are characterized by sequence-recognizing properties [20,22]. In an attempt to enhance the recognition potential of the sugar in the minor groove, the 4′-position appears suitable for introduction of a bulky substituent (e.g. additional sugar residues). Several anthracycline glycosides containing a disaccharide or trisaccharide chain have been described [23,24]. A structural feature common to natural disaccharides is the presence of an amino sugar as the carbohydrate moiety directly linked to the chromophore. A second sugar residue, bound by α [1–4] glycosidic linkage, is present in the natural compound 4′-*O*-α-daunosaminyl daunorubicin and in other semisynthetic derivatives [15]. Apparently, the second sugar does not provide improvement in cytotoxic or antitumor activity [15]. A nonaminated sugar residue directly attached to the aglycone is present in an inactive disaccharide analog in which the second sugar is linked at the 3′-position [25]. The inactivity of such a compound could have been predicted on the basis of evidence that the substituent at the 3′-position is involved in optimal drug interaction in the ternary complex and in determining the sequence selectivity of anthracycline-stimulated DNA cleavage [7]. Indeed, when the 3′-

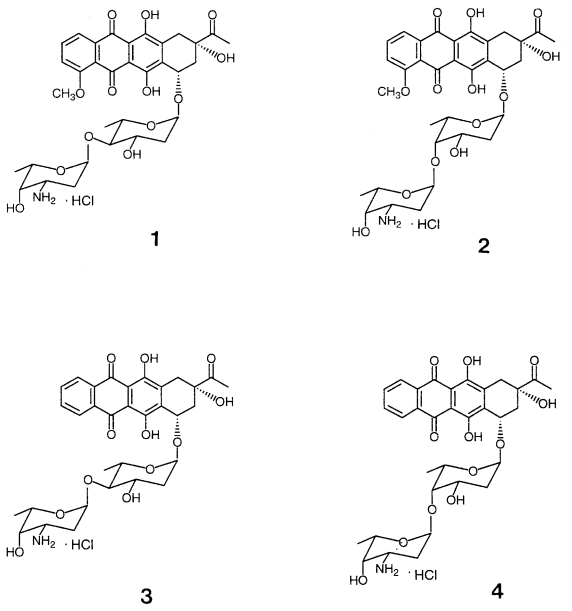

Fig. 2. Disaccharide analogs of daunorubicin (**1, 2**) and idarubicin (**3, 4**).

amino group is replaced by an hydroxyl group, the 3′-desamino-3′-hydroxy anthracycline analogs retain significant cytotoxic and antitumor activity [5,17], although the poor solubility of these compounds represents a relevant drawback for drug formulation and administration. Thus, in addition to possible advantages related to expanded recognition at the target site following introduction of the second sugar at the 4′-position, the development of disaccharide analogs is a useful strategy to overcome solubility problems as a consequence of removal of the charged amino group.

4. Influence of orientation of the second sugar on biological activity of disaccharide analogs

Detailed structure–activity studies performed with disaccharide analogs have documented striking configurational requirements of the sugar moiety for their biological activity [8,9]. Among daunorubicin analogs (Fig. 2, compounds **1** and **2**), the introduction of a second sugar residue dramatically reduces the cytotoxic potency. This effect could be ascribed, at least in part, to a reduction of intracellular drug accumulation as a consequence of increased hydrophilicity. In contrast, when the 4-methoxy group is removed (i.e. idarubicin analogs) (Fig. 2, compounds **3** and **4**), the axial configuration determined by the l-fucosyl residue confers a cytotoxic activity comparable or superior to that of natural antibiotics (doxorubicin and daunorubicin). The glycoside with the equatorial disposition was found to be substantially less effective in stimulating topoisomerase II-mediated DNA cleavage. The cytotoxic potency of the tested disaccharides closely reflects the ability of the drug to stimulate topoisomerase II-mediated DNA cleavage. The different topoisomerase poisoning activities of the disaccharide anthracyclines were not related to changes in DNA-binding ability. Thus, the mode of DNA interaction and/or the geometry of drug–DNA intercalation, rather than the DNA-binding affinity, appear to be critical factors for biochemical and pharmacological activity of anthracyclines. In particular, the dramatic influence of the orientation of the second sugar in the 4-demethoxy analogs suggests that the removal of the bulky 4-methoxy group in the intercalated ring system allows more flexible stacking of the planar aromatic system between base pairs, thus changing the fit of the sugar in the minor groove and favoring the stabilization of the ternary complex (drug–DNA–enzyme). This interpretation is consistent with the observation that idarubicin and, in general, 4-demethoxy derivatives are very potent as topoisomerase II inhibitors [5,26]. Again, these changes in topoisomerase poisoning activity could not be ascribed to DNA-binding affinity [26]. Taken together, such observations support the hypothesis that the external interactions of the non-intercalating domains of the drug are critical determinants of drug efficacy at the target level.

The importance of modifications at position 4′ of the sugar moiety of anthracyclines has already been documented in the pyrromycinone-based anthracyclines containing two or three sugar residues with the axial orientation (Fig. 3) [27]. The addition of a second sugar residue (2-deoxyfucose) at the 4′-position of the first aminated sugar of pyrromycin (i.e. musettamycin; Fig. 3, compound **6**) increases antitumor potency and inhibition of nucleolar RNA synthesis. The presence of a third sugar residue (also 2-deoxyfucose), as in marcellomycin (compound **7**), further increases the biological effects of the drug. The increase in the length of the oligosaccharide chain in this series of natural anthracyclines is correlated with an increase of binding affinity. Additionally, the nature of the terminal sugar residue significantly influences the DNA interaction. However, DNA-binding affinity of trisaccharide derivatives of the pyrromycinone series is not correlated with antitumor potency [27]. Natural glycosides of this series are potent topoisomerase I inhibitors [28]. Again, no precise correlations exist between affinity constants for DNA and ability to stimulate DNA cleavage [27,28]. Indeed, in spite of reduced DNA-binding affinity [29], rudolfomycin (containing rednosamine as the terminal sugar residue) is substantially more potent than marcellomycin as a topoisomerase I inhibitor [28]. Since the oligosaccharide chain lies in the DNA minor groove [29,30], the terminal saccharide may affect the precise interactions of the drug and/or cause structural DNA perturbations, influencing the function of the topoisomerase. Thus, as observed for idarubicin-related disaccharides as topoisomerase II inhibitors [8], the mode of DNA binding rather than the binding affinity is a critical determinant of topoisomerase I inhibition by anthracyclines.

The mechanism of action of natural anthracycline oligosaccharides (e.g. aclacinomycin A) is substantially different from that of daunorubicin- or doxorubicin-related monosaccharides, since the former could act as a topoisomerase I or

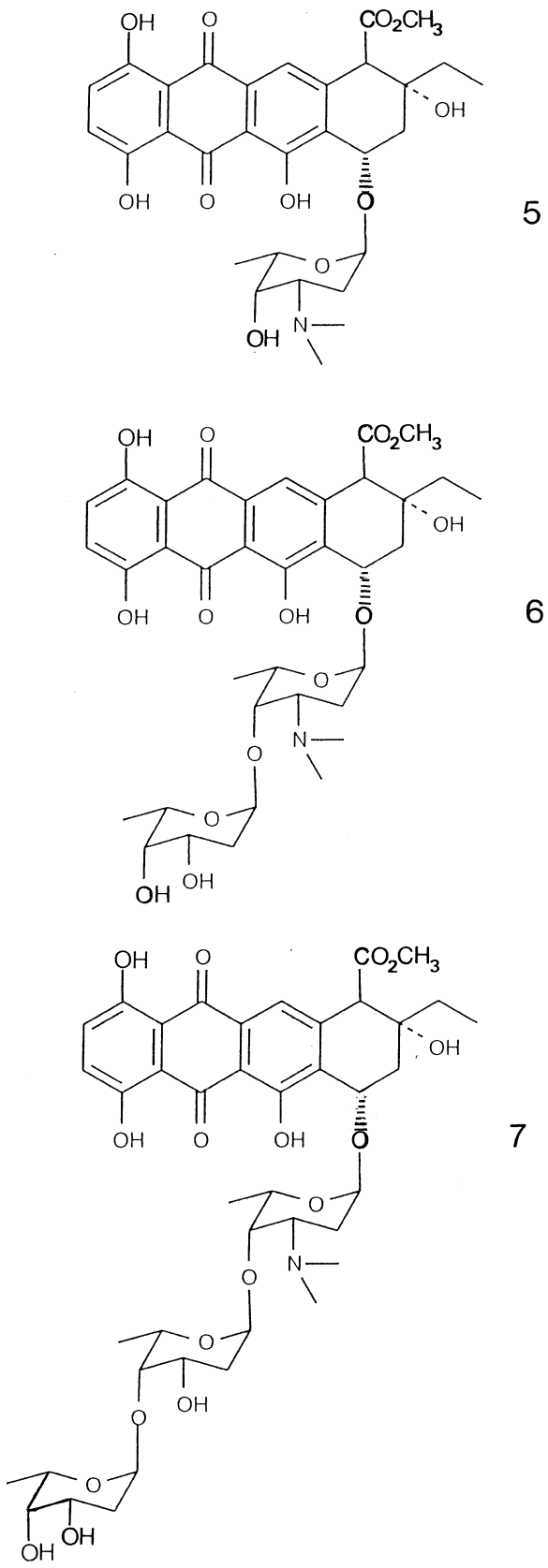

Fig. 3. Structures of natural oligodisaccharides: pyrromycin (**5**), musettamycin (**6**), and marcellomycin (**7**).

a dual topoisomerase I/II inhibitor [22,28]. Again, the ability to poison topoisomerase I has been ascribed to the saccharide moiety, which could interfere with specific enzyme functions involving the minor groove [28,31]. This interpretation is consistent with a specific inhibition of topoisomerase I by 3′-morpholinyl doxorubicin [32]. In contrast, the novel disaccharide analogs of idarubicin lacking the amino group in the first sugar were much more potent against topoisomerase II than topoisomerase I [9]. However, the idarubicin analog with the axial orientation was found to be more efficient in stabilizing topoisomerase I–DNA covalent complexes [9]. Thus, a possible contribution of topoisomerase I inhibition in the efficacy of anthracycline disaccharides should not be ruled out.

5. Conclusions

The most relevant pharmacological feature of the idarubicin disaccharides is the ability of the analog with the optimal (axial) orientation of the second sugar to significantly inhibit the growth of human solid tumor xenografts [8]. The antitumor efficacy of the disaccharide analog is comparable to that of doxorubicin. The activity of a daunorubicin-related analog against solid tumors is a somewhat unexpected feature in anthracyclines lacking the 14-hydroxy group. Indeed, the structurally related idarubicin (i.e. 4-demethoxy daunorubicin) is almost inactive against solid tumor models [8]. The molecular/cellular basis of the change in the pattern of tumor response to the novel disaccharide analog remains unknown. The evidence that optimal configuration of the disaccharide moiety in the idarubicin series confers activity in solid tumors stimulated the synthesis of 14-hydroxy analogs [10,33,34]. The effort led to the development of a doxorubicin disaccharide analog, daunosaminyl-2-deoxy-fucosyl doxorubicin (MEN 10755) [10]. The novel compound is more active than doxorubicin in human tumor models characterized by MDR-unrelated intrinsic resistance [10,11]. The improved antitumor efficacy observed with the novel analog has been tentatively related to an increased ability of the drug to induce apoptosis *in vivo* [10,35]. Indeed, MEN 10755 was more potent than doxorubicin as an inducer of apoptosis in all examined tumor models, and was more effective than doxorubicin in the treatment of *p53* mutant tumors [11]. Most of the tumors refractory to doxorubicin and responsive to MEN 10755 are characterized by overexpression of the Bcl-2 protein [11]. Although the precise role of this antiapoptotic protein in resistance to anthracyclines is still uncertain [35,36], a plausible explanation of the efficacy of MEN 10755 in Bcl-2-overexpressing tumors is the ability of the disaccharide analog to activate an apoptotic pathway, resulting in Bcl-2 inactivation. This interpretation is supported by a recent observation that response of the MX-1 breast carcinoma to effective treatment with MEN 10755 was associated with an early and marked phosphorylation of Bcl-2 [36]. This effect

was not detectable in doxorubicin-treated tumors, which were poorly drug responsive.

In spite of a reduced intracellular accumulation and a different subcellular distribution compared to doxorubicin, MEN 10755 exhibits comparable or superior cytotoxic activity [10]. This behavior at the cellular level parallels *in vivo* effects, since the superior antitumor activity could not be ascribed to drug accumulation in the tumor [37]. The DNA cleavage stimulated by the disaccharide analog appears to be longer lived than that stimulated by doxorubicin, thus supporting a more stable interaction of the disaccharide analog in the ternary complex, as we anticipated. Moreover, since the subcellular distributions of the two drugs are markedly different and the nuclear accumulation of the disaccharide analog is substantially lower than that of doxorubicin, a reduced concentration at target levels is also expected. This observation raises the possibility that additional extranuclear (i.e. topoisomerase-independent) effects may contribute to cellular response to disaccharide analogs.

In conclusion, the molecular and pharmacological features of disaccharide analogs support the interest of this novel series of anthracyclines. Optimization of the carbohydrate structure of anthracycline disaccharides, which may influence both drug–target interaction and cellular pharmacokinetics, could improve the efficacy and antitumor selectivity. This possibility is supported by the development of novel analogs of the disaccharide series.[1,2]

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