Metal Anthracycline Complexes as a New Class of Anthracycline Derivatives*

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Abstract

The anthracycline antibiotics adriamycin and daunorubicin are highly efficient antineoplastic agents; unfortunately their clinic use is limited by the development of a dose-limiting and potentially fatal cardiac toxicity.

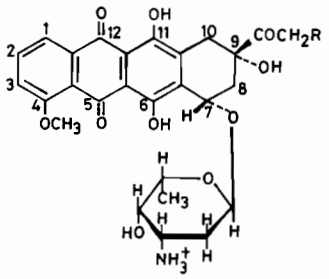
Although the mechanism of anthracycline cardiac toxicity remains not completely understood, recent studies have suggested that it may be related to the formation of semiquinone free-radical intermediates *in vivo* and it has been demonstrated that a component of mitochondrial NADH dehydrogenase actively reduces adriamycin. Thus, the hope of finding a non-cardiotoxic but yet active anthracycline antibiotic has prompted the development of a large number of semisynthetic analogs.

We have thus proposed the complexation of anthracycline with metal ions as a possible route to new derivatives. We have synthesized complexes of adriamycin, daunorubicin and carminomycin with Fe(II) and Pd(II). The stability constants of these complexes are very high and no release of metal ion is observed when they are injected in the plasma.

All these complexes (i) display antitumor activity against P-388 that compares with that of the free drug, (ii) unlike the free drug, they do not catalyze the flow of electrons from NADH to oxygen through NADH dehydrogenase, (iii) they do not exhibit cardiotoxicity when they are tested on cardiac cells in culture.

Introduction

The anthracycline antibiotics adriamycin (Adr), daunorubicin (Dr) and carminomycin (Cm) are highly efficient antineoplastic agents (Scheme 1). However, a major limitation to their use includes their acute and chronic toxicities. The chronic total dose-limiting toxicity is cardiotoxicity, which limits the duration of remissions in some situations [1]. Although the mechanism of anthracycline cardiac toxicity remains not completely understood, recent studies have suggested that the cytotoxic effects of these agents may be related to the formation of semiquinone free-radicals *in vivo* [2]. Both cardiac sarcosomes [3] and mitochondria [1, 3] can reduce Adr and Dr to their respective semiquinones, initiating a free-radical cascade.



Daunorubicin (Dr), R = H
Adriamycin (Adr), R = OH

Scheme 1.

The hope of finding a non-cardiotoxic yet active anthracycline antibiotic has prompted the search for new naturally occurring anthracyclines and the development of a large number of semisynthetic analogs. Thus, varying the number and the position of the hydroxyl groups on the aglycone moiety appears to greatly modify the redox chemistry of these compounds [4]. This modification of the redox properties is an important point, since recent investigations of anthracycline-induced cardiotoxicity have focused on the ability of the drug to be reduced by components of the NADH dehydrogenase system [1, 3].

The complexation of anthracycline by metal ions appears to be a route to get new compounds modified simultaneously at the quinone, the hydroxyl groups, and even in some cases at the amino sugar moiety. We thus set out to prepare metal anthracycline complexes exhibiting stability constants high enough for the complexes to reach the target without releasing the metal and of course exhibiting cytotoxicity that compares at least with that of the free drug. The study of the interactions of Adr, Dr and Cm with Pd(II) and Fe(III) ions has been undertaken in this context.

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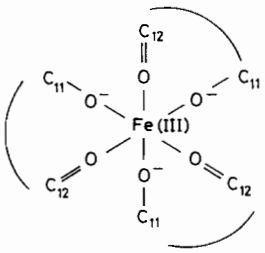
Results and Discussion

Adriamycin–Fe(III), Daunorubicin–Fe(III) and Carminomycin–Fe(III) Systems

Using potentiometric and spectroscopic measurements (absorption and circular dichroism (CD)) we have shown that Adr, Dr and Cm form three well-defined species in which three molecules of drug are chelated to one Fe(III) ion. This occurs with the release of one proton per molecule of drug. Magnetic susceptibility measurements indicate that the complexes thus obtained are in a high-spin form, suggesting that six oxygen atoms are bound to iron [5, 6]. The observations that 11-deoxyadriamycin [7] and aclacinomycin (which also lacks the hydroxyl group at C_{11}) are unable to complex strongly Fe(III) suggest that Fe(III) binds to anthracycline in a site involving the C_{12}-carbonyl and the C_{11}-phenolate oxygen forming a six-membered chelate ring. We thus proposed the following structure (Scheme 2).



Scheme 2.

The stability constants are defined by the following equilibria

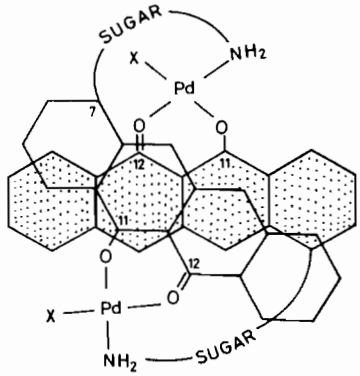
$$Fe^{3+} + 3HD \rightleftharpoons Fe(HD)_3$$

$$\beta_1 = \frac{[Fe(HD)_3]}{[Fe^{3+}][HD]^3}$$

where HD stands either for Adr, Dr or Cm deprotonated at the C_{11}-hydroxyl group. Taking into account that the pK value of this phenolic deprotonation is 10 [8] for Adr and Dr and 10.8 for Cm [6], we obtained $\beta_1 = 6 \times 10^{31}$ for $Fe(HAdr)_3$ and $Fe(HDr)_3$, and $\beta_1 = 8 \times 10^{33}$ for $Fe(HCm)_3$.

Adriamycin–Pd(II) and Daunorubicin–Pd(II) Systems

Adr forms a complex with Pd(II) in which two Pd(II) ions are bound to two molecules of drug (A_1 and A_2). One Pd(II) is bound to the oxygen on the carbons C_{11} and C_{12} of molecule A_1 and by the amino sugar of molecule A_2, whereas the second Pd(II) is bound to the oxygen on C_{11} and C_{12} of molecule A_2 and to the amino sugar of molecule A_1 (Scheme 3). The same complex is formed between



Scheme 3.

Pd(II) and daunorubicin [9]. The formation constant β is defined by the following equilibrium:

$$Pd^{2+} + HAd{-}NH_2 \rightleftharpoons (1/2)(Pd{-}HAd{-}NH_2)_2$$

$$\beta = \frac{[(Pd{-}HAd{-}NH_2)_2]^{1/2}}{[Pd^{2+}][HAd{-}NH_2]}$$

where $HAd{-}NH_2$ stands for adriamycin deprotonated at the C_{11}-hydroxyl group (pK = 10) and at the amino sugar (p$K \cong 8.6$). The stability constant thus obtained is $\beta = 1 \times 10^{22}$.

Complexes–Plasma Interaction

The first barrier encountered by the drug being plasma, it is an important point to determine the behavior of the complexes in face of the various components of plasma. The different complexes were added to plasma; no spectral modifications were observed even after several hours of incubation.

Antitumor Activity

The *in vitro* inhibition of P388 leukemia cell growth by the complexes was compared with that induced by the free drugs. They all displayed antitumor activity with $T/C \times 100 \cong 160$ for a dose of 5 mg/kg. No significant differences, in terms of therapeutic efficacy and general toxicity, were observed between the complexes and the free drugs.

Effect of the Complexes on Superoxide Production by NADH

To examine the role of oxygen radical metabolism in anthracycline-induced mitochondrial injury, we investigated the effect of the anthracycline metal complexes on superoxide anion formation by mitochondrial NADH dehydrogenase. This effect was compared with that of the free drugs. Adr, Dr and Cm increased superoxide formation by NADH dehydrogenase in a dose-dependent fashion that appeared to follow saturation kinetics. $Fe(HAd)_3$, $Fe(HCm)_3$, $(Pd{-}HAd{-}NH_2)_2$ and $(Pd{-}HDr{-}NH_2)_2$

did not increase superoxide formation over control levels. These data show that, although the flow of electrons from NADH to molecular oxygen through NADH dehydrogenase is enhanced significantly by the free drugs, it does not occur with the complexes.

Complexes–DNA Interaction

Since DNA has been postulated as one of the sites of action of anthracycline *in vivo*, we have examined the interaction of the complexes with DNA. The experiments were carried out under conditions for total binding of the free drug to DNA, *i.e.*, at a nucleotide-to-drug molar ratio higher than 7 [10]. The interaction between the iron complex and DNA is time dependent. From CD data we can state that after several days of incubation the free drug is intercalated between the base pairs after the release of Fe(III) from $Fe(HD)_3$. This process is most probably facilitated by the presence of DNA phosphate groups which will bind to ferric ion. On the contrary, almost no release of Pd(II) by the complex is observed even after 5 days of incubation.

Conclusions

The data reported in this paper appear to establish that Adr, Dr and Cm can bind Fe(III) and Pd(II) very strongly. Concerning the points that may be connected with cardiac toxicity, our data show that the complexes (unlike the free drugs) do not catalyze the flow of electrons from NADH to molecular oxygen through NADH dehydrogenase. This result could appear to be an improvement of the therapeutic index of anthracyclines. We can say that the complexation of anthracyclines by metal ions appears to be a route to obtain new compounds modified simultaneously at the quinone, at the hydroxyl groups, and even (in the case of Pd(II)) at the amino sugar moiety. This yields modifications of the redox properties and of the biological properties of these potent antitumor antibiotics.

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