review articles

Anthracycline antitumour agents

A review of physicochemical, analytical and stability properties

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Introduction

The anthracycline antitumour antibiotics daunorubicin (Cerubidine®) and doxorubicin (adriamycin, Adriblastina®), originating from Streptomyces peuceticus and the mutant strain Streptomyces peuceticus var. caesius, respectively, are considered to be the most effective antineoplastic agents used in current cancer chemotherapy. The structures of these compounds were elucidated by Arcamone et al. (Fig. 1)1-3 and the absolute stereochemical configurations were established later.4,5

Doxorubicin has a broad spectrum of activity and has established outstanding antitumour activities.6-20 The clinical applicability of daunorubicin is restricted to certain haematological malignancies in which it is as active as doxorubicin but lower in cost.12 Maximal effectiveness of anthracycline therapy is restricted mainly by drug induced dose limiting cardiotoxicity, caused by effects on cardiac mitochondria.12 Therefore, the total cumulative dose of doxorubicin should not exceed 450-550 mg/m².6,7,12 Many studies concerning the cardiotoxic properties of the anthracyclines have been conducted.79,16-32

Alternative dosage schedules,33-36 pre- or co-administration of radical scavengers9,32,40-46 and encapsulation of doxorubicin in liposomes47-55 have been proposed as a way to cope with the problem of cardiotoxicity. Furthermore, the toxic effects have urged the search for new, natural or semisynthetic derivatives with possibly reduced cardiotoxic properties.6,56-60 Among this group 4'-epidoxorubicin, 4-demethoxydaunorubicin, carminomycin and aclacinomycin A (Fig. 1 and 2) are promising members.61-64 These and other anthracycline cytostatics are now subjects of extensive research.65-89

The mechanism of the antineoplastic action of the anthracyclines is still uncertain, although an interaction with DNA, except for N-trifluoroacetyldoxorubicin-14-valerate (AD 32), is well recognized.6,12,18,90-98 Other mechanisms are also involved. This is clearly demonstrated by the observation that AD 32 does not bind to DNA,6 but still possesses profound antitumour activity. Other mechanisms of action are related to the generation of reactive oxygen species (superoxide, hydroxyl radicals, hydrogen peroxide).9,91-111 The reactive oxygen species may cause DNA strand scission, peroxidation of lipids and even cardiotoxicity. Bioreductive alklation of DNA by anthracyclines has also been proposed.99,112 Furthermore, it seems that anthracyclines, immobilized by polymer linkage, can be actively cytotoxic merely by interaction at the cell membrane.99,113,114 The observed complex formation of doxorubicin with negatively charged phospholipids115 also points in the direction of an interaction at the cell membrane level.

One of the reasons of treatment failure is the development of resistance to the chemotherapeutics, which may be the result of an enhanced active cellular drug efflux gaining importance over passive influx.12

The serious side effects observed in anthracycline therapy emphasize the need for an individual approach to the chemotherapy based on levels of the

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Key words

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Abstract

A review of physicochemical and analytical properties of anthracycline antitumour agents is presented. The following subjects are discussed: protolytic equilibria, partition and partition coefficients, self-association, adsorptive properties, metal complexation, spectroscopy and chromatography. Furthermore, the stability of anthracyclines in solutions, in pharmaceutical preparations and in biological media is discussed.
drug and metabolites in biological fluids, which requires the availability of sophisticated analytical methods, especially in combination therapy. In the quality control of anthracycline preparations the applied analytical method should be suitable for the assessment of the extent of degradation especially with respect to the aglycone fraction as these compounds are devoid of antitumour activity. Employment of the optimal analytical technique requires knowledge about the analytical and physicochemical properties of the anthracyclines, as improper handling of samples and the use of a non-optimal analytical technique may result in misinterpretations of experimental results. Familiarity with these properties (e.g. adsorption onto glass and stability during long-term infusions) is also essential for biochemical studies and for therapy.

Due to their tremendous clinical importance the anthracyclines have been the subject of many reviews. This review differs from the others, since it is especially focused on physicochemical and analytical properties of anthracycline cytostatics. Knowledge concerning these aspects is a prerequisite for successful research with these drugs.

**Protolytic equilibria**

The chemical and pharmacological properties of the anthracycline cytostatics strongly depend on the nature of the substituents of the compounds. Doxorubicin, as well as the related drugs, contains several prototropic functions, such as the amino group in the sugar moiety and two phenolic functions in the aglycone part of the molecule, whereas in concentrated acid one of the quinone carbonyl groups can accept a proton. Schemes provided by Sturgeon and Schulman and by Kiraly and Martin enable us to calculate both the microconstants for each of the individual protolytic reactions as well as the overall pKa values of the various overlapping groups (Table 1). The deprotonation scheme is depicted in Figure 3.

Although the assumption of interchangeability of the microconstants for the phenolic groups is questionable and evidence exists for an interaction of the aminosugar with the quinoidal moiety, these results (Table 1) are in reasonable agreement with each other. In the literature, however, a number of different pKa values are stated, mostly assigned to the amino function and the phenolic group at C11. The pKa(NH2) varies from 7.2 or 7.4 to 8.99, while the pKa(OH) varies from 8.53 to 10.16, apparently due to different methods of determination as well as different concentrations. Several authors noticed that the pKa values of the anthracyclines are dependent on the degree of self-association of the compounds.

Configurational changes in the daunosamine moiety cause changes in the pKa(NH2), as shown by DiMarco et al. and by Tanaka et al., while also the number of hydroxyl groups in the sugar moiety influences the pKa(NH2). Both DiMarco et al. and Siegried et al. showed that the reduction of the

**FIGURE 1**
Structures of most important anthracyclines

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**FIGURE 2**
Structure of aclacinomycin A