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Pharmacology and Chemotherapy

VOLUME 15

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Daunomycin: An Anthracycline Antibiotic Effective in Acute Leukemia

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I. Introduction

The Division of Cancer Treatment (DCT) of the National Cancer Institute (NCI) in its drug development function sponsors clinical trials with investigational anticancer drugs. These drugs are gathered from various sources, with about two-thirds coming from the DCT drug-screening program and about one-third from a cooperative drug exchange program conducted throughout the world. Among the interesting anticancer drugs developed abroad is daunomycin, an antitumor antibiotic of the anthracycline group. The drug was isolated in 1963, and in the last 13 years a large amount of preclinical and clinical data has accumulated. This information requires an updated analysis, particularly in light of the fact the drug has shown a substantial amount of activity in acute leukemia refractory to other antileukemic drugs.

II. Chemical and Pharmaceutical Aspects

A. Description

In 1961, Arcamone and his colleagues studied substances isolated from *Streptomyces* cultures that had been obtained from a soil sample from India. These substances had a marked influence on Ehrlich ascites tumors and Sarcoma 180 tumors in mice (Arcamone *et al.*, 1961).

Daunomycin is an antitumor antibiotic of the anthracycline group, which was isolated in the Farmitalia Research Laboratories in Milan, Italy, from cultures of *Streptomyces peucetius* by Grein *et al.* in 1963. The major part of the drug was found in the culture media of the mycelium and was isolated by means of solvent extraction, cation resins, and paper or column chromatography. The yield was 5–15 mg of product per liter of culture media. The soil sample was from Castel del Monte, near Puglie, and hence the name of the species *S. peucetius*.

The drug is classified as an anthracycline and has similar physicochemical characteristics to rhodomycins, cynerubines, pyrromicins, and rutilantines (DiMarco *et al.*, 1964b; Cassinelli and Orezzi, 1963). Other

names for the drug include daunorubicin HCl, rubidomycin, rubomycin C, Cerubidine, and NSC 82151.

Daunomycin consists of a pigmented aglycone (daunomycinone) in glycoside linkage with an amino sugar (daunosamine) (DiMarco *et al.*, 1964b); the structure is depicted in Fig. 1. Total synthesis of daunomycin was reported by Acton *et al.* in 1974.

B. Chemical Properties and Structure-Activity Relationship

Chemical properties necessary for identifying daunomycin are listed in Table I.

The structure–activity relationship of daunomycin has been commented on by Zee-Cheng and Cheng (1970). They found a common structural feature was shared by some nonalkylating antileukemic agents such as aminopterin, anthramycin, 5-azacytidine, camptothecin, cytosine arabinoside, emetine, demecolcine, riboside, methotrexate, streptonigrin, vinblastine, vincristine, and daunomycin. This structural feature is a triangulation between 1 nitrogen and 2 oxygen atoms with rather definite interatomic distances. For daunomycin, the N—O—O triangulation pattern is shown in Fig. 2 with the following interatomic distances:

$$N—O_1 = 7.05–10.20\ \text{Å}$$

$$N—O_2 = 8.36–11.40\ \text{Å}$$

$$O_1—O_2 = 2.70\ \text{Å}$$

The relationship of this triangulation pattern and antileukemic activity is substantiated by reports that neither the aglycone of daunomycin alone

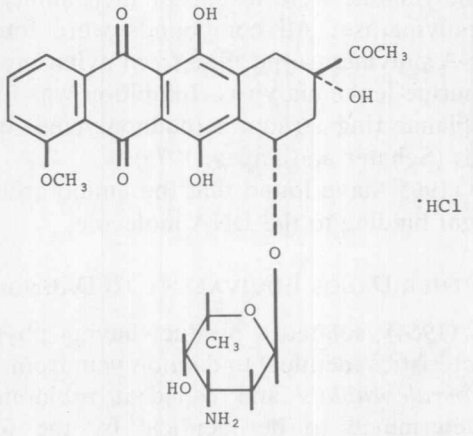

FIG. 1. The structure of daunomycin.

TABLE I

CHEMICAL PROPERTIES OF DAUNOMYCIN

Description
Red powder or thin red needles
Melting point, 188°–190°C
Molecular weight = 563.99 (anhydrous)
The pk_a equals 10.3. The color in aqueous solution varies according to pH of solution: pink at acid pH and blue at alkaline pH.
Elemental composition
Carbon, 57.5
Hydrogen, 5.36
Chlorine, 6.29
Oxygen, 28.4
Specific rotation
$[\alpha]_D$ = 253° (reproducibility difficult due to intense color of solution)
Solubility
In water = 450 mg/ml
Also soluble in methanol
Insoluble in chloroform, ether, and benzene
Thin-layer chromatography
Absorbent—SiO_2
Solvent system—*sec*-butanol/pyridine/water (1/1/1)
Detection—visible, UV

(daunomycinone) nor the amino sugar (daunosamine) possesses antileukemic activity.

Other portions of the molecule of daunomycin have been thought to be important for antitumor activity. Daunomycin, adriamycin, rubidazone, and daunomycinone were tested for their ability to inhibit oncornavirus DNA polymerase. All compounds were found to be potent inhibitors of DNA polymerase purified from avian myeloblastosis virus and Rauscher murine leukemia virus. Inhibition was found to be due to the tetracyclic planar ring structure (daunomycine) common to all of these compounds (Schafer and Papas, 1975).

Calendi *et al.* (1965) have found that the amino group of the sugar is necessary for tight binding to the DNA molecule.

C. OTHER DRUGS EQUIVALENT TO DAUNOMYCIN

Dubost *et al.* (1964) isolated a product having physicochemical and analytical characteristics identical to daunomycin from a culture broth of *Streptomyces coeruleorubidis* and called it rubidomycin. The compounds were determined to be identical by the following studies: elemental analysis; melting point; thin-layer chromatography; ultraviolet

spectrum; infrared spectrum; nuclear magnetic resonance; benzoate derivatives of both drugs; and X-ray diffraction patterns.

Rubomycin was produced from *S. coeruleorubidus* and was isolated in the Soviet Union. The drug represents a complex of two chemically related variants, namely, rubomycin B and rubomycin C. Rubomycin C is identical to daunomycin but rubomycin B is different from daunomycin (Gause, 1966).

D. Pharmaceutical Data

Daunomycin is commercially available in Europe but in the United States is available only as an investigational agent through the Investigational Drug Branch of the National Cancer Institute. The product is supplied as a lyophilized cake in vials containing 21.4 mg of daunomycin hydrochloride equivalent to 20 mg of daunomycin base. In addition, the vials also contain 100 mg of mannitol. The intact vials should be stored at room temperature (22°C) protected from light. When reconstituted with 4 ml of sodium chloride for injection, USP, each milliliter of solution contains 5 mg of daunomycin base.

Intact vials are stable for at least 3 years at room temperature (22°C). The reconstituted drug should be used within 8 hours or discarded since the lyophilized cake contains no preservatives.

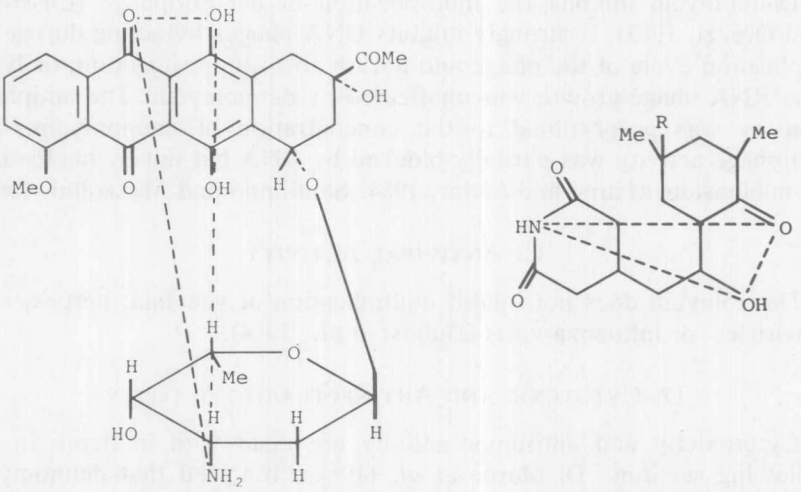

FIG. 2. Triangulation pattern of daunomycin. (From Zee-Cheng and Cheng, 1970, American Pharmaceutical Association. Adapted by permission of the copyright owner.)

III. Biological Properties

Daunomycin possesses an impressive number of biological effects.

A. Antibacterial Activity

Sanfilippo and Mazzoleni (1964) found that *Escherichia coli* Type B was sensitive to daunomycin but *E. coli* K12 was much less sensitive. They ascribed the differences in sensitivities to possible differences in cellular penetration of the daunomycin. Parisi and Soller (1964) found that daunomycin had no effect on growth of *E. coli* B or *E. coli* K12 in concentrations up to 80 μg/ml. When concentrations of up to 300 μg/ml of daunomycin were used, there was a lengthening of the division time to one-half more than normal. The drug has been found to be bacteriostatic against gram-positive organisms especially *Staphylococcus aureus* (Sanfilippo and Mazzoleni, 1964).

Pruzanski and Saito (1974) noted that daunomycin mixed with normal human or leukemic serum did not influence *in vitro* or *in vivo* levels of serum immunoglobulins, lysozyme, or total hemolytic complement. At low doses of daunomycin, there was slight inhibition of bacteriolytic activity of normal sera against *E. coli*. With high doses of daunomycin, there was enhancement of the bacteriolytic activity of normal serum.

B. Antibacteriophage Activity

Daunomycin inhibits the multiplication of bacteriophage (Cassinelli and Orezzi, 1963). It strongly inhibits DNA phages by acting during the replication cycle of the phage and not on absorption, injection, or lysis. The RNA phage growth was unaffected by daunomycin. The antiphage activity was proportional to the concentration of daunomycin. The antiphage activity was partially blocked by DNA but not by nucleotides or nucleosides (Parisi and Soller, 1964; Sanfilippo and Mazzolini, 1964).

C. Antiviral Activity

Daunomycin does not inhibit multiplication of vaccinia, herpes, adenoviruses, or influenza virus (Dubost *et al.*, 1964).

D. Cytotoxic and Antineoplastic Activity

Cytotoxicity and antitumor activity are dealt with in depth in the following sections. Di Marco *et al.* (1964a) reported that daunomycin had marked inhibitory activity on Ehrlich ascites and Sarcoma 180 ascites tumors in mice and also on hepatoma AH130 and Walker

carcinoma (see Section V). Rat fibroblast and HeLa and KB cell monolayers in cell cultures treated with daunomycin had marked alterations in the nucleus, nucleoli, and chromosomes with dramatic growth inhibition at 1 μg/ml. The antibiotic also stopped the mitotic activity in cells that have completed the DNA replication cycle and were in G_2 phase (Di Marco *et al.*, 1967).

E. Teratogenic Effect

Daunomycin has been teratogenic in chick embryos treated on the fourth day of incubation but has not been teratogenic in mice or rabbits. The type of malformations in the chick were largely joint deformities, syndactyly, and torsions (Dubost *et al.*, 1964).

F. Immunosuppressive Effect

Gericke and Chandra (1973) found that at low doses, daunomycin stimulates immune responses, with increases in plaque-forming cells in mouse spleen in animals sensitized with sheep red blood cells (SRBC) as well as increases in hemagglutinins to SRBC. At higher doses, there is distinct immunosuppression with the opposite effects in these two systems.

Umezawa *et al.* (1972) noted that timing of SRBC antigen administration was an important factor. If antigen was given 24 hours after daunomycin administration, the number of plaque-forming cells and the hemagglutination titers to SRBC increased, meaning increased immune response. If the antigen was given 24 hours before daunomycin administration, the number of plaque-forming cells decreased, indicating immunosuppression but the hemagglutination titers to SRBC remained the same.

The possibility has been considered that adriamycin is less immunosuppressive than daunomycin and that this difference is in part responsible for the greater therapeutic effects of adriamycin in certain transplantable tumor systems. The immunosuppressive effects of adriamycin and daunomycin were compared using complement-dependent and independent cellular toxicity as an *in vitro* assay of immunity. No significant differences in the effects of adriamycin and daunomycin were seen in that test system (Orsini and Mihich, 1975).

Schwartz (1974) stated that in P388 leukemia in mice, adriamycin has a greater antitumor activity than daunomycin. This therapeutic advantage is lost when the host is pretreated with whole-body radiotherapy. This result suggests that daunomycin is more immunosuppressive than adriamycin.