Cancer Chemother Pharmacol (1983) 11:91–93

Cancer Chemotherapy and Pharmacology

Prevention of adriamycin toxicity

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Summary. *Mice treated with lethal doses of adriamycin (A1) (IP) are rescued with a single IP dose of 3,5,5-trimethyl-2-morpholinon-3-yl radical dimer (TM3). The in vivo rescue is assumed to be analogous to the in vitro reaction of TM3 with A1 that produces the non-toxic 7-deoxy-adriamycinone (7dAone). TM3 prevents death if given within 60 min following A1 administration. Control A1-treated mice died by 8 days (median survival time) whereas TM3 rescued A1-treated mice had a median survival time of greater than 60 days.*

Introduction

Adriamycin (A1) is an antitumor anthracycline antibiotic isolated from *Streptomyces peucetius* [15]. It has shown a wide spectrum of activity against a variety of human and animal tumors and is considered the most effective chemotherapeutic agent at the present time for the treatment of human breast cancer [5]. Like most anticancer drugs, A1 has a number of toxicities which limit its use in treatment of cancer. These toxicities include myelosuppression and gastrointestinal effects as well as an unusual myocardial toxicity. The biochemical mechanism responsible for cardiac damage is not clearly understood but is thought to be related to the formation of free radicals involving the quinone nucleus of A1, molecular oxygen, and the myocardial cell's own metabolic capacity in concert with NADPH [1, 2, 14]. Experiments have shown the production of superoxide ion and other reactive oxygen metabolites in tissue exposed to quinoid anti-cancer drugs, supporting this hypothesis [6–8, 10].

Attempts to alleviate or prevent A1 toxicity have centered around this free radical model and some experimental success has been achieved [12–14, 16]. For example, it has been reported that pretreatment with the radical scavenger *d*-α-tocopherol, vitamin E, reduces myocardial damage to mice subsequently treated with A1 [12–14, 16]. Other approaches have involved slight changes in the basic structure of the anthracycline molecule, with some favorable decrease in myocardial toxicity but with lessened therapeutic efficacy as well [17, 18].

We dedicate this paper to the memory of Dr. Solomon Garb of the AMC Cancer Research Center and Hospital

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We have observed (Fig. 1) that 3,5,5-trimethyl-2-morpholinon-3-yl radical dimer (TM3) reacts with A1 in vitro almost instantaneously to produce the insoluble and pharmacologically inactive 7dAone [3]. It is our belief that the use of such a specifically reactive reagent may be useful to eliminate A1 in vivo as a rescue maneuver. From this viewpoint we initiated the in vivo testing of TM3.

Materials and methods

Mice used were C57 black × DBA/2F$_1$ hybrid females, between 13 and 15 weeks old, having a mean weight of 15.4 g. All injections were IP.

Doxorubicin HCl (10 mg) and lactose (50 mg) (Adriamycin, Adria Labs) was dissolved in 5 ml of 0.9% sterile saline to a final concentration of 2 mg/ml A1.

3,5,5-Trimethyl-2-morpholinon-3-yl radical dimer (TM3) was dissolved in dimethyl sulfoxide (DMSO, 100%, Sigma Chemicals, St. Louis, USA) to a concentration of 20 mg/ml.

TM3 was prepared by the method of Koch et al. [9], which consists of a photodimerization of 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one, in turn prepared from 2-amino-2-methylpropanol and ethyl pyruvate.

Mice were given a single 25 mg/kg dose of A1, sufficient to cause death to half of them within 8 days. Randomized groups of mice were then given a 'rescue dose' (100 mg/kg) of TM3 at varying intervals after the initial injection of A1.

Results

Mice given A1 alone (group 3) had a median survival time of 8 days (Table 1). Mice given the TM3 injection within 60 min of the A1 dose (groups 4–6) were afforded significant protection from toxicity, having a median survival time of greater than 60 days. Mice treated with TM3 after an interval of 24 h were not protected and had a median survival of 9 days (group 7). When mice were given DMSO 15 min after A1 injection (group 9) the toxicity appeared to be worse than in the controls (group 3); however, the difference between the median survival times of these two groups was not significant. Mice treated with TM3 at the 100 mg/kg dose in DMSO showed no toxic effects (group 8). The reaction products of TM3, pyruvic acid, and 2-amino-2-methylpropanol, are also non-toxic (data not shown).

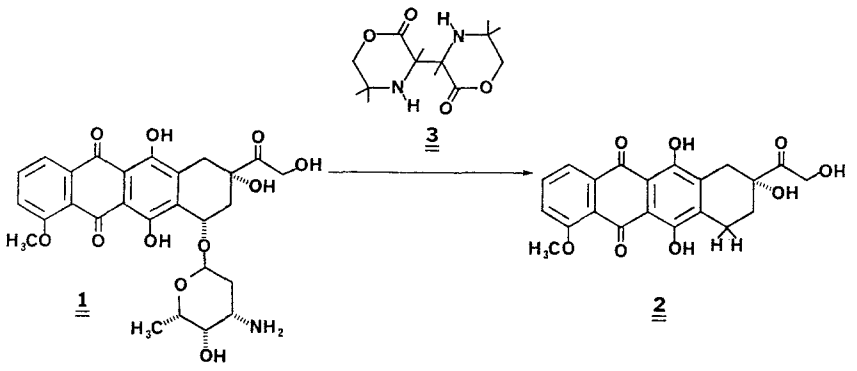

Fig. 1. In vitro reaction of A1 (*1*) and TM3 (*3*) to produce 7 dAone (*2*)

Table 1. Survival of mice in the various experimental groups

Group no.	Treatment IP	No. of mice	Time[c]	Median survival time	*P* Value[d]
1	Saline	15	–	60 days	0.005
2	DMSO	15	–	60 days	0.05
3	A1[a]	15	–	8 days	–
4	A1 + TM3[b]	15	15 min	60 days	0.05
5	A1 + TM3	15	30 min	60 days	0.05
6	A1 + TM3	15	60 min	60 days	0.01
7	A1 + TM3	15	24 h	9 days	NS
8	Saline + TM3	15	15 min	60 days	0.005
9	A1 + DMSO	15	15 min	4.5 days	NS

[a] A1 dose was 25 mg/kg in 0.2 ml saline
[b] TM3 dose was 100 mg/kg in 0.1 ml of DMSO
[c] TM3 (or DMSO) was administered after A1 injection
[d] Statistical significance compared with group 3, using Wilcoxon rank sum analysis

Discussion

Attempts to alleviate or diminish the toxic effects of A1 have dealt principally with the reduction of the radical by-products of A1 [12–14, 16]. Our approach may eliminate the source of the free radicals, i.e., excess A1. Work in our laboratories has shown that TM3 reacts directly with A1 to form the pharmacologically inactive 7-dAone. This reaction is irreversible and very rapid [3]. Previous work has shown that tumor tissue in mice reaches a maximum, dose-dependent level of A1 within 5 min of administration [11]. Subsequent treatment with TM3 may destroy available A1 in the animal. TM3 may also scavenge any free radicals which may have been formed [3]. Death is prevented in mice treated with high doses of A1, provided TM3 is administered within 60 min of the A1 dosing; and the animals survive to at least 60 days.

It is not yet known whether TM3 crosses cell membranes; and thus we do not know whether TM3 interferes with the action of A1 once the antibiotic has entered the tumor cells. Preliminary results with tumor-bearing animals suggest that there is no interference with A1 antitumor activity, and high-dose A1 treatment with rescue is currently being evaluated in a number of animal tumor models. These and other aspects of TM3 pharmacology must await further investigation.

Acknowledgements. This investigation was supported in part by USPHS grant no CA 24665 awarded to THK by the National Cancer Institute, Department of Health and Human Services.

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Received November 3, 1982/Accepted May 4, 1983