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Naphthazarin Derivatives (VII): Antitumor Action against ICR Mice Bearing Ascitic S-180 Cells

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Various analogues of 5,8-dimethoxy-1,4-naphthoquinone (DMNQ) such as 2- or 6-(1-hydroxyiminoalkyl)-DMNQs were prepared and evaluated for the antitumor action. (1-Hydroxyiminoalkyl)-DMNQ derivatives expressed greater antitumor action than (1-hydroxyalkyl)- or acyl-DMNQ derivatives. Moreover, 6-(1-hydroxyiminoalkyl)-DMNQ derivatives expressed higher antitumor action than 2-sudstituted ones, suggestive of a steric effect. Some of 6-(1-propyloxyalkyl)-DMNQ derivatives with an alkyl group of butyl to octyl moiety showed T/C values of >400%

Key words: S-180 tumor, Antitumor activity, Structure-activity relationship

INTRODUCTION

Naphthazarin (DHNQ, 5,8-dihydroxy-1,4-naphthoquinone) is an important structural component in various antitumor agents such as adriamycin analogues and mitoxantrone. It is also the main pharmacophore of shikonin and its analogues (Sankawa *et al.*, 1977; Kim *et al.*, 1990; Papageorgiou *et al.*, 1999). Utilizing 5,8-dimethoxy-1,4-naphthoquinone (DMNQ), a dimethylated product of naphthazarin, as a lead compound, we synthesized 2-(1-hydroxyalkyl)- or 6-(1-hydroxylalkyl)-DMNQ derivatives, which exhibited great potency in the inhibition of DNA topoisomerase-I (TOPO-I) and the cytotoxic activity against L1210 cells (You *et al.*, 1998b). In comparison, it was found that 6-substituted DMNQ derivatives were more active than 2-substituted ones, indicating that a steric effect may be responsible for the difference of the bioactivities (You *et al.*, 1998b; Baik *et al.*, 1997; Song *et al.*, 1999a). In addition, it was found that 2-or 6-acyl-DMNQ derivatives were more potent than 2- or 6-(1-hydroxyalkyl)-DMNQ derivatives in cytotoxicity, presumably due to the enhanced electrophilicity in the quinonoid moiety of the former anaologues (Song *et al.*, 2000b). Further, in an attempt to improve the solubility of DMNQ derivatives in aqueous system, they were transformed to oximes, 2- or 6-(1-hydroxyiminoalkyl)-DMNQ derivatives. However, *in vitro* test, the oximes did not show any improvement of bioactivities in comparison with the acyl DMNQ derivatives (Song *et al.*, 2000a).

In the present study, we assessed the antitumor action of various DMNQ derivatives including 2- or 6-(1-hydroxyiminoalkyl)-DMNQ derivatives in mice bearing S-180 cells in peritoneal cavity, and the structure-antitumor activity was disscussed.

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MATERIALS AND METHODS

Animals and cells

Male ICR mice were purchased from Daehan Laboratory Animal Co. (Korea) and used when they weighed from 18 to 22 grams. The mice were acclimated for at least four days to the animal facilities, which were maintained at 23 ± 1°C and 12 h cycle of light/dark. Feed and water were freely accessible to the mice. S-180 cells were kindly provided by Dr. J. O. Lee, Korea Research Institute of Chemical Technology.

Materials

Naphthazarin derivatives were prepared in our previous experiments (Song *et al.*, 2000a, Song *et al.*, 2000b) and used without further purification. All other chemicals not indicated were purchased from Sigma Chemical Co. (St. Louis, MO).

Antitumor activity in ICR mice bearing Sarcoma 180 cells

The following procedure was due to the protocol of National Cancer Institute USA, 1972. The test sample dissolved in a predetermined amount of 50% PEG200

were stored 4°C. Sarcoma 180 cells (0.1 ml per mouse) suspended in saline (1×10^7 cells/ml) were inoculated intraperitoneally to male ICR mice (National Cancer Institute, 1972). 24 h after the transplantation, mice were divided so that each group contains 8 mice. The sample was administered into the intraperitoneal cavity of the mouse daily for 7 days. The rate of growth inhibition (T/C, %) was calculated by following equation;

$$T/C(\%) = \frac{\text{Average survival period in the test group}}{\text{Average suvivial period in the control group}} \times 100$$

RESULTS AND DISCUSSION

2- or 6-Acyl-5,8-dimethoxy-1,4-naphthoquinones

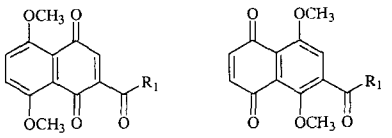
Earlier studies indicated that in the inhibition of TOPO-I and the cytotoxicity against L1210 cells, 2- or 6-acyl-DMNQ isomers were more potent than 2- or 6-(1-hydroxyalkyl)-DMNQ isomers, and 6-acyl DMNQ derivatives were more active than 2-acyl derivatives (Song *et al.*, 2000b). From these results, it was supposed that the electrophilicity of the quinone moiety was one of decisive factors for the bioactivities of naphthazarin derivatives. In related studies, we examined the antitumor action of various naphthazarin analogues in ICR mice bearing S-180 cells in peritoneal cavity (You *et al.*, 1998a).

Among acylated isomers (Table I), it was generally observed that 6-acyl-DMNQ derivatives (T/C, 106~247%) displayed a higher T/C value than 2-acyl-DMNQ isomers (T/C, 106~156 %); 6-pentanoyl-DMNQ (240 %) *vs.* 2-pentanoyl-DMNQ (145%). The lower antitumor activity of 2-acyl-DMNQ derivatives, compared to that of 6-acyl-DMNQ derivatives, could be ascribed to the steric hindrance of alkyl chain in the quinonoid moiety as had been already observed in the cytotoxicity and the inhibition of TOPO-I (Song *et al.*, 1999a; Song *et al.*, 2000b). Interestingly, there was an optimal size of acyl group for maximal T/C values ranging from hexanoyl to octanoyl group for 2-acyl-DMNQ derivatives (142~156%) and from butanoyl to heptanoyl for 6-acyl-DMNQ derivatives (201~247%). Overall, smaller or larger acyl groups lowered the antitumor activity. This phenomenon might be explained by a metabolic or pharmacokinetic view; the naphthoquinones with a smaller acyl group might be inactivated at a faster rate by the first-pass effect in the liver of the test animal, while those with a larger acyl group might be difficult of access to the foci of the cancer cell. Presumably, the partition coefficient could be one of factors for the explanation (Dohme *et al.*, 1926).

2- or 6-(1-Hydroxyiminoalkyl)-5,8-dimethoxy-1,4-naphthoquinones

In the subsequent experiment to improve the water solubility of DMNQ derivatives, 2- or 6-acyl DMNQ derivatives were transformed into corresponding oximes, 2- or 6-(1-hydroxyiminoalkyl)-DMNQ derivatives according to the procedure reported previously (Song *et al.*, 2000a).

Table I. Effect of intraperitoneal administration of 2- or 6-acyl-5,8-dimethoxy-1,4-naphthoquinones on the life span of ICR mice bearing Sarcoma 180 cells



2-isomer 6-isomer

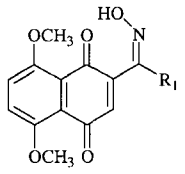
R_1	2-isomer		6-isomer	
	T/C (%)	Survival ratio[a] (50 days)	T/C (%)	Survival ratio[a] (50 days)
Formyl	129	0/8	141	0/8
Acetyl	138	0/8	156	1/8
Propanoyl	133	0/8	189	1/8
Butanoyl	142	0/8	201	2/8
Pentanoyl	145	0/8	240	2/8
Hexanoyl	151	0/8	247	2/8
Heptanoyl	156	0/8	230	3/8
Octanoyl	152	0/8	198	1/8
Nonanoyl	131	0/8	141	0/8
Decanoyl	119	0/8	138	0/8
Undecanoyl	106	0/8	109	0/8
Tridecanoyl	106	0/8	106	0/8
Isohexanoyl	134	0/8	236	4/8
Adriamycin			240	4/8

Dose of naphthoquinones: 15 μmole/kg/day, dose of adriamycin: 1.15 μmole/kg/day
[a]Survival ratio: ratio of the number of mice survived for more than 50 days to 8 test animals

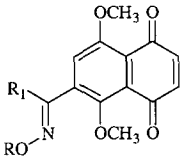
The structures and the T/C values of 2-(1-hydroxyiminoalkyl)- or 6-(1-hydroxyiminoalkyl)-DMNQs are shown in Table II. Compared to acyl-DMNQ derivatives (Song *et al.*, 2000b), hydroxyiminoalkyl-DMNQ analogues exhibited higher T/C values; 2-(1-hydroxyiminoalkyl)-DMNQ derivatives (T/C, 179~241%) vs. 2-acyl-DMNQ derivatives (T/C, 106~156%) and 6-(1-hydroxyiminoalkyl)-DMNQ derivatives (T/C, 191~288%) vs. 6-acyl-DMNQ derivatives (T/C, 106~247%). The higher antitumor activity of oxime derivatives might be explained in part by the enhancement of the water solubility (Song *et al.*, 2000a).

In addition, 6-(1-hydroxyiminoalkyl)-DMNQ derivatives (T/C, 191~288%) seemed to show higher antitumor action than 2-(1-hydroxyiminoalkyl)-DMNQ isomers (179~ 241%). The lower antitumor activity of 2-(1-hydroxyiminoalkyl)-DMNQ derivatives could be due to the steric hindrance of the side chain, as had been observed with 2-(1-hydroxyalkyl)- or 2-acyl-DMNQ derivatives (Song *et al.*, 2000

Table II. Effect of intraperitoneal administration of 2- or 6-(1-oxyiminoalkyl)-5,8-dimethoxy-1,4-naphthoquinones on the life span of ICR mice bearing S-180 cells



2-Isomer



R = H; 6-Isomer
R = Propyl; 6-Propisomer

R_1	2-isomer		6-isomer		6-propisomer	
	T/C (%)	Survival ratio[a] (50 days)	T/C (%)	Survival ratio[a] (50 days)	T/C (%)	Survival ratio[a] (50 days)
H	179	0/8	191	0/8	385	6/8
Methyl	186	0/8	203	1/8	410	7/8
Ethyl	190	0/8	218	2/8	375	6/8
Propyl	201	1/8	231	3/8	351	5/8
Butyl	228	2/8	240	4/8	329	4/8
Pentyl	232	3/8	247	4/8	423	7/8
Hexyl	239	4/8	250	5/8	>450	8/8
Heptyl	241	4/8	265	6/8	>450	8/8
Octyl	236	3/8	280	6/8	453	7/8
Nonyl	226	2/8	288	7/8	>450	8/8
Decyl	219	1/8	279	6/8	401	6/8
Dodecyl	211	1/8	261	6/8	404	6/8
i-pentyl	229	1/8	241	4/8	398	6/8
ADR[b]	240	4/8				

Dose; 18 μmole/kg/day for 2-isomers, 15 μmole/kg/day for 6-isomers and 6-propisomer and 15 μmole/kg/day for ADR, [a]Survival ratio; ratio of number of mice survived for more than 50 days or 80 days to 8 test animals. ADR[b]; adriamycin

b). Added to this, the antitumor effect of 2- or 6-(1-hydroxyiminoalkyl)-DMNQ derivatives was dependent on the size of alkyl chain; among 2-(1-hydroxyiminoalkyl)-DMNQ derivatives, the ones (T/C vlaue, 236~241%) bearing hexyl to octyl group exhibited higher antitumor action than other derivatives. And of 6-(1-hydroxyiminoalkyl)-DMNQ analogues, the highest antitumor action was expressed by those (T/C values, 279~288%) containing octyl to dodecyl group.

Likewise, it was found that the survival ratio, expressed as the number of mice which survived more than 50 days among 8 animals tested, had remarkably increased through the introduction of oxime group; compared to corresponding acyl-DMNQ derivatives (Table I). 2-(1-Hydroxyiminoheptyl)-DMNQ (R_1, hexyl) and 2-(1-hydroxyiminooctyl)-DMNQ (R_1, heptyl) derivatives showed a higher survival ratio (4/8), which is the same as that of adriamycin (ADR). Moreover, 6-(1-hydroxyiminoalkyl)-DMNQ isomers with butyl to dodecyl group showed the survival ratio of 4/8. The highest survival ratio (7/8) was demonstrated by 6-(1-hydroxyiminodecyl)-DMNQ displaying the highest T/C value of 288%. Based on these data, the antitumor action seems to be governed by the hydrophilicity of side chain, which might affect the bioavailability of the naphthoquinone derivatives.

6-(1-Propyloxyiminoalkyl)-5,8-dimethoxy-1,4-naphthoquinones

Oximes are known to be vulnerable to biooxidation which regenerates their oxo-forms. It had been reported that amphetamine, incubated with microsome of rat or rabit liver, was transformed via oxime into corresponding phenylacetone (Parli *et al.*, 1971). We have tried to follow up the metabolic pathway of the naphthoquinone derivatives in liver microsome of ICR mice. 9-(1-Hydroxyiminoethyl)-DMNQ as a representative substance was incubated with the microsome of ICR mice at 37°C, and the time-dependent conversion of the compound was investigated using HPLC [Shimadzu LC-10AD; column, Interstil ODS-3; detection, UV 254 nm; injection, 10 μl; flow rate, 2 ml/min; eluent, 65 parts of 0.1 M sodium acetate buffer (pH 4.4)/35 parts of 62.5% methanol]. The peak with a retention time of 10.55min corresponds to 6-(1-hydroxyiminoethyl)-DMNQ, the intensity of which decreased rapidly after 5 min incubation. The peak at 9.58 min, corresponding to 6-(1-oxoethyl)-DMNQ, increased first and then began to decrease after 5 min. The other peaks at 21.00

and 27.00 min, which were not identified at present, steadily increased with the elapse of time. Based on the HPLC data, it was supposed that 6-(1-hydroxyimino-alkyl)-DMNQ derivatives underwent a biooxidation in microsome to form corresponding 6-acetyl-DMNQ derivatives as actual cytotoxic substances *in vivo* system.

In an attempt to retard the possible biooxidation and promote the transport of the oximes to cancer cells more efficiently, the hydroxyl group of oxime was propylated to yield 6-(1-propyloxyiminoalkyl)-DMNQ derivatives. Their T/C values were shown in Table II. Although the propylation of the oximes did not improve the cytotoxic action against L1210 cells (Song *et al.*, 2000a), it potentiated the antitumor activity to a great extent; 6-(1-hydroxyimino-alkyl)-DMNQ derivatives (T/C, 191~288%) vs. 6-(1-propyloxyiminoalkyl)-DMNQ derivatives (T/C, 329 ~ >450%) at a dose of 15 μmole/kg/day. Noteworthy, the derivatives possessing alkyl group (R_1) longer than pentyl group exhibited T/C values of > 400%. In particular, 6-(1-propyloxy-iminoheptyl)- (R_1, hexyl), 6-(1-propyliminooctyl)-DMNQ (R_1, heptyl) and 6-(1-propyloxyiminodecyl)-DMNQ (R_1, nonyl) derivatives showed T/C values of > 450%, much higher than the T/C value (240%) of ADR at a dose of 1.15 μmole/kg/day. For the antitumor activity of 6-(1-propyl-oxyiminoalkyl)-DMNQ derivatives, there was also a size dependence of alkyl chains with the optimal R_1 groups being pentyl (423%), hexyl (>450%), heptyl (>450), octyl (453%) and nonyl (>450%) moieties.

Additionally, the survival ratio was remarkably enhanced by the propylation; in particular, 6-(1-propyloxyiminoheptyl)-(R_1, hexyl), 6-(1-propyliminooctyl)-(R_1, heptyl)- and 6-(1-propyloxyiminodecyl)-DMNQ (R_1, nonyl) derivatives, which possessed high T/C values (>450%), displayed the survival ratio of 100%. In other words, all of the animals of the test groups survived 80 days after drug administration. The high antitumor action of these oximes might be explained by the assumption that the propylation might prevent the oximes from the degradation by first-pass effect in the liver, so that they could be transported in an elevated concentration to the cancer cells. Besides, the size of alkyl groups (R_1) also seemed to be important for the maximal antitumor action. The size dependence of alkyl group was more prominent for 6-(1-propyloxyimino-alkyl)-DMNQ derivatives than 6-acyl-DMNQ or 6-(1-hydroxyiminoalkyl)-DMNQ derivatives. Therefore, it was assumed that the hydrophobicity of alkyl group and the hydrophilicity of oxime compounds might participate in the antitumor action in a cooperative manner.

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