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NAPHTHAZARIN DERIVATIVES (II)[1] : FORMATION OF GLUTATHIONE CONJUGATE, INHIBITION OF DNA TOPOISOMERASE-I AND CYTOTOXICITY

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Abstract: 6-(1-Hydroxyalkyl)-5,8-dimethoxy-1,4-naphthoquinones, expressing a higher reactivity in conjugation with glutathione, showed a greater potency in the inhibition of DNA topoisomerase-I and the cytotoxicity against L1210 cells than 2-(1-hydroxyalkyl)-DMNQ derivatives, implying the participation of electrophilic arylation in the bioactivities. In further study 6-(1-Hydroxyalkyl)-5,8-dimethoxy-1,4-naphthoquinones with an alkyl group of shorter chain length ($C_2 \sim C_6$) exerted a greater bioactivities than those with longer chain length($>C_6$). © 1999 Elsevier Science Ltd. All rights reserved.

Introduction

The presence of 5-hydroxy group or 5,8-dihydroxy group, which facilitates the tautomerism in the structure of 1,4-naphthoquinone, should reduce the electrophilicity of naphthoquinone ring[2]. Meanwhile, according to our recent study[1], the methylation of naphthazarin to form 5,8-dimethoxy-1,4-naphthoquinone (DMNQ) resulted in the increase of electrophilicity in quinonoid moiety, as evidenced by the greater reactivity of glutathione with DMNQ, compared with naphthazarin, which might be ascribed to the disappearance of tautomerism. Thus, it was suggested that the electrophilicity might be responsible for the chemical reactivity of DMNQ.

In the present study we have measured the rate for the formation of glutathione conjugates, the inhibitory effect on DNA topoisomerase-I (TOPO-I) and the cytotoxicity against L1210 cells for the series of 2-(1-hydroxyalkyl)- and 6-(1-hydroxyalkyl)-DMNQ derivatives. The chemical reactivity and bioactivities will be correlated to ensure a structure-activity relationship.

Results and Discussion

2-(1-Hydroxyalkyl)- and 6-(1-hydroxyalkyl)-DMNQ derivatives, shown in Tables 1 and 2, were synthesized according to Terada's[3] and Baik's methods[4], respectively. The respective GSH conjugate, which was used as a specimen for the HPLC comparison, was synthesized as described in a previous report[5].

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Relationship between the formation of GSH conjugate, the inhibition of DNA topoisomerase-I and the cytotoxicity of naphthoquinone derivatives: The capability of forming glutathione conjugates was expressed as a criterium to evaluate the electrophilicity of DMNQ derivatives. Each DMNQ derivative(1.0 mM) in 100 μL methanol was added to 0.5 mM of glutathione in 100 μL of 0.1M potassium phosphate buffer (pH 7.4) containing 0.05 mM EDTA at 37℃, and after 5 min incubation the amount of the conjugate formed was measured by a HPLC. As shown in Table 1, although the GSH conjugate of 2-(1-hydroxyalkyl)-DMNQ derivatives was not produced using 0.5 mM glutathione under the reaction conditions, a considerable amount of 3-glutathionyl-2-(1-hydroxyalkyl)-DMNQ derivative was yielded in the presence of excess glutathione(5 mM). Fig.1 indicates that the formation of 3-glutathionyl-DMNQ derivatives was time-dependent at a millimolar ratio of [quinone] / [GSH] = 1:5. The order for the rate of the conjugate formation was 2-(1-hydroxypentyl)- > 2-(1-hydroxyethyl)- > 2-(1-hydroxyhexyl)-3-glutathionyl-DMNQ, implying that the conjugate formation was not dependent on the size of alkyl group. This finding led to a suggestion that the electrophilic reaction could be important for the metabolism and bioactivity of 2-(1-hydroxyalkyl)- DMNQ derivatives in liver tissue abundant in GSH.

Compared to 2-(1-hydroxyalkyl)-DMNQ derivatives, 6-substituted ones showed a higher reactivity with GSH (Table 2), consistent with the higher electrophilicity of quinone moiety in 6-(1-hydroxypentyl)-DMNQ than 2-(1-hydroxypentyl)- DMNQ. As suggested previously[1], the greater reactivity of quinone moiety in 6-(1-hydroxyalkyl)-DMNQ may be ascribed to the exposure of quinone moiety to nucleophiles. In addition, the steric hindrance could be the reason for the lower reactivity of 2-(1-hydroxyalkyl)-DMNQ derivatives. Noteworthy, the reactivity of 6-(1-hydroxyalkyl)-DMNQ derivatives with GSH decreased with an increasing size of alkyl substituent at C-2; 6-(1-hydroxymethyl)-DMNQ (0.304 mM) vs. 6-(1-hydroxyheptyl)-DMNQ (0.182 mM) as an example. This difference of the reactivity might be due to the steric hindrance of the substituents, as had been reported for the side chain of basic DMNQ derivatives[1].

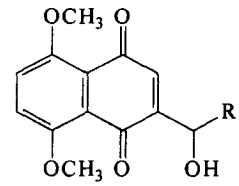
Next, the inhibitory effect of DMNQ derivatives on TOPO-I was examined as described before[6,7]. 2-(1-Hydroxyalkyl)-DMNQ derivatives did not inhibit the enzyme, while 6-substituted naphthoquinones showed a considerable inhibitory action, implying that the electrophilicity and steric hindrance at the quinonoid moiety is important for the inhibition of TOPO-I. The inhibitory effect of 6-(1-hydroxyalkyl)-DMNQ derivatives on TOPO-I was dependent on the size of alkyl groups (C_1-C_9); of derivatives tested; the one bearing hexyl group was the most potent with an IC_{50} value of 30 μM. The elongation of alkyl moiety beyond six carbons rather decreased the potency.

When the cytotoxicity against L1210 cells was determined using Thayer's method[8], it was observed that 6-substituted analogues were more cytotoxic against L1210 cells than 2-substituted ones. 6-Substituted DMNQ derivatives possessing the alkyl groups (R) of shorter chain length such as ethyl (ED_{50}, 0.085 μg/mL), propyl (ED_{50}, 0.043 μg/mL), butyl (ED_{50}, 0.057 μg/mL) and pentyl group (ED_{50}, 0.066 μg/mL) were highly cytotoxic. However, the extension of alkyl moiety over six carbons rather decreased the bioactivity. Thus, the size of alkyl moiety appeared to be commonly important for the inhibition of both TOPO-I and the cytotoxicity, although there was no parallel correlation between two bioactivities. From these results, it is supposed that a common mechanism for the inhibitory effect of 6-substituted DMNQ derivatives on TOPO-I and their cytotoxicity against L1210 cells could be electrophilic arylation as implied from the conjugation of 6-substituted DMNQ derivatives with GSH. Thus, the electrophilicity of quinone moiety is suggested to be an important factor for the bioactivity of substituted naphthoquinone derivatives. One interesting result is that 2-(1-hydroxyalkyl)-DMNQ derivatives, sterically hindered, still showed a considerable cytotoxic activity, implying that besides the arylation of the cellular nucleophiles, other mechanisms might be responsible for the cytotoxic activity of the 2-substituted naphthoquinones. In previous study, the so-called bioreductive activiation might was proposed to play a role in cytotoxicity of 2-(1-hydroxyalkyl)-DMNQ derivatives)[1] and 2-(1-hydroxyalkyl)-anthracene-1,4,9,10-tetraones[9]; according to the proposed mechanism, first, quinone moiety is reduced by cellular quinone reductase to hydroquinone species, which in turn is transformed to a quinone methide. This quinone methide functions as a Michael acceptor for cellular nucleophiles[10].

Summary

6-(1-Hydroxyalkyl)-DMNQ derivatives, showing a greater reactivity in the conjugation with GSH, were more potent in the inhibition of TOPO-I and the cytotoxicity against L1210 cells, compared with 2-(1-hydroxyalkyl)-DMNQ derivatives. Thus, the electrophilicity of the quinone moiety of DMNQ derivatives appears to govern the bioactivities. In addition, steric hindrance of alkyl side chain certainly lowered the reactivity and bioactivities of DMNQ derivatives, especially 2-substituted ones. Meanwhile, a long time exposure of 2-substituted DMNQ derivatives to a high concentration of GSH resulted in the formation of GSH conjugates to a considerable extent, and the delayed electrophilic reaction could be responsible for their cytotoxic activity. In addition, the bioreductive alkylation is supposed to be an alternative action mechanism of 2-(1-hydroxyalkyl)-DMNQ derivatives.

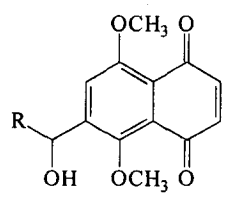
Table 1. Relationship between formation of GSH conjugate, DNA topoisomerase-I inhibition and cytotoxicity of 2-(1-hydroxyalkyl)-5,8-dimethoxy-1,4-naphthoquinone



R	GSH-conjugate(mM)[a]		TOPO- I [b]	L1210
	0.5	5	IC_{50} (μM)	ED_{50} (μg/mL)[d]
H	N.D.[c]	0.130±0.02	N.D	0.524±0.048
Methyl	N.D	0.125±0.02	>1500	0.487±0.053
Ethyl	N.D	0.084±0.01	N.D	0.516±0.018
Propyl	N.D	0.116±0.02	N.D	0.409±0.022
Butyl	N.D	0.131±0.01	N.D	0.412±0.009
Pentyl	N.D	0.124±0.02	N.D	0.378±0.054
Hexyl	N.D	0.072±0.01	N.D	0.732±0.047
Heptyl	N.D	0.060±0.01	N.D	1.043±0.082

[a]Conc. of the conjugate formed during 5min incubation of quinone (1 mM) with GSH (0.5mM or 5 mM).
[b]DNA topoisomerase-I from calf thymus. [c]Not detected. [d]ED_{50} values , refer to 8.

Table 2. Relationship between formation of GSH conjugate, DNA topoisomerase-I inhibition and cytotoxicity of 6-(1-hydroxyalkyl)-5,8-dimethoxy-1,4-naphthoquinones



R	GSH-conjugate (mM)[a]	TOPO- I [b] IC_{50} (μM)	L1210 ED_{50}(μg/mL)[d]
H	0.314±0.042	N.D.[c]	0.062±0.028
Methyl	0.304±0.070	110.63±0.80	0.132±0.065
Ethyl	0.295±0.054	121.83±5.82	0.085±0.023
Propyl	0.278±0.009	129.70±3.48	0.043±0.016
Butyl	0.310±0.081	83.14±21.47	0.057±0.009
Pentyl	0.280±0.047	39.00±3.21	0.066±0.014
Hexyl	0.182±0.039	30.10±4.32	0.220±0.051
Heptyl	0.137±0.035	43.93±0.24	0.274±0.049

[a] Conc. of the conjugate formed during 5min incubation of quinone (1 mM) with GSH (0.5mM).
[b] DNA topoisomerase-I from calf thymus. [c]Not detected. [d]ED_{50} values , refer to 8.

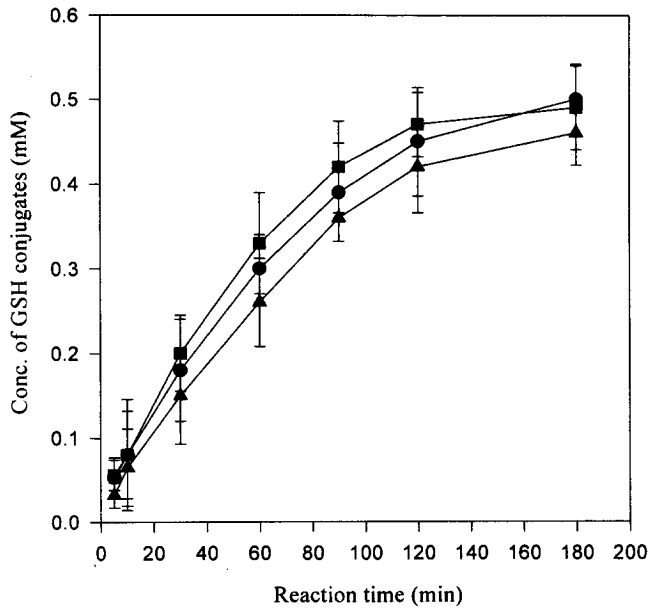


Figure 1. Time dependent formation of GSH conjugates; 3-glutathionyl-2-(1-hydroxyethyl)-(●), 3-glutathionyl-2-(1-hydroxypentyl)-(■) and 3-glutathionyl-2-(1-hydroxyhexyl)-DMNQ (▲), [Quinone] / [GSH] = 1 : 5 mM

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