# MECHANISM OF INHIBITION OF REVERSE TRANSCRIPTASE BY QUINONE ANTIBIOTICS

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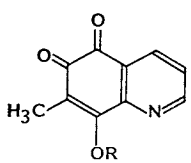
(Received for publication June 26, 1987)

Streptonigrin is a potent inhibitor of avian myeloblastosis virus (AMV) reverse transcriptase in a non-competitive manner by direct interaction with an enzyme molecule[1]. In addition to streptonigrin, inhibition of AMV reverse transcriptase was observed with another quinone antibiotic, sakyomicin A[2]. These observations prompted us to extend our search for inhibitors of AMV reverse transcriptase to various carbocyclic and heterocyclic quinones[3~5]. Several naphthoquinone, quinoline quinone and isoquinoline quinone derivatives were found to inhibit AMV reverse transcriptase to the same extent as streptonigrin. The inhibitory activities against AMV reverse transcriptase of these quinone compounds including both streptonigrin and sakyomicin A were well correlated with their catalytic activities in the oxidation of NADH by rat liver mitochondria or *Clostridium kluyveri* diaphorase, which were dependent on their potential to accept electrons from NADH catalyzed by diaphorase and autoxidation to quinones with the simultaneous generation of hydrogen peroxide by transferring electrons to molecular oxygen. 1,4-Benzoquinone was an exception to this general concept, however, showing inhibition of reverse transcriptase without any marked effect on the oxidation of NADH by *C. kluyveri* diaphorase in terms of generation of hydrogen perioxide.

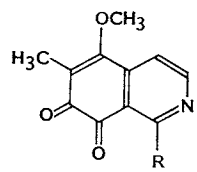
Contrary to the earlier proposition by WICK and FITZGERALD[6] that the generation of semiquinone and/or oxygen radical triggered the reactions resulting in the inactivation of reverse transcriptase, we demonstrated that the induction of semiquinone and accompanying superoxide anion had no effect on the inhibition of AMV reverse transcriptase by quinones including, in particular, sakyomicin A[7].

In the preceding papers[3~5], we proposed that the naphthoquinone and quinoline quinone moieties were the minimum requisites for the biological activities of sakyomicin A and streptonigrin, respectively, based on the biological properties of quinoline quinones, naphthoquinones and 1,4-benzoquinone. Interestingly, isoquinoline quinones were as potent as inhibitors of AMV reverse transcriptase as quinoline quinones. Furthermore, inhibition of AMV reverse transcriptase was not found to differ whether *ortho*- or *para*-quinoline (or isoquinoline) quinones were employed. Saframycin A is also a quinone antibiotic with a 1,2,3,4,5,8-hexahydroisoquinoline-5,8-dione structure. However, this antibiotic proved to be devoid of inhibitory activity against AMV reverse transcriptase. In

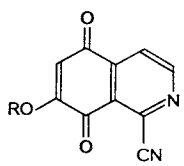
Fig. 1. Structures of the quinone compounds.



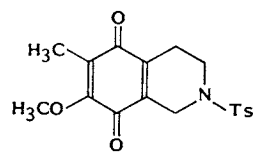
**1** R = $CH_3$
**2** R = $C_2H_5$



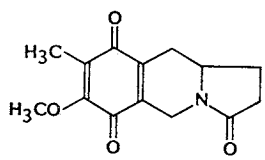
**3** R = H
**4** R = CN



**5** R = $C_2H_5$
**6** R = $(CH_2)_3CH_3$



**7**
Ts = *p*-Toluenesulfonyl



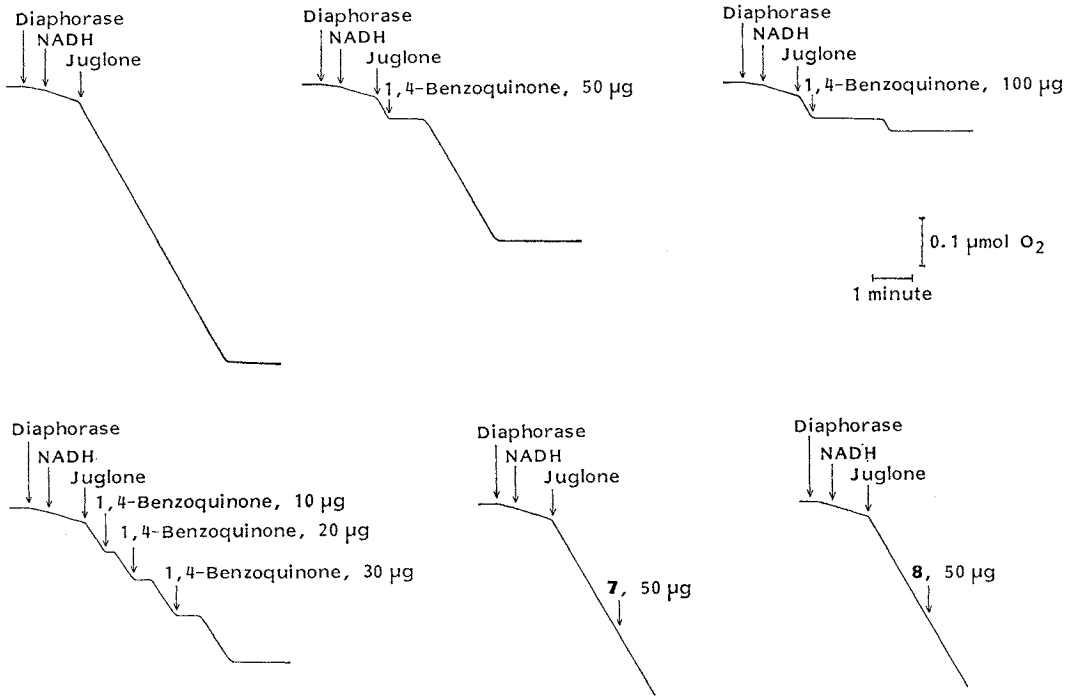
**8**

Fig. 2. Effect on the oxidation of NADH by *Clostridium kluyveri* diaphorase.

The oxidation of NADH by bacterial diaphorase was measured in 3 ml of a basal medium (225 mM sucrose, 5 mM potassium phosphate and 10 mM Tris-HCl, pH 7.4).

The quinones were dissolved in DMSO at 5 mg/ml and used at the dosage indicated in the figure.

The other additions (stock solution): Diaphorase (900 units/ml in $H_2O$), 9.0 units; NADH (100 mM in $H_2O$), 1.0 $\mu$mol; juglone (5 mg/ml in DMSO), 10 $\mu$g.




order to understand this observation, a comparative study on the biological activities of various quinones shown in Fig. 1, 1,4-benzoquinone and 1,4-dihydrobenzoquinone was conducted.

Diaphorase (*C. kluyveri*, EC 1.6.99.) was obtained from Oriental Yeast Co., Ltd., Tokyo and NADH was purchased from Sigma Chemical Co., MO. 1,4-Benzoquinone and 1,4-dihydrobenzoquinone were the products of Nakarai Chemicals Ltd., Kyoto and Wako Pure Chemical Ind., Ltd., Osaka, respectively. 8-Methoxy-7-methyl-5,6-dihydroquinoline-5,6-dione (**1**) and 5-methoxy-6-methyl-7,8-dihydroisoquinoline-7,8-dione (**3**) were prepared as reported previously[8]. The synthesis of quinoline quinone (**2**), isoquinoline quinones (**4**~**6**), 1,2,3,4,5,8-hexahydroisoquinoline-5,8-diones (**7**~**8**) will be reported separately. All other chemicals were commercial products of analytical grade. The details of assay methods for AMV reverse transcriptase and growth of L5178Y cells were described previously[9,10]. Hydrogen peroxide was determined by the method of TRINDER[11] with some modifications[12]. Oxygen consumption was measured with a Clark type electrode (Yellow Spring Instrument Co., Yellow Spring, Ohio)[13].

Recently we observed that juglone-dependent oxygen consumption accompanying the oxidation of NADH catalyzed by *C. kluyveri* diaphorase temporarily ceased following the addition of 1,4-benzoquinone (Fig. 2). Meantime, the amount of NADH consumed to give hydrogen peroxide was less than in the initial reaction mixture. The amount of missing NADH was stoichiometric to that of 1,4-benzoquinone added to the reaction mixture, implying that the reduction of 1,4-benzoquinone, which was not followed by autoxidation, presumably substituted for oxygen consumption mediated by juglone in a continuous fashion. When the conversion of 1,4-benzoquinone to 1,4-dihydrobenzoquinone was completed, electrons were again transferred to juglone which in turn donated electrons to

Table 1. Comparison of biological properties of quinones.

| Compound | $ED_{50}$ ($\mu$g/ml)[a] ($H_2O_2$) | $ID_{50}$ ($\mu$g/ml) | |
|---|---|---|---|
| | | L5178Y[b] | RDDP[c] |
| **1** | 1.8 | 16 | 2.0 |
| **2** | 0.12 | 8 | 7.9 |
| **3** | 3.5 | 0.17 | 2.0 |
| **4** | 4.1 | 0.72 | 1.9 |
| **5** | 0.74 | 0.17 | 3.6 |
| **6** | 1.5 | 0.55 | 0.52 |
| **7** | >100 | 0.046 | >20 |
| **8** | >100 | 0.21 | >20 |
| 1,4-Benzoquinone | >100 | 0.51 | 13 |
| 1,4-Dihydrobenzoquinone | >100 | 0.49 | >160 |

[a] The concentration required to get 50% the maximum value under the assay conditions employed[12].
[b] The growth of murine lymphoblastoma L5178Y cells.
[c] RNA-directed DNA polymerase (AMV reverse transcriptase).

molecular oxygen. This hypothesis was further supported by the fact that cessation of oxygen consumption lasted longer if more 1,4-benzoquinone was added to the reaction mixture.

In marked distinction to quinoline quinones and isoquinoline quinones, the derivatives of 1,2,3,4,5,8-hexahydroisoquinoline-5,8-dione, **7** and **8**, were neither efficient electron acceptors nor inhibitors of AMV reverse transcriptase as in the case of 1,4-dihydrobenzoquinone, while 1,4-benzoquinone exhibited inhibition of AMV reverse transcriptase without showing any marked effect on the generation of hydrogen peroxide coupled with the oxidation of NADH catalyzed by *C. kluyveri* diaphorase (Table 1). As noted above, 1,4-benzoquinone substitutes for juglone, which is reflected in a level slope in Fig. 2. Under the same conditions, **7** and **8** failed to stop juglone-dependent oxygen consumption, suggesting that both **7** and **8** were inactive as electron acceptors. In Table 1, it is obvious that those inactive as electron acceptors are defective in inhibition of AMV reverse transcriptase despite the total independence of cytotoxicity against murine lymphoblastoma L5178Y cells from the other properties. Thus, we propose that oxidation of AMV reverse transcriptase, in other words, the reduction of quinones should be enough to adversely affect the enzyme activity. The autoxidative feature of quinones might result in progressive inactivation of AMV reverse transcriptase by supplying electron acceptors continuously.

Acknowledgment

This work was supported in part by a Grant-in-Aid for the General Scientific Research from the Ministry of Education, Science and Culture, Japan.

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