β-D-RIBOFURANOSYL-1,4-BENZOQUINONE - ANTIBACTERIAL AGENT WITH SHOWDOMYCIN-LIKE MODE OF ACTION

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SUMMARY. β-D-Ribofuranosyl-1,4-benzoquinone is toxic in wild-type E. coli while mutants deficient in constitutive nucleoside permease are resistant; the toxic action may be abolished by 2 -chloro-2'-deoxyuridine known to inhibit nucleoside permease. α-D-Ribofuranosyl-1,4-benzoquinone and 4(β-D-ribofuranosyl)-1,2-benzoquinone are inactive. 1,4-Dihydroxy-2-β-D-ribofuranosylbenzene does not interact with nucleoside permease. It appears that nucleoside analogs with 1,4-benzoquinone ring are transported by nucleoside permease and their mode of action resembles that of showdomycin.

Nucleoside permease of E. coli B interacts not only with natural nucleosides except guanosine, but will recognize other β-D-ribofuranosides bearing some pyrimidine or purine base analogs; the corresponding α-anomers or L-ribofuranosides are always inactive (1). The bactericidal action of the antibiotic showdomycin, i.e. 2(β-D-ribofuranosyl)maleimide, is due to its maleimide moiety which blocks sulfhydryl groups of proteins essential for the uptake of many nutrients and for other vital cellular functions (2,3). To reach the target sulfhydryl groups showdomycin must be brought into the cells by nucleoside-transporting system and mutants deficient in this system are resistant to the drug (4). We now wish to report our findings indicating that β-D-ribofuranosyl-1,4-benzoquinone has similar properties, being recognized by constitutive nucleoside permease as an analog of uridine.

MATERIALS AND METHODS. Showdomycin (5) and both anomers of D-ribofuranosyl-1,4-benzoquinone were prepared and identified as described (6). 1,2-Dihydroxy-4-β-D-ribofuranosylbenzene was synthesized using the procedure analogous to that described for the preparation of 1,4-dihydroxy-2-α-D-ribofuranosylbenzene (6), starting with 1,2-bis-(2-bromoethoxy)benzene; the details of the synthesis will be described elsewhere; the crystalline 1,2-dihydroxy-4-β-D-ribofuranosylbenzene had α_D^{25}-40.4 (c 0.7 in water) and its m.p. was 166-168 °C. To obtain the corresponding o-quinone the oxidation was performed in solution with tyrosinase and air oxygen. Tyrosinase was purified from Psalliota sp. (7). The formation and decomposition of the o-quinones was assayed polarographically (7).

E. coli B (Shm^s) and its mutant resistant to showdomycin and 5-azacytidine (Shm^r) were used (8,9). The bacteria were grown in glucose-mineral salts medium (10). The bactericidal action was tested by incubating a suitably diluted bacterial culture (10^7 cells/ml) at 37 °C or 25 °C with shaking; samples were taken in intervals for dilution and plating; to stop the action of the inhibitors the medium used for the first dilution step contained 0.1 mM dithiothreitol. Growth inhibition was measured turbidimetrically after 90-min incubation of exponentially growing bacterial culture at 37 °C with shaking. Inactivation of nucleoside-transporting system was detected by measuring the uptake of [2-^{14}C] deoxycytidine (43 mCi/mmole, final conc. 2 μM) in one-min pulses, using rapid-filtration technique (11); the bacteria were incubated with the inhibitors for 10 min prior the test, then filtered and resuspended in fresh medium without the inhibitors.

RESULTS. In E. coli B (Shm^s) β-D-ribofuranosyl-1,4-benzo-

quinone (200/uM) was bactericidal; the bacteria, however, were killed at least ten-times more slowly than with unsubstituted 1,4-benzoquinone. The corresponding α-anomer was completely non-toxic under the same conditions. The Shm^r mutant, bearing a genetic defect in constitutive nucleoside-transporting system (9), was resistant to β-D-ribofuranosyl-1,4-benzoquinone, although its sensitivity to unsubstituted 1,4-benzoquinone was equal to that of the Shm^s strain (Fig.1).

At about 50/uM β-D-ribofuranosyl-1,4-benzoquinone had a pronounced bacteriostatic effect on Shm^s bacteria. Growth inhibition could be completely abolished by adding 2′-deoxy-2′-chlorouridine, a compound shown previously (1) to block

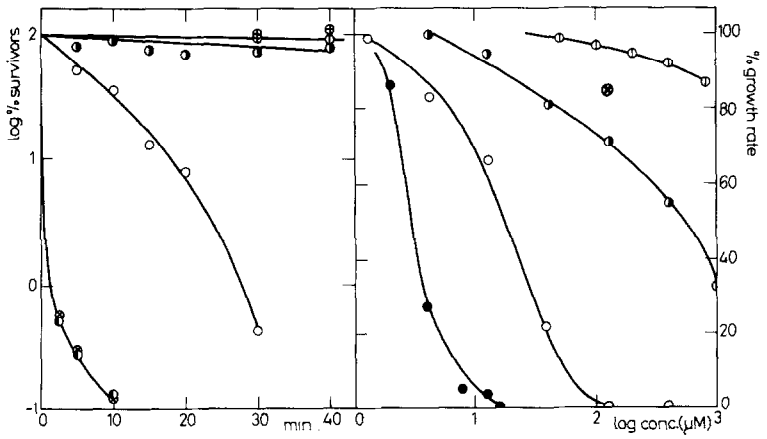

Fig. 1 (left. Killing curves of E. coli B (Shm^s) and the Shm^r mutant by 1,4-benzoquinone and the anomers of D-ribofuranosyl-1,4-benzoquinone at 200/uM

Fig. 2 (right). Growth inhibition of E. coli B (Shm^s) and the Shm^r mutant by quinonoid compounds
⊗———⊗ 1,4-benzoquinone, Shm^s; ◐———◐ same compound, Shm^r;
○———○ β-D-ribofuranosyl-1,4-benzoquinone, Shm^s;
◑———◑ same compound, Shm^r;
◑———◑ α-D-ribofuranosyl-1,4-benzoquinone, Shm^s;
⊕———⊕ same compound, Shm^r; ●———● showdomycin, Shm^s;
⊗ β-D-ribofuranosyl-1,4-benzoquinone + 2′-deoxy-2′-chlorouridine, 300/uM, Shm^s.

the nucleoside-transporting system. The α-anomer had no effect on growth rate, even at much higher conc. 1,4-Dihydroxy-2-β-D-ribofuranosylbenzene was non-toxic and attempts to demonstrate its interaction with constitutive nucleoside permease by means of showdomycin-detoxication test (1) gave negative results.

In order to test the antibacterial activity of 4(β-D-ribofuranosyl)-1,2-benzoquinone, which could not be prepared in solid state, 1,2-dihydroxy-4-β-D-ribofuranosylbenzene was oxidized directly in the medium with tyrosinase and air oxygen, using catechol as control. While both these compounds were non-toxic themselves, catechol oxidized with tyrosinase killed the bacteria in less than 1 min, but no effect was observed with oxidized 1,2-dihydroxy-4-β-D-ribofuranosylbenzene. Polarographic control has shown that in the medium used in these tests (pH 7.0) the half life time of 1,2-benzoquinone was 1.3 min and that of 4(β-D-ribofuranosyl)-1,2-benzoquinone 3.0 min at 25 °C. Therefore the lack of bactericidal activity of the latter could be due to its instability combined with slow penetration into the cells. At pH 5.9 and 25 °C, however, the half time of 4(β-D-ribofuranosyl)-1,2-benzoquinone was 21.5 min, yet even under these conditions no bactericidal effect could be demonstrated (Fig.3). Consequently 4(β-D-ribofuranosyl)-1,2-benzoquinone is inactive per se, not because of its low stability.

Both 1,4-benzoquinone and 1,2-benzoquinone as well as β-D-ribofuranoside-1,4-benzoquinone inactivated the system responsible for the uptake of deoxycytidine in a manner similar to that of maleimide and showdomycin (Tab. 1). Therefore it seems most probable that the mechanism of action of

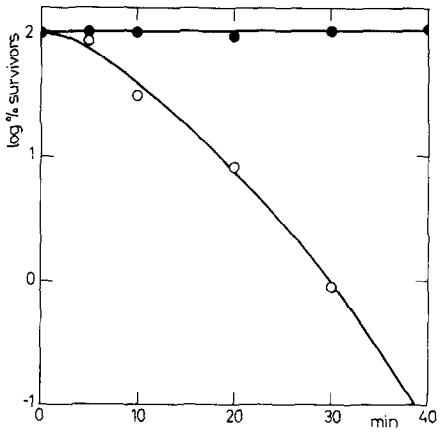

Fig. 3. Killing curves of E. coli B (Shm^S) at pH 5.9 and 25°C
○——○ β-D-ribofuranosyl-1,4-benzoquinone;
●——● 1,2-dihydroxy-4-β-D-ribofuranosylbenzene, oxidized with tyrosinase and air oxygen.

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both groups of compounds is similar, namely blocking of sulfhydryl groups of proteins essential for the transport of nutrients (4, 12).

DISCUSSION. The action of benzoquinone-D-ribofuranosides on E. coli is characterized by following features: 1) the presence of functional constitutive nucleoside permease is required and the action may be antagonized by nucleoside analogs competing for the permease system; 2) the β-configuration of the ribofuranoside is required; 3) the 1,4-benzoquinone ribofuranoside, but not the 1,2-benzoquinone ribofuranoside is active; 4) the action of the ribofuranoside is much slower than with free quinones.

β-D-Ribofuranosyl-1,4-benzoquinone provides another example of a C-glycoside recognized by constitutive nucleoside permease; the presence of a nitrogen atom in the cyclic aglycone moiety is not required, but the carbonyl group in α-position with respect to the N- or C-atom engaged in the

TABLE I

Inactivation of deoxycytidine-uptaking system of the cells of E. coli (Shm^S) with maleimide, quinones and their ribofuranosyl derivatives

Inactivating agents	Conc. (μM)			
	18	56	180	560
	Rate of deoxycytidine uptake[a] (%)			
Maleimide	8	3	2	n.d.
Showdomycin	2	1	1	1
1,4-Benzoquinone	10	5	2	1
β-D-Ribofuranosyl-1,4-benzoquinone	125	27	4	1
α-D-Ribofuranosyl-1,4-benzoquinone	n.d.	130	115	92
Catechol	n.d.	105	104	102
Catechol + tyrosinase	3	2	2	1
1,2-Dihydroxy-4-β-D-ribofuranosylbenzene	n.d.	100	95	103
Same compound + tyrosinase	98	132	138	124

[a] The rate of uptake of deoxycytidine in the untreated control was 0.2 μM/min/10^8 bacteria (100 %).

n.d., not determined

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formation of glycosidic bond is essential. Comparison with other analogs (1) indicates that this is a necessary but not a sufficient condition.

Although β-D-ribofuranosyl-1,4-benzoquinone is a weaker bactericidal agent than showdomycin, its redox properties could occasionally offer some advantage; e.g. it should be possible to administer the less toxic reduced form, which could then be oxidized enzymatically in situ.

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