J. Pharm. Pharmacol. 1984, 36: 467–470
Communicated October 28, 1983

Rifampicin curing of plasmids in *Escherichia coli* K12-rifampicin resistant host

E. E. OBASEIKI-EBOR, *Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria*

The in-vitro rifampicin curing of R-plasmids JR225, BN102, BN106 and F'-plasmid F'*lac*$^+$/R386 in *Escherichia coli* K12 rifampicin-resistant host J62-2 is described. The minimum rifampicin curing concentration and the frequency of curing of plasmids from the rifampicin-resistant host cell J62-2 and the isogenic rifampicin-sensitive J62-1 host cell were similar. However, rifampicin did not cure some test R-plasmids such as R222 and RP4 from rifampicin-resistant and rifampicin-sensitive host cells, indicating that it is the R-plasmid itself and not the host strain that determines rifampicin curing.

Chemical agents such as acridine dyes (Mitsuhashi et al 1961; Watanabe & Fukasawa 1961), sodium dodecylsulphate (Tomoeda et al 1968), dihydrofolate reductase and thymidylate synthetase inhibitors (Pinney & Smith 1971; Bremer et al 1973) have been used to eliminate R-plasmids and sex-factors from bacteria. Rifampicin has been reported to eliminate plasmids from rifampicin-sensitive bacteria such as *Staphylococcus aureus* (Johnson & Richmond 1970) and *E. coli* (Bazzicalupo & Tocchini-Valentini 1972), and is a well known chemotherapeutic agent used in the therapy of mycobacterial and staphylococcal infections (Morrison-Smith 1975; Nessi & Fowst 1979; Jensen 1975). Rifampicin inhibits the synthesis of RNA by interacting specifically with the β-subunit of RNA polymerase (di Mauro et al 1969), and was recently found to cause the segregation of R-plasmid in a rifampicin-resistant strain (Obaseiki-Ebor 1983), leading us to this study of the effect of rifampicin on R-plasmids in rifampicin-resistant *E. coli*.

Materials and methods

Bacterial strains and bacteriophage

Escherichia coli W677 (JR225) *lac*$^-$, *gal*$^-$, *leu*$^-$, *thr*$^-$ possessing the R-plasmid JR225 conferring resistance to gentamicin, kanamycin, tobramycin, neomycin, ampicillin and cephalosporin (Simpson & Breeze 1981) was obtained from Dr J. Davies, Biogen, S.A., Switzerland. R-plasmids BN102 conferring resistance to nitrofurantoin, sulphonamide and tetracycline and BN106 conferring resistance to nitrofurantoin, trimethoprim, ampicillin and chloramphenicol were obtained from *E. coli* clinical isolates in University of Benin Teaching Hospital, Benin City, Nigeria. *E. coli* K12 M176, *lacB*$^-$, *his*$^-$, *lys, trp*, Sm^R, Tc^R possessing F'*lac*$^+$. R-plasmid R222 conferring resistance to ampicillin, streptomycin, tetracyclkine, sulphonamide (Crumplin & Smith 1981) was obtained from Professor J. T. Smith, School of Pharmacy, University of London. *E. coli* K12 J62-2 (F$^-$, *pro*$^-$, *lac*$^-$, *trp*$^-$, *his*$^-$; with chromosomal rifampicin resistance, Coetzee et al 1972). *E. coli* K12 J62-1 (F$^-$, *pr*$^-$, *lac*$^-$, *trp*$^-$, *his*$^-$, with chromosomal nalidixic acid resistance); *E. coli* K12 RP1 harbouring plasmid RP4 which confers resistance to ampicillin, tetracycline and kanamycin and male specific bacteriophage, MS2, were provided by Dr A. S. Breeze, Department of Pharmacy, Heriot-Watt University, Edinburgh.

Media used. Diagnostic sensitivity test agar, DST (Oxoid); L-broth: Difco trypton, 10g; Difco yeast extract, 5g; sodium chloride, 10g and distilled water, 1 l, pH 7·2. Davis & Mingioli (1950) basal minimal salts agar media with glucose as a carbon source.

Antibacterial agents and chemicals. Rifampicin, nalidixic acid, gentamicin and nitrofurantoin were purchased from Sigma Chemical Co., streptomycin and ampicillin from Glaxo laboratories.

Acridine dye was provided by Dr H. Bialy presently of the Department of Biology, The University of New Mexico, Albuquerque, New Mexico, USA.

Determination of the minimum inhibitory concentrations (MIC) of the antibacterial agents. About 10^5–10^6 c.f.u. ml^{-1} of normal saline (0·9% NaCl) dilutions of overnight test cultures were spotted onto series of overdried DST agar plates containing increasing concentrations of the test antibacterial agent. The plates were incubated at 37 °C for about 24 h and observed for growth. The lowest concentration of the antibacterial agent preventing visible growth was regarded as the MIC.

Curing of R-plasmids with rifampicin. 1 ml overnight cultures of R$^+$ cells were subcultured in 10 ml L-broth. The culture was decimally diluted to 10^{-5} in L-broth containing varying concentrations of rifampicin and incubated at 37 °C for 6 h. Suitably diluted aliquots were plated on DST agar plates. The colonies (about 200 in each determination) were tested for their antibiotic resistance by replica-plating on DST agar containing 20 μg ml^{-1} of either ampicillin, gentamicin, nitrofurantoin or streptomycin. Frequency of curing was

taken as percentage of cured cells in 200 rifampicin treated cells per hour for each rifampicin concentration of the test culture inoculum.

Infection with R-plasmid. The method of Datta & Hedges (1972) was used. The R-plasmids were introduced into the recipient strains J62-1 and J62-2, from the donor strains by mixing equal volumes of exponential cultures (c.f.u. 10^3 cells ml^{-1}) and incubating the mixed culture at 37 °C for 6 h. Transconjugants were selected on Davis & Mingioli (1950) glucose minimal agar containing 5, 10 or 20 μg ml^{-1} of streptomycin or nitrofurantoin respectively, as the appropriate recipient strain growth requirements and the counterselective antibiotic.

Infection with male specific bacteriophage MS2. The bacteriophage was used to confirm the loss of R-plasmid. R^- cells are resistant to MS2 bacteriophage and do not form plaques on infection with the phage. Infection was by the modified method of Miller (1972). Suitable dilutions of the MS2 bacteriophage lysates were mixed with the test and control strains in soft agar onto tryptone agar plates containing 4 mM $MgCl_2$. The plates were incubated at 35 °C for 10–15 h. Formation of plaques indicated sensitivity to the phage and presence of R-plasmid in the host cell.

Results

Curing of R-plasmids with rifampicin. R-plasmids JR225, BN102, BN106 and F'-plasmid F'*lac*$^+$ were cured from the rifampicin-resistant mutants and the nalidixic acid-resistant mutants by rifampicin, but R-plasmids R222 and RP4 were not. Curing was most often obtained between the 4 to 6 h of rifampicin treatment and decreased thereafter (Table 1). During the exponential growth phase of the rifampicin-treated cells, the average percentage rate of curing by rifampicin (1, 2·5 μg ml^{-1}) was about 16 and 48% h^{-1} respectively (calculated as the mean of the ratio of cured cells to uncured cells h^{-1}). In general, curing was dependent on the concentration of rifampicin, culture inoculum and the growth phase of the test R-plasmid culture (Figs. 1–3, Table 1).

Subinhibitory concentrations of rifampicin, 1–2·5 μg ml^{-1}, cured JR225, BN102, BN106, F'*lac*$^+$ from rifampicin-resistant and -sensitive strains but the rate of curing increased according to the concentration of rifampicin. Curing was phenotypically indicated by the loss of the R-plasmid resistance spectrum and confirmed by the resistance of the cured cells to MS2 bacteriophage.

Although rifampicin was unable to cure R-plasmids R222 and RP4 from J62-1 and J62-2, acridine dye at a concentration of 100 μg ml^{-1} pH 7·5 in L-broth cured the R-plasmids from J62-1 and J62-2.

Table 1. Curing of R-plasmid JR225 from J62-2 rifampicin resistant host with varying concentrations of rifampicin.

Exp. no.	Inoculum size	Rifampicin concn	Period of incubation (h)	Viable count cells ml^{-1}	No. of colonies cured/ no. of colonies tested	% Frequency of curing
1	1×10^3	0	2	$2{\cdot}0 \times 10^5$	0/90	0
			4	$5{\cdot}0 \times 10^6$	0/90	0
			6	$2{\cdot}5 \times 10^7$	0/90	0
			12	$5{\cdot}0 \times 10^8$	0/90	0
			24	$6{\cdot}0 \times 10^9$	0/90	0
		1·0	2	$2{\cdot}0 \times 10^5$	6/90	7
			4	$6{\cdot}0 \times 10^5$	10/90	11
			6	$3{\cdot}0 \times 10^6$	20/90	22
			12	$5{\cdot}5 \times 10^7$	24/90	27
			24	$2{\cdot}2 \times 10^9$	36/90	40
		2·5	2	$1{\cdot}4 \times 10^4$	10/60	17
			4	$3{\cdot}2 \times 10^5$	36/40	57
			6	$3{\cdot}8 \times 10^6$	40/60	67
			12	$2{\cdot}0 \times 10^8$	45/60	75
			24	$5{\cdot}0 \times 10^8$	49/60	82
		5·0	2	$3{\cdot}0 \times 10^3$	12/60	20
			4	$4{\cdot}2 \times 10^4$	36/60	60
			6	$2{\cdot}1 \times 10^4$	49/60	82
			12	$2{\cdot}0 \times 10^3$	52/60	87
		10	2	$2{\cdot}0 \times 10^3$	15/60	25
			4	$2{\cdot}0 \times 10^3$	37/60	62
			6	$1{\cdot}0 \times 10^2$	51/60	85
			12	—	—	—
2	5×10^3	0	6	$6{\cdot}0 \times 10^5$	0/60	0
		5	6	$4{\cdot}0 \times 10^4$	55/90	61
3	1×10^5	0	6	$3{\cdot}0 \times 10^8$	0/60	0
		5	6	$1{\cdot}2 \times 10^6$	49/90	53
4	1×10^7	0	6	$4{\cdot}2 \times 10^8$	0/90	0
		5	6	$2{\cdot}4 \times 10^6$	36/90	40

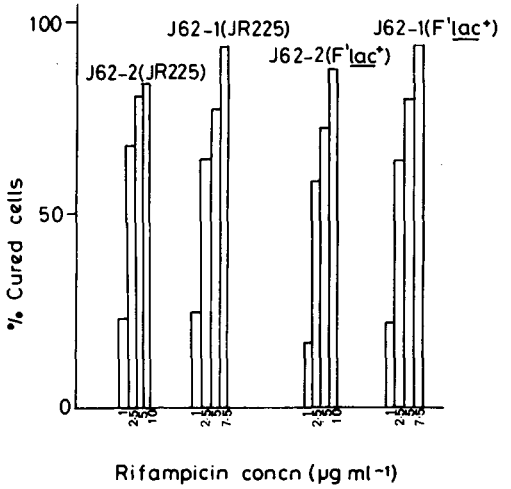

FIG. 1. Curing of R-plasmid JR225 and F'-plasmid F'*lac*+/R386 from rifampicin-sensitive, J62-1, and rifampacin-resistant, J62-2, hosts by varying concentrations of rifampicin after 6 h of growth.

Reversion of cured cells and infection of cured cells with R-plasmid. Reversion of the cured cells to antibiotic resistance was investigated by determining the susceptibility of the cured cells to ampicillin, gentamicin, streptomycin and nitrofurantoin compared to J62-1 and J62-2 as the R^- reference strains. The MIC of the test antibacterial agents to the cured cells and the reference F^- strains were similar. The cured cells were reinfected with R-plasmids JR225 and R222, with about the same frequency of 79–83% as obtained from the infection of J62-1 and J62-2 recipient strains.

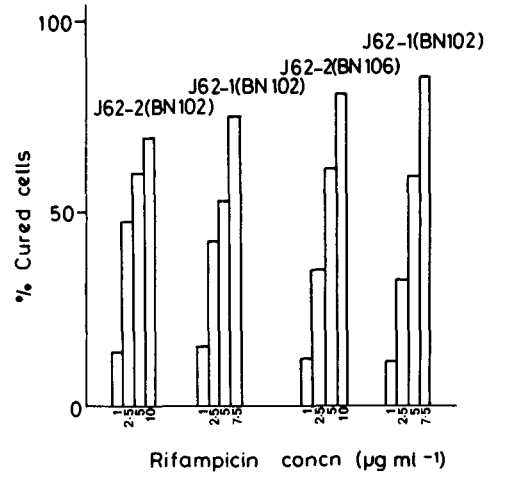

FIG. 2. Curing of R-plasmids BN102 and BN106 from rifampicin-sensitive, J62-1, and rifampicin-resistant, J62-2, hosts by varying concentrations of rifampicin, after 6 h of growth.

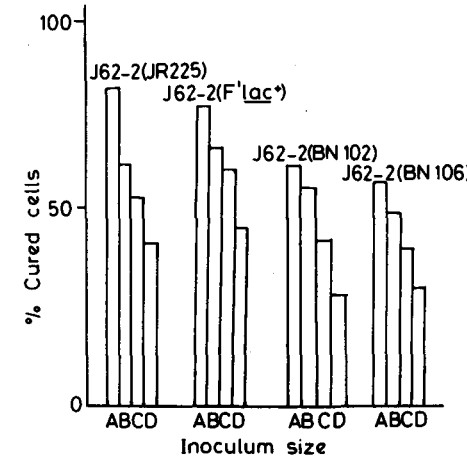

FIG. 3. Effect of inoculum size (c.f.u. ml^{-1}) on the rifampicin curing of the plasmids from rifampicin-resistant host, J62-2, by 5 μg ml^{-1} rifampicin after 6 h of growth: A, 1×10^3; B, 5×10^3; C, 1×10^5; D, 1×10^7.

Rifampicin susceptibility of the cured and uncured cells. The levels of the rifampicin susceptibility of J62-1 (JR225), J62-2 (JR225), J62-1 (F^+*lac*$^+$), J62-2 (F^+*lac*$^+$) cells and the respective cured cells were compared. The MIC of rifampicin to the rifampicin-sensitive and rifampicin-resistant strains were the same irrespective of the presence of the R-plasmids or F'-plasmids.

Discussion

The results indicate that rifampicin curing requires actively growing cells and does not affect R-plasmid reinfection of the cured cell, and that the R-plasmid susceptibility profile to its curing action varies, e.g. R-plasmids such as R222 and RP4 were not eliminated from host cells. Rifampicin curing might be impairing R-plasmid mRNA synthesis without affecting the rifampicin-resistant host cell RNA synthesis.

Rifampicin was reported to eliminate F'*lac*$^+$ plasmid from rifampicin-sensitive *E. coli* without affecting the F'*lac*$^+$ in rifampicin-resistant *E. coli* (Bazzicalupo & Tocchini-Valentini 1972). These results show that F'*lac*$^+$ plasmid could be cured either from rifampicin-sensitive or -resistant strains. Chromosomal resistance of the host cell does not seem to affect the curing effect of rifampicin, as the rate of curing JR225, F'*lac*$^+$. BN102 and BN106 from rifampicin-sensitive and rifampicin-resistant strains was similar. These results suggest that susceptibility of R-plasmids to rifampicin curing could be a property of the R-plasmid itself and explain why. R-plasmid BN171, conferring resistance to trimethoprim, sulphonamide, ampicillin, tetracycline and chloramphenicol, was only segregated in rifampicin-resistant host cell J62-2, by rifampicin (Obaseiki-Ebor 1983).

Receipt of University of Benin Staff research grant is acknowledged for supporting this work.

REFERENCES

Bazzicalupo, P., Tocchini-Valentini, G. P. (1972) Proc. Nat. Acad. Sci. USA 69: 298–300

Bremer, K., Pinney, R. J., Smith, J. T. (1973) J. Pharm. Pharmacol. 25: Suppl. 131P–132P

Coetzee, J. N., Datta, N., Hedges, R. W. (1972) J. Gen. Microbiol. 72: 543–552

Crumplin, G. C., Smith, J. T. (1981) J. Antimicrob. Chemother. 7: 379–388

Davis, B. D., Mingioli, E. S. (1950) J. Bacteriol. 60: 17–28

Datta, N., Hedges, R. W. (1972) J. Gen. Microbiol. 72: 349–355

di Mauro, E., Synder, L., Marino, P., Lambert, A., Coppo, A., Tocchini-Valentini, G. P. (1969) Nature (London) 222: 533–536

Jensen, K. (1975) in: Williams, J. D., Geddes, A. M. (eds) Chemotherapy, Vol. 1, Clinical aspects of infections. Plenum Press, New York and London, pp 43–47

Johnson, J. H., Richmond, M. A. (1970) J. Gen. Microbiol. 60: 137–139

Miller, J. H. (1972) Experiments in Molecular Genetics, Cold Spring Harbour Laboratory, Cold Spring Harbour, New York

Mitsuhashi, S., Harada, K., Kamuza, M. (1961) Nature (London) 189: 947

Morrison-Smith, J. (1975) J. Antimicrob. Chemother. 1: 353–354

Nessi, R., Fowst, G. (1979) J. Int. Med. Res. 7: 179–186

Obaseiki-Ebor, E. E. (1983) J. Pharm. Pharmacol. 35: 130–131

Pinney, R. J., Smith, J. T. (1971) Genetic Res. 18: 173–177

Simpson, A. M., Breeze, A. S. (1981) J. Appl. Bacteriol. 50: 469–474

Tomoeda, M., Inuzuka, M., Kubo, N., Nakamura, S. (1968) J. Bacteriol. 95: 1078–1089

Watanabe, T., Fukasawa, T. (1961) ibid. 81: 679–683

J. Pharm. Pharmacol. 1984, 36: 470–472
Communicated November 30, 1983

Effect of omeprazole and polyethylene glycol-400 on antipyrine elimination by the isolated perfused rat liver

LORRAINE K. WEBSTER, D. BRIAN JONES, GEORGE W. MIHALY, RICHARD A. SMALLWOOD*, *Gastroenterology Unit, University of Melbourne Department of Medicine, Austin Hospital, Heidelberg 3084, Victoria, Australia*

The effect of the substituted benzimidazole, omeprazole, a potent inhibitor of gastric acid secretion, on the hepatic elimination of antipyrine was studied in the rat isolated perfused liver. Bolus dosage (10 mg in 100 ml perfusate) and infusions (1 μg ml^{-1} perfusate concentrations) of omeprazole in its solvent, polyethylene glycol-400 (PEG-400), reduced antipyrine clearance by approximately one third ($P < 0.05$). PEG-400 alone caused a 15% decrease in antipyrine clearance ($P > 0.10$), indicating that the effect seen with omeprazole was at least partly due to the vehicle of dissolution. A significant but mild choleresis was noted in all preparations ($P < 0.01$) exposed to PEG-400. We conclude that the effect of omeprazole on hepatic drug elimination in patients warrants further study.

Omeprazole, a substituted benzimidazole, is a potent inhibitor of gastric acid secretion, acting by blocking the ($H^+ + K^+$) ATPase in the secretory canalicular membrane of the parietal cell (Olbe et al 1982). Benzimidazole derivatives have been shown to inhibit hepatic mixed function oxidases (MFO) (Murray et al 1982) and the potential inhibition of the metabolism of concurrently administered drugs by omeprazole may therefore be clinically important. In addition, the i.v. formulation for omeprazole may use polyethylene glycol (PEG-400) as the solvent, although the action of PEG-400 on the MFO enzyme system is unknown.

* Correspondence.

We have previously employed the rat isolated perfused liver to examine the effects of H_2-receptor antagonists on MFO activity (Mihaly et al 1982). Antipyrine undergoes extensive multi-pathway metabolism by hepatic MFO enzymes, and its clearance is widely accepted as an index of hepatic drug metabolizing enzyme activity (Vuitton et al 1981). The isolated liver is particularly suitable for the short term study of hepatic drug elimination, since the volume of the system is held constant, liver blood flow is precisely controlled and other routes of elimination present in the intact animal are excluded. The present study compares the effect of single doses and infusions of PEG-400 and omeprazole in PEG-400 on antipyrine elimination by the rat isolated perfused liver.

Materials and methods

Livers of male Sprague-Dawley rats (190–240 g) under ether anaesthesia were isolated by standard techniques (Gollan et al 1981), and perfused in a constant flow (16 ml min^{-1}) recirculating system at 37 °C (Mihaly et al 1982). The main indices of liver viability were steady oxygen consumption (1·5–2·0 μmol g^{-1} liver min^{-1}), sustained bile production (0·4–1·0 ml h^{-1}), constant perfusion pressure (6–8 cm water), and normal appearance on light microscopy.

Omeprazole (H168/68, 5-methoxy-2- [[(4-methoxy-