

SYNERGISTIC ACTION OF DIMETHOXYPHENYL-PENICILLIN AND CEPHALORIDINE AGAINST GRAM NEGATIVE BACTERIA

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It has been reported that dimethoxyphenyl-penicillin (DMP-PC) acts as powerful competitive inhibitor of hydrolysis of 7-Amino-Cephalosporinic acid (7-ACA) by the enzyme from a strain of *Pseudomonas aeruginosa*, though it itself was not hydrolysed at an appreciable rate. (L. D. SABATH *et al.*, Nature. 204: 1006, 1964) In the combination of these drugs synergistic action can be obtained from this strain

in vitro. In view of this idea, a few other investigations, using methicillin (DMP-PC) or Methylchlorophenylisoxazolyl-PC (MCI-PC) as competitive inhibitor and other penicillin derivatives or 7-ACA together, have been carried out, employing gram negative bacteria. (J. M. T. HAMILTON MILLER *et al.*, Nature. 201: 867, 1964) (R. SUTHERLAND *et al.*, Nature. 201: 868, 1964) We have been working *in vitro* on similar action of DMP-PC and Cephaloridine (CER) against many clinical organisms isolated from urinary tract infections which were highly resistant to CER. Among 120 strains of gram negative bacilli isolated from urinary tract infections at Keio University Hospital, resistant more than 250 mcg/ml of CER on plates, 16 strains were selected. The strains tested were 2 *E. coli*, 6 *Klebsiella*, 2 *Providencia*, 6 *Citrobacter*. *Pseudomonas* strains were eliminated because they produce heavy threads-like masses while shaking, making optical measurement of growth difficult. Penassay broth 'Difco' was used in both of precultures and shaking cultures. Overnight cultures in penassay broth were the inoculates. They were diluted into the media in 'L' tubes which were shaken at 37°C in the incubator. Bacterial growth was followed by optical density at 530 m μ with an electrophotometer.

Fig. 1 Typical bacteriolysis curves in combined culture

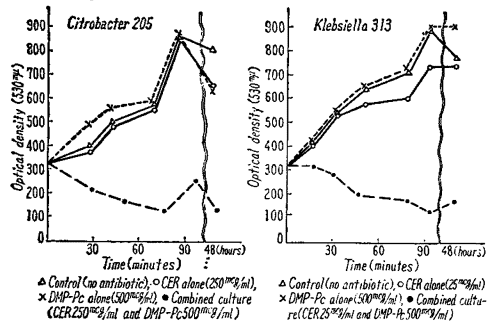


Table 1 The synergistic action of CER and DMP-PC against selected isolates.

MIC of CER (tube dilution method)	synergistic action			
	observed	No. of strains	not observed	No. of strains
500 \leq mcg/ml	<i>Klebsiella 313</i> <i>Providencia 702</i>	2	<i>E. coli 76</i> <i>Klebsiella 325</i> <i>Providencia 701</i>	3
250 \leq mcg/ml	<i>Citrobacter 205</i> <i>Citrobacter 209</i>	2	<i>Klebsiella 308</i>	1
100 \leq mcg/ml	<i>Citrobacter 208</i>	1	<i>Citrobacter 203</i>	1
50 \leq mcg/ml	<i>Klebsiella 307</i>	1	<i>E. coli 50</i>	1
25 \leq mcg/ml	<i>Citrobacter 207</i>	1	<i>Klebsiella 309</i> <i>Klebsiella 310</i>	2
10 \leq mcg/ml	<i>Citrobacter 206</i>	1		
Total		8		8

Drug free growth, growth in the tubes containing DMP-PC or CER alone were the controls. Concentrations of CER used were the highest ones which allow uninhibited growth in the preliminary experiments. 500 mcg/ml of DMP-PC were employed as potent enzyme inhibitor throughout this experiment. Bacteriolysis as a result of synergistic action was found in 8 strains among 16 isolates. (Table 1) Typical lysis curve is shown in Fig. 1. The *in vivo* experiments followed by clinical application are now being progressed.