ENHANCEMENT OF 5-FLUOROURACIL UPTAKE INTO THE BACTERIAL CELL BY PIPERACILLIN

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The antibacterial activity of 5-fluorouracil, an antineoplastic agent, was found to be enhanced by piperacillin, a β-lactam antibiotic, resulting in a synergism. It was considered that the synergism was caused by an increase of 5-fluorouracil uptake into the bacterial cell. On the other hand, amount of piperacillin bound to penicillin binding protein (PBP) did not change when 5-fluorouracil was added. The morphological change of the cell was due to the 5-fluorouracil when synergism appeared between 5-fluorouracil and piperacillin.

5-Fluorouracil, an anti-neoplastic agent, has been reported to have antibacterial activity against Gram-negative bacteria[1]. This activity is considered to be due to an inhibitory action against thymidylate synthetase[2]. Further, as reported previously[3], some antineoplastic agents exhibited synergism with piperacillin, a β-lactam antibiotic. In this study, regarding to 5-fluorouracil particularly, we observed that the incorporation of 5-fluorouracil into the bacterial cell is promoted by piperacillin.

Materials and Methods

Chemicals

5-Fluorouracil (Fuji Chemical Co., Ltd.) and piperacillin (Toyama Chemical Co., Ltd.), as well as 5-fluoro[6-^{3}H]uracil (Amersham International, England, 2.1 Ci/mmol) and benzyl[^{14}C]penicillin potassium (Amersham, 58.9 mCi/mmol) were used.

Bacterial Strains

Escherichia coli TK-54, *Klebsiella pneumoniae* Y-40, *Pseudomonas aeruginosa* S-340, and *P. aeruginosa* S-344 were used. These strains were clinical isolates.

Antibacterial Activity and Synergy Studies

Minimal inhibitory concentrations (MICs) of piperacillin, 5-fluorouracil, and these drugs in combination were determined by the 2-fold dilution method using Mueller-Hinton agar (Eiken Co., Ltd.). The bacteria were cultured overnight in Mueller-Hinton broth (Difco Laboratories) at 37°C, and one loopful of diluted pre-culture (1/100) was inoculated on the agar plate (approximately 10^{4} cfu). After overnight incubation at 37°C, the MICs were determined. To compare the combination effect, we calculated the fractional inhibitory concentration (FIC) index for each strain according to the following formulas[4,5]. FIC=MIC of the drug in combination/MIC of the drug alone. FIC index= the sum of the FICs for each drug in combination. Synergy was defined as an FIC index ≤ 0.5, and antagonism as an FIC index >2.

Incorporation of 5-Fluoro[^{3}H]uracil into the Bacterial Cell

The strains were grown in Brain-Heart Infusion broth (Eiken Co., Ltd.) at 37°C for 18 hours.

The culture broth was inoculated into 200 ml fresh Brain-Heart Infusion broth, and incubated until the concentration reached about 10^8 cells per ml ($OD_{530}=0.35$). Immediately after that, 50 μl of 5-fluoro[6-^{3}H]uracil (0.2 μCi/ml) and different concentration of piperacillin were added to 5 ml of culture. After being kept at 37°C for 10, 20, 30 and 60 minutes, the cells were collected by centrifugation (7,000 rpm, 20 minutes, 4°C), and washed with 0.01 M phosphate buffer (pH 7.0). The cells were dissolved with 1 ml of Soluen 100 (Packard). The incorporation of 5-fluoro[^{3}H]uracil was determined by liquid scintillation counting (Packard TRL-CABB 460 CD).

Binding of Piperacillin to Penicillin Binding Protein (PBP)

As described above, 200 ml of fresh culture of *E. coli* TK-54 was prepared. One ml of piperacillin and, in some experiments, 5-fluorouracil were added to the culture. After being kept at 37°C for 20 minutes, the cells were collected by centrifugation (7,000 rpm, 20 minutes, 4°C). The subsequent processing was carried out at 4°C. The cells washed twice with 0.01 M phosphate buffer (pH 7.0). Then, the cells, suspended in 0.01 M phosphate buffer (pH 7.0) with 0.01 M EDTA, were sonicated. The preparation was centrifuged at 7,000 rpm for 50 minutes. The supernatant was collected and ultra-centrifuged (42,500 rpm, 40 minutes). The pellet of membrane protein was collected. The protein concentration was determined by the method of LOWRY *et al.*[6]. The solubilized (2% Triton X-100) membranes were incubated at 30°C with benzyl[^{14}C]penicillin potassium for 10 minutes. PBP was visualized after SDS-polyacrylamide gel electrophoresis[7] and fluorography[8]. The densitometry was carried out with a Fuji FD A-4 scanner.

Morphological Effect

Morphological effect was observed on *E. coli* TK-54. The concentration of piperacillin was 0.05 μg/ml, and of 5-fluorouracil 50 μg/ml (alone) or 3.13 μg/ml (in combination with piperacillin).

Results

Antibacterial Activity and Synergy Studies

The interaction of 5-fluorouracil and piperacillin against *E. coli* TK-54, *K. pneumoniae* Y-40 and *P. aeruginosa* S-340, S-344 is shown in Table 1.

An FIC index of all strains used were <0.5. Synergism was thus observed between 5-fluorouracil and piperacillin in all cases.

Incorporation of 5-Fluoro[^{3}H]uracil into the Bacterial Cell

The 5-fluoro[^{3}H]uracil uptake into the cell was observed at 10, 20, 30 and 60 minutes. At 20 minutes, 5-fluoro[^{3}H]uracil uptake was about 1.4×10^3 dpm per 10^6 cells. The uptake was increased by the presence of piperacillin at sub-MIC (0.05 μg/ml) (3.9×10^3 dpm per 10^6 cells) (Fig. 1).

When 5-fluoro[^{3}H]uracil incorporation at 20 minutes was examined for other strains in the presence of piperacillin, the uptake was increased about 2.0~3.0-fold as compared with 5-fluoro[^{3}H]-

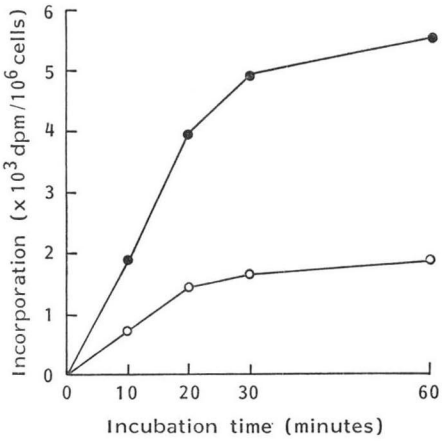
Table 1. Interaction between piperacillin and 5-fluorouracil.

Strain	Piperacillin MIC[a]		5-Fluorouracil MIC		FIC index
	Alone	In combination with 5-fluorouracil	Alone	In combination with piperacillin	
Escherichia coli TK-54	0.1	0.025	25	3.13	0.38
Klebsiella pneumoniae Y-40	50	3.13	100	12.5	0.25
Pseudomonas aeruginosa S-340	6.25	0.78	≥400	12.5	≤0.16
P. aeruginosa S-344	12.5	1.56	200	6.25	0.16

[a] μg/ml.

Fig. 1. Enhancement by piperacillin of 5-fluoro-[6-^{3}H]uracil incorporation in *Escherichia coli* TK-54 cells.

●: Piperacillin 0.05 μg/ml, ○: control.

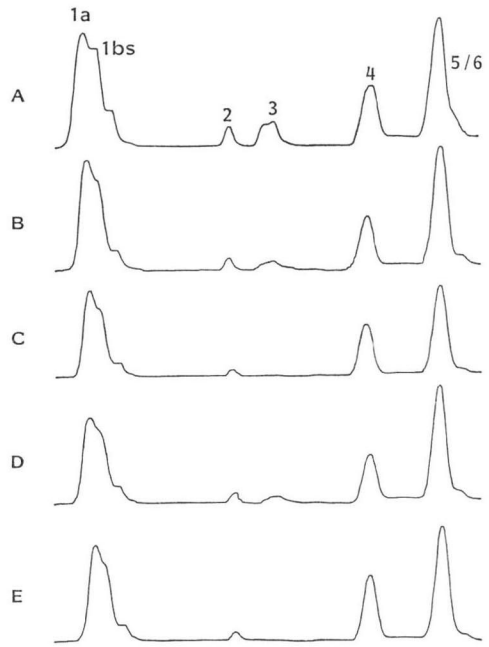


uracil alone (Table 2).

For *P. aeruginosa* S-340, the 5-fluoro[^{3}H]-uracil uptake into the cell was 1.4×10^3 dpm per 10^6 cells in the absence of piperacillin. Sub-MIC (1.56 μg/ml) of piperacillin enhanced the uptake to 4.1×10^3 dpm per 10^6 cells.

Fig. 2. Affinity of piperacillin to penicillin binding protein of *Escherichia coli* TK-54.

A: Control, B: piperacillin 0.025 μg/ml, C: piperacillin 0.05 μg/ml, D: piperacillin 0.025 μg/ml+5-fluorouracil 6.25 μg/ml, E: piperacillin 0.05 μg/ml+5-fluorouracil 6.25 μg/ml.



Binding of Piperacillin to PBP

The affinity of piperacillin to PBP was examined in the presence of 5-fluorouracil, and compared with that of piperacillin alone. The result is shown in Fig. 2.

According to the concentration of piperacillin, the binding amount of benzyl[^{14}C]penicillin potassium to PBP 1a, 1bs and 3 decreased. That is to say, piperacillin bound to PBP increased. But the binding of piperacillin was little affected by sub-MIC (6.25 μg/ml) of 5-fluorouracil.

Table 2. Effect of piperacillin on the incorporation of 5-fluoro[6-^{3}H]uracil into bacterial cells.

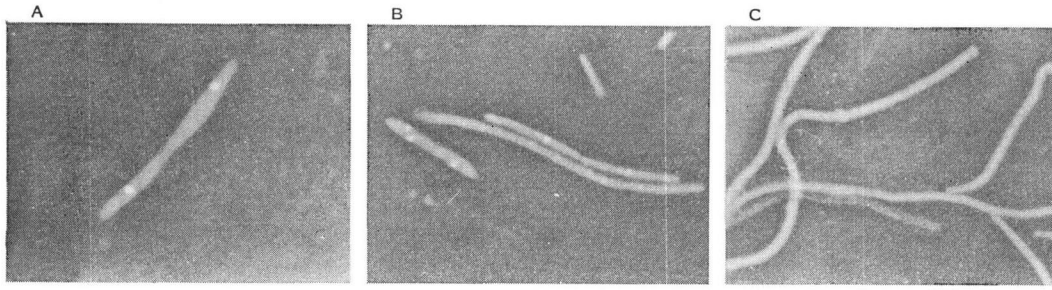
Strain	Piperacillin concentration[a]					
	0	0.00625	0.0125	0.025	0.05	
Escherichia coli TK-54	1,353±68[b]	2,038±35	2,069±35	3,601±90	3,603±233	
Strain	Piperacillin concentration[a]					
	0	0.39	0.78	1.56	3.13	6.25
Klebsiella pneumoniae Y-40	1,339±51	—	1,903±81	2,259±202	2,679±368	3,054±62
Pseudomonas aeruginosa S-340	1,378±162	1,816±44	2,515±189	4,102±63	3,644±266	—
P. aeruginosa S-344	1,804±92	—	2,106±69	2,155±104	4,102±63	3,644±266

[a] Final concentration in test tube (μg/ml).

[b] dpm per 10^6 cells.

Fig. 3. Morphological effect on *Escherichia coli* TK-54 by 5-fluorouracil alone or in combination with piperacillin.

A: 5-Fluorouracil 50 μg/ml, B: 5-fluorouracil 3.13 μg/ml+piperacillin 0.05 μg/ml, C: piperacillin 0.05 μg/ml.



Morphological Effect

When 2×MIC (50 μg/ml) of 5-fluorouracil was added, the cells were changed into round shape and slight elongation, and subsequently killed. When the combination of piperacillin (0.05 μg/ml) and 5-fluorouracil (3.13 μg/ml) was added, the cells were more elongated or changed into round shape. On the other hand, the cells were still more elongated by piperacillin alone (0.05 μg/ml) (Fig. 3). 5-Fluorouracil did not act at all on the cells at the concentration of 3.13 μg/ml.

Discussion

5-Fluorouracil, an antineoplastic agent, has antibacterial activity itself[1], and exhibits synergism with β-lactam antibiotics[3]. However, MANTEN and TERRA[9] described antagonism or indifference but no synergism by combination of mitomycin C or actinomycin with β-lactam antibiotics, when tested against *E. coli* and other Gram-negative bacteria.

In our experiments, 5-fluorouracil exhibited some antibacterial activity, and strong synergism with piperacillin. Furthermore, in a strain in which synergism was recognized, the incorporation of 5-fluoro[^{3}H]uracil into the cell was enhanced by piperacillin. On the other hand, the binding amount of piperacillin to PBP was not changed by 5-fluorouracil. The reason for the increase of 5-fluorouracil uptake is not known. However, it was considered that the increase of 5-fluorouracil uptake in short periods, resulted in a lowering of the partial MIC. The degree of the increase tend to raise near the MIC of piperacillin. As to the morphological effect, when 5-fluorouracil was combined with piperacillin, the characteristic morphology change by 5-fluorouracil, rounding and slight elongation, appeared at a concentration in which 5-fluorouracil alone did not act. Piperacillin caused a change to still more elongated shape at this concentration. This result proved that the 5-fluorouracil uptake into the bacterial cell was enhanced by piperacillin. The increase in permeability by penicillin was often discussed, for example, regarding to the case of penicillin and aminoglycoside antibiotics, but there is no report for permeability relating to the case of penicillin and 5-fluorouracil. The molecular weight of 5-fluorouracil was 130.08. So it is considered that 5-fluorouracil can pass through the porin of Gram-negative bacteria. It is necessary to shed light on the reason for the increase of 5-fluorouracil uptake.

Cancer patients are compromised hosts and are liable to infections with Gram-positive and Gram-negative bacteria or other organisms. Consequently, it was considered to be interesting that 5-fluorouracil used in cancer therapy exhibits synergism with an antibacterial agent, and that the synergism results in an increase of the antibacterial activity of 5-fluorouracil.

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