NOTES

SYNERGISM BETWEEN EFROTOMYCIN AND BOTTROMYCIN

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Efrotomycin[1] is a narrow spectrum antibiotic which is most active *in vitro* against representative species of *Pasteurella, Moraxella* and *Corynebacterium*[2]. The *in vivo* activity, however, is much better than one would expect from the *in vitro* data[2]. Experimental test systems indicate efrotomycin has a potential in selected animal infections and as a growth permittent. For example, STUTZ *et al.*[3] have shown efrotomycin to be effective as a growth permittant for grower swine. FOSTER and HARRIS[4] have reported the drug to show promise for prophylactic control of swine dysentery in experimental infections incited by *Treponema hyodysenteriae*. Some antibiotics which act by inhibition of protein synthesis have been tested in our laboratory in combination with efrotomycin. This paper will be confined to experiments with combinations of efrotomycin and bottromycin[5].

The *in vitro* antibacterial activities of efrotomycin and bottromycin alone and in combination were determined as described previously[2]. Briefly, the surface of agar plates containing drugs were spot inoculated with 10^{-3} broth dilutions of 16-hour broth cultures. Growth was observed after 24 hours at 37°C. Sodium efrotomycin and the tertiarybutylamide of bottromycin were used in these experiments. The data are summarized in Table 1 which shows only the point of maximum synergy. Our use of the term synergy is in agreement with that of KERRY *et al.*[6] The synergistic effect of the two antibiotics is apparent when at least one of the drugs is active against the test organism. Synergy, where observed, was reciprocal.

Table 1. *In vitro* antibacterial activity of efrotomycin and bottromycin alone and in combination.

Test organism	MIC[a] μg/ml		ΣFIC[b]	Test organism	MIC[a] μg/ml		ΣFIC[b]
	E	B			E	B	
Bordetella bronchiseptica F1728	200	100	0.38	*Escherichia coli* 3386	>400	>200	G
Bordetella bronchiseptica 74	200	100	0.38	*Klebsiella pneumoniae* 3068	200	200	<0.50
Bordetella bronchiseptica B	200	100	0.38	*Pasteurella hemolytica* 67	25	6.25	0.62
Bordetella bronchiseptica 48	100	100	0.31	*Pasteurella hemolytica* 6	25	12.5	0.38
Bordetella bronchiseptica 25	400	100	0.38	*Pasteurella hemolytica* 13	12.5	12.5	<0.53
Bordetella bronchiseptica 26	200	100	0.50	*Pasteurella multocida* 86	3.12	3.12	0.75
Bordetella bronchiseptica 65	200	200	0.50	*Pasteurella multocida* 89	12.5	6.25	0.62
Corynebacterium renale 3164	6.25	6.25	<0.06	*Pasteurella multocida* 2869	25	6.25	0.50
Enterococcus sp. 198	>400	3.12	<0.31	*Pasteurella multocida* 1590	6.2	3.12	0.56
Erysipilothrix rhuziopathiae 87193	400	1.56	0.16	*Pasteurella multocida* 2871	12.5	6.25	0.50
Escherichia coli 3307	400	200	0.50	*Pasteurella multocida* 2873	12.5	6.25	0.62
Escherichia coli 3317	>400	6.25	<0.19				

(to be continued)

Table 1. (Continued)

Test organism	MIC[a] μg/ml		ΣFIC[b]	Test organism	MIC[a] μg/ml		ΣFIC[b]
	E	B			E	B	
Pasteurella multocida 2909	12.5	6.25	0.50	*Staphylococcus aureus* 2957	>400	3.12	<0.38
Pseudomonas aeruginosa 3210	>400	>200	G	*Staphylococcus aureus* Smith	>400	3.12	<0.38
Pseudomonas aeruginosa 3301	>400	>200	G	*Streptococcus agalactiae* 1934	12.5	0.39	0.38
Salmonella cholerae-suis	>400	>200	<0.75	*Streptococcus pneumoniae* 3273	25	0.39	0.25
Salmonella decatur	>400	>200	G	*Streptococcus pyogenes* 3332	1.56	0.097	0.62
Salmonella schottmuelleri 3010	400	200	0.50	*Streptococcus pyogenes* 1685	25	0.78	0.14
Salmonella typhimurium 3404	>400	>200	G	*Streptococcus pyogenes* C203	12.5	0.39	0.28
Serratia marcescens 1543	400	>200	G	*Yersinia pseudotuberculosis* 275	25	100	0.62

a: MIC=minimal inhibitory concentration.
b: ΣFIC=sum of fractional inhibitory concentration at the point of maximum synergy. Synergy≦0.7[6].
G=growth

Table 2. Efficacy of varied concentrations of efrotomycin given alone or in combination with two fixed concentrations of bottromycin against a *Bordetella bronchiseptica* infection[a].

Efrotomycin mg/dose	No. of survivors/total infected mice treated with		
	Efrotomycin alone	Efrotomycin plus bottromycin[b]	
		10 mg/dose	5 mg/dose
0	0/12	5/12	0/12
0.125	NT[c]	4/6	NT
0.25	0/12	9/12	6/12
0.5	0/12	11/12	5/12
1.0	1/12	12/12	8/12
2.0	4/12	11/12	11/12
4.0	10/12	12/12	11/12
ED_{50}[d]	2.47	0.163+ 10 mg bottromycin	0.551+ 5 mg bottromycin

a=Infection produced by intraperitoneal injection of a broth dilution of a 16-hour culture. Treatment given by gavage 0 and 6 hours after infecting. Data are combined from two tests.
b=Impure material which contained approximately 40% bottromycin by weight.
c=Not tested
d=ED_{50}, calculated by method of REED and MUNCH,[16] is given as mg efrotomycin per dose.

Table 3. Efficacy of varied concentrations of bottromycin alone and in combination with two fixed concentrations of efrotomycin against a *Bordetella bronchiseptica* infection[a].

Bottromycin[b] mg/dose	No. of survivors/total infected mice treated with		
	Bottromycin alone	Bottromycin plus efrotomycin	
		1 mg/dose	0.5 mg/dose
Test 1[c]			
0	0/6	0/6	0/6
2.5	NT[d]	4/6	1/6
5	1/6	5/6	3/6
10	3/6	6/6	5/6
20	1/6	6/6	4/6
Test 2[e]			
0	0/6	0/6	0/6
4	0/6	0/6	0/6
8	0/6	3/6	0/6
16	0/6	5/6	1/6

a=Infecting challenge and treatment done as in Table 2. Mice in both tests 1 and 2 received approximately 14 LD_{50} doses of *B. bronchiseptica*.
b=Material which was approximately 40% bottromycin by weight.
c=ED_{50} for efrotomycin alone was 3.59 mg×2 doses.
d=Not tested
e=ED_{50} for efrotomycin alone was 2.83 mg×2 doses.

The efficacy of combined oral therapy with efrotomycin and bottromycin in *Bordetella bronchiseptica* infections has been studied by administration of a constant concentration of one drug while varying the concentration of the other drug.

Data from experiments in which bottromycin was held constant in combination with different concentrations of efrotomycin are summarized in Table 2. Both concentrations of bottromycin in combination with varied doses of efrotomycin gave a marked reduction in the amount of efrotomycin required for 50% protection (ED_{50}).

The results of experiments in which fixed concentrations of efrotomycin were combined with different concentrations of bottromycin are summarized in Table 3. We were unable to determine the ED_{50} for bottromycin alone because of the lack of pure drug. However, with the impure sample available for testing, it seems probable that the ED_{50} is considerably greater than two oral doses of 20 mg per mouse. This dosage level cannot be considered toxic as it was well tolerated by mice which received oral doses of efrotomycin. Bottromycin alone gave an erratic dose response in the first experiment and did not protect any of the infected mice in the second trial even though the challenge infections in both tests were approximately the same. However, in both tests combination of bottromycin with 1 mg doses of efrotomycin resulted in many more survivors than with bottromycin alone. The data in Table 3 support the *in vivo* synergy of efrotomycin and bottromycin. Insufficient supply of bottromycin prevented further *in vivo* trials with *B. bronchiseptica* or other infections.

The best explanation for the observed synergy of efrotomycin and bottromycin is that they act at close metabolic sites. At the beginning of this study, the mode of action of kirromycin was known[7]. This agent inhibits peptide bond formation by acting on elongation factor (EF-Tu)[8]. Efrotomycin, which is closely related structurally to kirromycin[9], since has been found to inhibit EF-Tu dependent reactions[10]. Bottromycins are a group of closely related antibiotics[11]. One of these, bottromycin A_2, was reported prior to this study to be an inhibitor of protein synthesis[12]. The antibiotic also was shown to inhibit translocation of peptidyl-tRNA by interacting with the large ribosomal subunit[13,14]. More recently bottromycin A_2 has been reported to cause release of aminoacyl or peptidyl-tRNA from the A site[15]. Thus, these closely related activities of efrotomycin and bottromycin could result in synergy.

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