IN VITRO ACTIVITY OF U-57930E AGAINST ANAEROBIC BACTERIA AND ITS COMPARISON WITH CLINDAMYCIN, AMPICILLIN, CARBENICILLIN AND TETRACYCLINE

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The *in vitro* activity of U-57930E, a pipecolic acid amide of clindamycin, was compared with those of clindamycin, ampicillin, carbenicillin and tetracycline against 321 anaerobic clinical isolates. The MIC (μg/ml) of U-57930E that inhibited 95% *Bacteroides fragilis, Peptococcus prevotii, B. melaninogenicus* and *P. asaccharolyticus* was 0.0625; 0.03125 for *Peptostreptococcus anaerobius, B. vulgatus, Propionibacterium* and *Peptococcus* species. Clindamycin, on the other hand, gave MIC values of 0.5 μg/ml for *B. fragilis, P. prevotii* and *P. asaccharolyticus*, 0.25 for *Propionibacterium* sp. All strains of *Clostridium perfringens* were inhibited by 0.5 μg/ml of U-57930E. Both clindamycin and U-57930E showed similar MIC values for all strains of *Fusobacterium nucleatum* and *Propionibacterium acnes* tested. The MIC values for ampicillin, carbenicillin and tetracycline were within the expected range. U-57930E had a 4~8 fold lower MIC than clindamycin and is significantly active against anaerobic bacteria.

The frequency and severity of infections caused by anaerobic bacteria have generated interest in development and evaluation of antimicrobial agents for their treatment. The commonly used antimicrobials include benzylpenicillin, ampicillin, carbenicillin, clindamycin, lincomycin, chloramphenicol, tetracycline and metronidazole. Since *Bacteroides fragilis*, the most frequently isolated organism from anaerobic infections is generally resistant to penicillins, the treatment regimen would include other broad spectrum antibiotics. Two antibiotics that have shown consistent clinical promise against *B. fragilis* and other anaerobes are clindamycin and chloramphenicol. However, these and other chemotherapeutic agents have been associated with serious side effects, especially antibiotic associated colitis[1~4]. This has led to a search for effective yet nontoxic or less toxic drugs for the eradication of anaerobes.

U-57930E is a new clindamycin analog which was tolerated well in rodents (LEWIS *et al.*, Abst. Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA. 1980, #66). We have recently investigated the *in vitro* activity of U-57930E against a variety of anaerobic bacteria and compared it with clindamycin, ampicillin, carbenicillin and tetracycline. This report presents results of these studies.

Materials and Methods

Test Organisms

Three hundred twenty-one isolates of anaerobic bacteria comprising of Gram-positive cocci, Gram-

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negative bacilli, Gram-positive spore-forming and nonspore-forming bacilli were used in this study. All organisms were isolated from clinical specimens at the Microbiology Laboratories of Oklahoma Memorial Hospital and Clinics, Children's Memorial Hospital and Veterans' Administration Medical Center, Oklahoma City, Oklahoma. These isolates were identified by standard and established procedures in each laboratory[5,6]. All strains were grown on enriched blood agar (Brucella agar base) containing hemin (5 μg/ml) and vitamin K_1 (10 μg/ml) at 35°C for 48 hours in an anaerobic chamber (Coy Manufacturing Co., Ann Arbor).

Antibiotics

Laboratory standard powders of various antibiotics were provided as follows: U-57930E and clindamycin (Upjohn Co., Kalamazoo); carbenicillin (Roerig, New York); tetracycline and ampicillin (Bristol Lab., Syracuse). Stock solutions were prepared by dissolving measured amounts of antibiotics in sterile distilled water and stored at −70°C. Just prior to use, the solutions were thawed, appropriate dilutions were made and incorporated into media.

Antimicrobial Susceptibility Testing

Agar dilution tests to determine MIC's were performed according to the method of MARTIN, GARDNER and WASHINGTON[7]. Sets of 3 agar plates were prepared for each dilution of antibiotic. The media was supplemented with vitamin K (10 μg/ml), hemin (5 μg/ml) and 5% defibrinated sheep blood. Just prior to pouring the plates, 2 ml of appropriate dilution of each antibiotic was added to 18 ml of supplemented medium to obtain a final antibiotic concentration of 0.0156 to 128 μg/ml. All plates were prereduced for 6 hours by placing them in the anaerobic chamber. The test organisms were grown for 48 hours in enriched thioglycollate broth and the cell density was adjusted equivalent to a 0.5 McFarland standard. The STEER's replicator technique was used for inoculation on plates[8]. The inoculated plates were allowed to dry and incubated at 35°C in anaerobic chamber for 48 hours. The plates were read at 24 and 48 hours. The minimum inhibitory concentration (MIC) was read as the least concentration of antibiotic showing no growth on the plate.

Quality control of media and reagents was performed according to standard laboratory procedures. *Bacteroides fragilis* ATCC 25285 and *Clostridium perfringens* ATCC 13124 were used as control organisms for testing MIC's.

Results

A total of 321 clinical anaerobic isolates were used to evaluate the *in vitro* activity of U-57930E, and the results were compared with those of clindamycin, ampicillin, carbenicillin and tetracycline. Table 1 illustrates the comparative activity of U-57930E and clindamycin against anaerobic bacteria. At a concentration of 0.25 μg/ml U-57930E inhibited over 95% of the strains of anaerobic isolates tested. The only exceptions were *Propionibacterium acnes* (25%), *Bacteroides oralis* (44%), *Fusobacterium nucleatum* (94%) and *Clostridium perfringens* (92%). More than 95% of the different strains of *Bacteroides fragilis, B. vulgatus, B. asaccharolyticus, B. melaninogenicus* subsp. *intermedius* and *Fusobacterium* sp. were inhibited by 0.0625 μg/ml of U-57930E. The susceptibility patterns of *B. oralis* and *F. nucleatum* were almost identical at 0.5 μg/ml. Clindamycin was less active than the new derivative and inhibited over 90% of most *Bacteroides* species and *F. nucleatum* at a concentration of 0.5 μg/ml. Four *Fusobacterium* strains remained resistant at this concentration.

At 0.5 μg/ml, U-57930E inhibited 98.4% of the 321 clinical isolates tested. On the other hand clindamycin at 0.5 μg/ml inhibited 90.6% of these bacteria. The activities of ampicillin, carbenicillin and tetracycline against the anaerobes were determined throughout the study. Results are shown in Table 2. Ampicillin was found to be effective against most of the strains except *B. fragilis* at 16 μg/ml or less. Carbenicillin inhibited all but 50% of *B. fragilis* at 16 μg/ml or less. Most of the anaerobes tested demonstrated the expected patterns of susceptibility and resistance to tetracycline[9].

Table 1. MIC of U-57930E and clindamycin (CC) against anaerobic bacteria.

Organisms	No. tested	Cumulative numbers (%) inhibited by various concentrations (μg/ml)									
		0.03125		0.0625		0.1250		0.250		0.50	
		U-57930E	CC	U-57930E	CC	U-57930E	CC	U-57930E	CC	U-57930E	CC
B. fragilis	54	28 (52)	0	51 (95)	1 (2)	52 (96)	38 (70)	53 (98)	42 (78)	53 (98)	51 (95)
B. oralis	23	0	0	0	0	2 (8)	0	10 (44)	8 (35)	22 (95)	21 (91)
B. vulgatus	23	22 (96)	0	22 (96)	0	22 (96)	15 (65)	22 (96)	17 (74)	22 (96)	19 (83)
B. asaccharolyticus	5	1 (20)	0	5 (100)	0	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)
B. melaninogenicus subsp. *intermedius*	41	5 (12)	0	39 (95)	2 (5)	40 (97)	37 (90)	41 (100)	40 (97)	41 (100)	41 (100)
Fusobacterium sp.	9	8 (81)	0	9 (100)	0	9 (100)	0	9 (100)	1 (11)	9 (100)	5 (55)
F. nucleatum	16	0	0	0	0	4 (25)	4 (25)	15 (94)	14 (87)	15 (94)	15 (94)
C. perfringens	22	0	0	1 (5)	0	15 (70)	12 (55)	20 (92)	13 (60)	22 (100)	16 (75)
Propionibacterium sp.	4	4 (100)	0	4 (100)	0	4 (100)	2 (50)	4 (100)	4 (100)	4 (100)	4 (100)
P. acnes	16	0	0	0 (00)	0	2 (16)	2 (16)	4 (25)	3 (19)	12 (70)	12 (70)
Peptococcus asaccharolyticus	50	47 (94)	0	49 (98)	0	50 (100)	32 (64)	50 (100)	47 (94)	50 (100)	48 (96)
P. prevotii	16	6 (26)	0	15 (95)	1 (6)	15 (95)	10 (62)	16 (100)	12 (80)	16 (100)	15 (95)
Peptococcus sp.	3	3 (100)	0	3 (100)	0	3 (100)	1 (33)	3 (100)	2 (66)	3 (100)	2 (66)
Peptostreptococcus anaerobius	29	28 (96)	0	28 (96)	0	29 (100)	24 (82)	29 (100)	25 (87)	29 (100)	27 (95)
Peptostreptococcus intermedius	10	10 (100)	0	10 (100)	5 (50)	10 (100)	8 (80)	10 (100)	9 (90)	10 (100)	10 (100)
B. fragilis ATCC 25285				1 (100)							1 (100)
C. perfringens ATCC 13124	1		0	0	0	1 (100)			1 (100)		

Table 2. MIC values of ampicillin, carbenicillin and tetracycline.

Organism	No. tested	Cumulative strains (%) inhibited by: (μg/ml)															
		Ampicillin					Carbenicillin					Tetracycline					
		2	4	8	16	32	4	8	16	32	64	0.5	1	2	8	16	32
B. fragilis	54			41	60	81			50	70	72				46	70	95
B. oralis	23			52	80	92			100						91	96	100
B. vulgatus	23			56	79	92			100						87	91	100
B. asaccharolyticus	5	80	80	80	100		80	80	100			60	80	100			
B. melaninogenicus subsp. *intermedius*	41	75	83	90	97	97	80	90	97	97	100	63	75	97	100		
F. nucleatum	16			100					94	100					94	100	
Fusobacterium sp.	9			100					100						100		
C. perfringens	22	90	100				90	100				75	85	92	95	95	100
Propionibacterium sp.	4	100					100					50	100				
P. acnes	16	94	100				100					31	80	100			
P. asaccharolyticus	50	100					100					36	50	80	80	80	94
P. prevotii	16	100					100					37	62	87			94
Peptococcus sp.	3	100					100					33	33	100			
P. anaerobius	29	100					100					41	52	70	83	90	100
P. intermedius	10	100					100					40	60	80	90	100	

Discussion

Antibiotic associated colitis is regarded as a severe gastrointestinal disease, which has been the subject of considerable concern in recent years[1,10,11]. The evidence was based on animal models in which it was shown that the antimicrobial agents given to hamsters produced lethal colitis[2,12,13]. Recent implications of clindamycin, ampicillin, cephalosporins, tetracycline and metronidazole associated colitis in humans[14] prompted an intensive search for alternative antimicrobial agents. Initial animal studies on U-57930E appear to offer promise (personal communication). This pipecolic acid amide analog of clindamycin was found to be more inhibitory against the clinical anaerobic isolates than the 5 antibiotics investigated in this study. DHAWAN, BANSAL and THADEPALLI (Abst. Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL. 430, 1981) also reported it to be more effective than clindamycin and cefoxitin against 138 clinical isolates of *Bacteroides*. They found that U-57930E was highly inhibitory towards strains of *B. fragilis*, including those that were cefoxitin resistant, and reported that 91% of strains of *B. fragilis* had an MIC of 4 μg/ml. Their findings were similar to ours except that we found the MIC values to be lower. This discrepancy may result from the variation in bacterial population, hospital environment or methodology used in MIC determinations.

Our work confirms the activities of the more frequently used antibiotics, such as ampicillin, carbenicillin, tetracycline and clindamycin, against anaerobic bacteria[3,9,15]. Of all the antibiotics tested *in vitro* U-57930E and clindamycin were found to be most effective. However, U-57930E showed consistently lower MIC's than clindamycin or equivalent ones against all the strains of anaerobes tested. It was also found to be superior to ampicillin, carbenicillin and tetracycline in its ability to inhibit anaerobic organisms at lower concentrations. Clinical studies are warranted to determine its potential in the treatment of anaerobic infections and its possible side effects in humans, if any.

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