ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1995, p. 94–98 Vol. 39, No. 1
0066-4804/95/$04.00+0

Comparison of Concentrations of Two Doses of Clavulanic Acid (200 and 400 Milligrams) Administered with Amoxicillin (2,000 Milligrams) in Tissues of Patients Undergoing Colorectal Surgery

CLAUDE MARTIN,[1]* MARIE-NOËLLE MALLET,[2] BERNARD SASTRE,[3] XAVIER VIVIAND,[1] ANNIE MARTIN,[4] PHILIPPE DE MICCO,[2] AND FRANÇOIS GOUIN[4]

Anesthesia Department and Trauma Center, Hôpital Nord, 13915 Marseille Cedex 20,[1] and Microbiology Department, Hôpital Salvator,[2] and Surgical Department,[3] and Anesthesia Department,[4] Hôpital Sainte-Marguerite, 13274 Marseille Cedex 9, France

Received 10 September 1993/Returned for modification 7 February 1994/Accepted 24 October 1994

The concentrations of clavulanic acid and amoxicillin were determined in sera and different abdominal tissues of 17 patients who underwent elective colorectal surgery. Patients were randomly allocated to two groups. At the time of induction of anesthesia, patients in group 1 were given 200 mg of clavulanic acid with 2,000 mg of amoxicillin and patients in group 2 received 400 mg of clavulanic acid with 2,000 mg of amoxicillin. In both groups, the initial dose was administered again after 2 h. Blood samples were collected to determine peak and trough antibiotic levels. Serial blood samples were also collected at predetermined periods (opening and closure of the abdominal cavity and surgical anastomosis). Abdominal wall fat, epiploic fat, and colonic wall tissue samples were collected simultaneously. Antibiotic concentrations were determined by high-performance liquid chromatography. Increasing the dose of clavulanic acid to 400 mg resulted in significantly higher peak and trough levels in serum ($P < 0.03$). Following the injection of 400 mg, mean concentrations of clavulanic acid in the fatty tissues were significantly increased at the time of opening ($P < 0.02$). The concentrations of clavulanic acid and amoxicillin in fatty tissues were 17 to 52% and 12 to 23% of the levels in sera, respectively. In the colonic wall, the concentrations of clavulanic acid and amoxicillin were 52 to 63% and 49 and 27% of the levels in sera, respectively. In sera, clavulanic acid given at a dose of 200 or 400 mg reached or exceeded the concentrations found to be effective in vitro to reduce the MICs of amoxicillin from the resistant to the susceptible category for 90% of the potential pathogens. In most of the tissues investigated, increasing the dose of clavulanic acid to 400 mg resulted in a significantly higher number of samples with concentrations found to be effective in vitro (72 versus 11%; $P < 0.05$). In conclusion, increasing the dose of clavulanic acid to 400 mg resulted in higher levels in sera and improved penetration into the abdominal tissues in patients undergoing colorectal surgery.

Colorectal surgery is a "contaminated-aseptic surgery," and prophylactic antibiotics are widely used prior to and during surgery, and the importance of the prophylactic antibiotics in reducing postoperative infections and mortality has been demonstrated (2, 3, 13, 22). Antibiotics are used to prevent both wound infections and peritonitis which may be caused by intraoperative contamination. Antibiotics are selected on the basis of the ability to eradicate the bacteria most likely to contaminate a surgical wound: *Escherichia coli* and various members of the family *Enterobacteriaceae*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacteroides fragilis*, and other anaerobic bacteria (2, 3, 13, 22). The basic concept underlying the optimal use of antibiotic prophylaxis is that an adequate amount of an appropriate antibiotic should be present in the blood and tissues before a bacterial challenge. It seems clear that during a surgical procedure, adequate antibiotic concentrations should be achieved at all sites of potential infection. Also, antibiotic activity should be maintained throughout the procedure, from incision to closure (4, 6, 8, 13, 14, 18, 19).

Amoxicillin-clavulanic acid has been widely used as a prophylactic antibiotic in abdominal and gynecological surgery (11, 24). It is effective in the prevention of wound infections in operations in which the most likely pathogens are gram-negative, anaerobic, or mixed bacteria. However, after colorectal surgery, postoperative infections have been reported after the use of amoxicillin-clavulanic acid (median, 12%) (24). In in vitro studies, it has been found that 2 mg of clavulanic acid per ml or less is required to reduce the MICs of amoxicillin from the resistant to the susceptible category for 90% of the *E. coli*, *B. fragilis*, *Proteus* and *S. aureus* strains tested (1, 5, 25, 26).

The current study was conducted during colorectal surgery to determine the levels in sera and different abdominal tissues of clavulanic acid and amoxicillin given prior to and during surgery. We wanted to confirm the presence of an effective antibacterial concentration of the antibiotics at potential sites of infection. Therefore, two doses of clavulanic acid (200 and 400 mg) were administered with 2,000 mg of amoxicillin.

MATERIALS AND METHODS

Patients and drugs. This study received the approval of the ethical committee of our institution, and all patients gave informed consent. Seventeen patients were scheduled for elective colorectal surgery. All patients had normal hepatic function (serum bilirubin, <10 μmol/liter) and renal function (creatinine clearance, >100 ml/min/1.74 m^2). None had a history of allergic reaction to β-lactam antibiotics. None presented any clinical sign (normal body temperature, normal physical examination) or laboratory sign (normal leukocyte count) of infection or had received antibiotic treatment in the preceding 3 weeks.

Patients were randomly assigned to one of two groups. Group 1 (six males, three females; 59 ± 19 years old; body weight, 63 ± 11 kg) was given 200 mg of

* Corresponding author. Phone: 33-91-96-86-50. Fax: 33-91-96-28-18.

TABLE 1. Concentrations of amoxicillin and clavulanic acid in serum[a]

Dose	Group 1						Group 2					
	Amoxicillin at 2,000 mg		Clavulanic acid at 200 mg		Clavulanic acid-amoxicillin ratio at:		Amoxicillin at 2,000 mg		Clavulanic acid at 400 mg		Clavulanic acid-amoxicillin ratio at:	
	Peak concn (μg/ml)	Trough concn (μg/ml)	Peak concn (μg/ml)	Trough concn (μg/ml)	Peak	Trough	Peak concn (μg/ml)	Trough concn (μg/ml)	Peak concn (μg/ml)	Trough concn (μg/ml)	Peak	Trough
1	176 ± 75	24 ± 21	12.0 ± 5.3	2.3 ± 2.1	0.07	0.09	220 ± 83	25 ± 4	42 ± 15[b]	5.4 ± 3[b]	0.19[b]	0.21[b]
2	192 ± 83		13.0 ± 7.0		0.07		211 ± 68		35 ± 18[b]		0.17[b]	

[a] The first dose was given at induction of anesthesia, and the second dose was given 2 h later. Values are means ± standard deviations.
[b] $P < 0.03$ compared with clavulanic acid at 200 mg.

clavulanic acid with 2,000 mg of amoxicillin by intravenous bolus injection. Group 2 (three males, five females; 57 ± 16 years old; body weight 66 ± 10 kg) was given 400 mg of clavulanic acid with 2,000 mg of amoxicillin. In both groups, antibiotics were administered at the time of induction of anesthesia and the initial dose was administered again 2 h later.

Specimen collection. Blood samples (10 ml each) were collected from a central venous catheter before and 10 min (first peak level) and 2 h (trough level) after the first injection and 10 min (second peak level) after the second injection. Blood samples were kept on ice and were centrifuged in a refrigerated centrifuge within 30 min of collection. Sera were placed into polypropylene tubes.

Several samples of the selected tissues were collected during surgery. Abdominal wall fat and epiploic fat were obtained at the times of opening and closure of the abdominal cavity; colonic wall samples were obtained at the time of surgical anastomosis. The exact time of sample collection was registered, and a corresponding blood sample was taken. Tissue sample sizes were 1 cm^3 or larger. Attached blood was removed from all tissue samples by cleaning with dry, sterile gauze. Tissue samples were placed into sterile polypropylene tubes. Serum and tissues samples were assayed immediately following surgery.

Specimen preparation procedures. For determination of clavulanic acid in serum, 400 μl of the sample was mixed with 0.1 M ammonium citrate and 3.0 M imidazole (pH 6.8). After mixing (vortex; 30 s), the sample was deproteinized by addition of 1.0 ml of acetonitrile. After mixing (15 s) and centrifugation 10 min at 3,000 rpm (Sorvall centrifuge), the acetonitrile was removed by extraction with 3.0 ml of dichloromethane. The solution was then vortexed (30 s) and centrifuged for 5 min at 3,000 rpm. The aqueous phase (50 μl) was injected onto a high-performance liquid chromatography (HPLC) system.

For determination of amoxicillin in serum, 500 μl of a sample was stabilized with 500 μl of 0.2 M ammonium acetate (pH 7.0). After mixing and centrifugation, the sample was treated in the same way as the clavulanic acid samples. The aqueous phase (50 μl) was injected onto the HPLC system.

For determination of clavulanic acid and amoxicillin in tissues, samples were weighed and 300 mg was homogenized with an Ultra-Turrax homogenizer at 4.0°C for 45 s. Centrifugation of the homogenate yielded a clear supernatant which was treated as serum samples were.

HPLC systems (10, 17). Clavulanic acid concentrations were determined by using a dual-column HPLC system with UV detection. The precolumn (LiChrospher RP 18 E; 5-μm pore size; 25 by 4 mm [inside diameter]) was connected to the analytical column (LiChrospher RP 18 E; 5-μm pore size; 125 by 4 mm [inside diameter]). The mobile phases consisted of 4% acetonitrile and 96% 0.01 M potassium dihydrogen phosphate buffer (pH 3.2). The flow rate was 1.3 ml/min, and detection of clavulanic acid was obtained at 311 nm.

Amoxicillin concentrations were determined with the same HPLC systems. The mobile phase consisted of 3% acetonitrile and 97% 0.02 M disodium monophosphate buffer (pH 6.8). The flow rate was 1.0 ml/min, and detection of amoxicillin was obtained at 225 nm. The lower limit of clavulanic acid detection was 0.1 μg/ml for tissue and serum samples. The lower limits of amoxicillin detection were 0.1 and 0.2 μg/ml for serum and tissue samples, respectively. The percentages of recovery of clavulanic acid were 100% ± 4% and 91% ± 3% for serum and tissue samples, respectively. For clavulanic acid, the within-day and between-days (3 days) levels of interassay precision were 1.9 and 2.4% (10 μg/ml) and 1.1 and 2.1% (2 μg/ml) for serum samples, respectively, and 4.3 and 7.0% (3 μg/g) and 5.2 and 8.4% (0.5 μg/g) for tissue samples, respectively. For amoxicillin the within-day and between-days (3 days) levels of interassay precision were 0.5 and 2.0% (100 μg/ml) and 3.9 and 4.8% (25 μg/ml) for serum samples, respectively, and 4.6 and 7.2% (15 μg/g) and 5.9 and 8.8% (1 μg/g) for tissue samples, respectively.

Evaluation of tissue penetration. Tissue/serum concentration ratios were obtained by dividing the antibiotic concentration in the tissue sample by the concentration in the corresponding serum sample. Samples of specific tissues were analyzed according to collection time: opening and closure of the abdominal cavity and surgical anastomosis of the colon. For each defined period, the ratios of the concentrations of the two compounds (clavulanic acid/amoxicillin) were obtained for the different tissue samples and the corresponding serum samples. The mean ratio ± the standard deviation was calculated for both groups.

Statistical analysis was performed with Student's *t* test for unpaired values and the chi-square test. A *P* value of less than 0.05 was considered significant.

RESULTS

Concentrations in serum. Clavulanic acid and amoxicillin concentrations in serum are presented in Table 1. Use of the higher dose (400 mg) resulted in a significant increase in peak

TABLE 2. Concentrations of amoxicillin and clavulanic acid in abdominal tissues and serum of patients undergoing colorectal surgery at different periods of the surgical procedure

Group and drug (dose [mg])	Mean drug concn ± SD at:							
	Opening[a]			Surgical anastomosis[b]		Closure[c]		
	Abdominal wall fat (μg/g)	Epiploic fat (μg/g)	Serum (μg/ml)	Colonic wall (μg/g)	Serum (μg/ml)	Abdominal wall fat (μg/g)	Epiploic fat (μg/g)	Serum (μg/ml)
1								
Amoxicillin (2,000)	16 ± 8	17 ± 11	95 ± 60	24 ± 12.5	57 ± 35	14.1 ± 8.0	14.0 ± 9.1	89 ± 84
Clavulanic acid (200)	2.2 ± 2.7	1.8 ± 1.3	7.2 ± 3.3	2.7 ± 2.3	4.3 ± 3.1	2.1 ± 2.3	3.2 ± 4.3	5.1 ± 3.9
2								
Amoxicillin (2,000)	11.0 ± 2.0	11.0 ± 5.0	110 ± 47	17.2 ± 10.1	72 ± 47	12.0 ± 7.1	15.3 ± 5.5	71 ± 47
Clavulanic acid (400)	6.0 ± 4.4[d]	3.7 ± 1.8[d]	23.3 ± 21[d]	2.5 ± 1.9	4.5 ± 2.9	4.2 ± 3.2	3.5 ± 2.7	7.5 ± 3.4

[a] For groups 1 and 2, the opening times in relation to the start of antibiotic administration were 34 ± 15 and 30 ± 10 min, respectively.
[b] For groups 1 and 2, the surgical anastomosis times in relation to the start of antibiotic administration were 126 ± 37 and 116 ± 28 min, respectively.
[c] For groups 1 and 2, the closure times in relation to the start of antibiotic administration were 157 ± 40 and 162 ± 38 min, respectively.
[d] $P < 0.02$ compared with clavulanic acid at 200 mg.

TABLE 3. Tissue/serum concentration ratios of clavulanic acid and amoxicillin at different periods of the surgical procedure in patients undergoing colorectal surgery

Group and drug (dose [mg])	Mean serum/tissue drug concn ratio ± SD at:				
	Opening[a]		Surgical anastomosis[b] (colonic wall)	Closure[c]	
	Abdominal wall fat	Epiploic fat		Abdominal wall fat	Epiploic fat
1					
Amoxicillin (2,000)	0.19 ± 0.09	0.20 ± 0.10	0.49 ± 0.17	0.17 ± 0.12	0.17 ± 0.09
Clavulanic acid (200)	0.31 ± 0.12	0.29 ± 0.09	0.63 ± 0.24	0.33 ± 0.15	0.32 ± 0.13
2					
Amoxicillin (2,000)	0.12 ± 0.11	0.12 ± 0.13	0.27 ± 0.16	0.15 ± 0.10	0.23 ± 0.13
Clavulanic acid (400)	0.27 ± 0.15	0.17 ± 0.11	0.52 ± 0.23	0.52 ± 0.31	0.43 ± 0.17

[a] The opening times in relation to the start of antibiotic administration were 34 ± 15 and 30 ± 10 min for groups 1 and 2, respectively.
[b] The surgical anastomosis times in relation to the start of antibiotic administration were 126 ± 37 and 116 ± 37 min for groups 1 and 2, respectively.
[c] The closure times in relation to the start of antibiotic administration were 157 ± 40 and 162 ± 38 min for groups 1 and 2, respectively.

and trough clavulanic acid concentrations in serum ($P < 0.03$). Clavulanic acid/amoxicillin concentration ratios ranged between 0.07 and 0.09 with 200 mg and increased significantly to 0.17 and 0.21 with the 400-mg dose ($P < 0.03$).

Antibiotic concentrations in tissues. Clavulanic acid and amoxicillin concentrations in tissue at various periods during surgery are shown in Table 2. In group 1, stable concentrations were observed in fatty tissues when comparing the opening and closure periods. This was related to the injection of the second dose of antibiotics. Amoxicillin concentrations were higher in the colonic wall than in fatty tissues. Use of the 400-mg dose of clavulanic acid resulted in greater penetration into the fatty tissues (Table 2). This was statistically significant at the time of opening.

Tissue/serum concentration ratios. Tissue/serum concentration ratios are shown in Table 3. For amoxicillin, ratios ranged from 0.12 to 0.23 in fatty tissues and from 0.27 to 0.49 in the colonic wall. For clavulanic acid, ratios ranged from 0.17 to 0.52 in fatty tissues and from 0.52 to 0.63 in the colonic wall. The mean tissue/serum concentration ratios of clavulanic acid in the fatty tissues tended to show time dependency (Table 3). They increased from 0.17 and 0.27 at time of opening to 0.43 and 0.52 at the time of closure of the abdominal cavity.

Clavulanic acid-to-amoxicillin concentration ratios. Clavulanic acid-to-amoxicillin concentration ratios are presented in Table 4. In group 1, they ranged from 0.10 to 0.22 in tissues and from 0.06 to 0.09 in serum. With the 400-mg dose (group 2), the ratios ranged from 0.25 to 0.51 in tissues and from 0.11 to 0.21 in serum. The ratios were significantly increased at the time of opening and at surgical anastomosis (Table 4).

Effective (>2-μg/ml) antibacterial concentrations. In all plasma samples, clavulanic acid and amoxicillin exceeded the concentrations found to be effective in vitro. Effective concentrations of amoxicillin were found in all tissue samples. The number of patients with effective clavulanic acid concentrations in tissue is shown in Table 5. In group 1, with the 200-mg dose, 5 (11%) of 45 samples showed clavulanic acid concentrations of >2 μg/g, compared with 29 (72%) of 40 ($P < 0.05$) when the 400-mg dose was used.

DISCUSSION

Previous studies have established the basic principles of antibiotic prophylaxis in surgical procedures, and the main points are that (i) the antibiotic must reach the tissues involved before surgery allows bacterial contamination, and (ii) the drug must attain and maintain concentrations in serum and tissues high enough to inhibit the growth of contaminating pathogens (4, 6, 8, 13, 18).

This study addressed the tissue penetration of a β-lactam–β-lactamase inhibitor combination with the β-lactamase inhibitor being given at two different doses: 200 and 400 mg. The absolute concentrations of drugs in tissues, the time course of these concentrations and the extent of penetration are of interest. Penetration was evaluated by measuring the tissue-to-plasma concentration ratios at specific collection times.

The data for clavulanic acid (200 mg) and amoxicillin (2,000 mg) are in agreement with results previously reported (7, 9, 12, 27). Specific differences between tissues and the two compounds were observed. The extent of amoxicillin penetration into fat was 10 to 20% of the levels in serum, lower than the penetration of clavulanic acid at 200 mg (30%). Although the two compounds share the β-lactam structure, the difference in chemical composition probably explains the difference in af-

TABLE 4. Clavulanic acid/amoxicillin concentration ratios at different periods of the surgical procedure in patients undergoing colorectal surgery

Group	Mean clavulanic acid/amoxicillin concn ratio ± SD at:							
	Opening			Anastomosis		Closure		
	Abdominal wall fat	Epiploic fat	Serum	Colonic wall	Serum	Abdominal fat wall	Epiploic fat	Serum
1[a]	0.13 ± 0.05	0.11 ± 0.06	0.09 ± 0.04	0.10 ± 0.05	0.08 ± 0.04	0.14 ± 0.06	0.22 ± 0.12	0.06 ± 0.03
2[b]	0.51 ± 0.17[c]	0.33 ± 0.17[c]	0.21 ± 0.11[c]	0.15 ± 0.07	0.07 ± 0.04	0.35 ± 0.17	0.25 ± 0.11	0.11 ± 0.06

[a] Patients in group 1 received 2,000 mg of amoxicillin and 200 mg of clavulanic acid.
[b] Patients in group 2 received 2,000 mg of amoxicillin and 400 mg of clavulanic acid.
[c] $P < 0.02$.

TABLE 5. Patients with adequate clavulanic acid levels (>2 μg/g) in different tissues and at different periods during the surgical procedure[a]

Group[b]	No. of patients with adequate clavulanic acid levels in tissue/total no. of patients					
	Abdominal wall fat		Epiploic fat		Colonic wall	Total
	Opening	Closure	Opening	Closure		
1	0/9	1/9	0/9	1/9	3/9	5/45
2	6/8	5/8	7/8	5/8	6/8	29/40[c]

[a] See the text for an interpretation of these data.
[b] Group 1 ($n = 19$) received two 200-mg clavulanic acid doses 2 h apart, and group 2 ($n = 8$) received two 400-mg clavulanic acid doses 2 h apart.
[c] $P < 0.05$.

finity for fatty tissues. The exact mechanism that causes this important finding needs further investigation. This is at variance with a study on the tissue penetration of piperacillin-tazobactam in which the penetration of both compounds into fat was almost the same (10% of the levels in plasma) (14). In the present study, the extend of penetration of both compounds was maintained throughout the surgical procedure probably because of the second injection performed after 120 min. This emphasizes the need for repeated injections of drugs with shorter half-lives (4, 8, 13, 18, 19). Had this second injection not been performed, decreased concentrations of both compounds would probably have been measured in fatty tissues, with an increased risk of postoperative infection.

Penetration of clavulanic acid and amoxicillin into the colonic wall was higher than that into fatty tissues. This is probably explained by the fact that the blood flow to the gastrointestinal tract is higher than that to the fat. Once again, the extent of penetration of clavulanic acid was higher than that of amoxicillin. Interestingly, the extent of penetration of clavulanic acid into fatty tissues increased with time from ratios of 0.17 to 0.27 to ratios of 0.43 to 0.52. From these data, it is clear that no single mechanism governs the extent of penetration of clavulanic acid into fatty tissues. Further studies are needed to establish the relationship among blood flow, water content, and the physicochemical properties of the agent.

Prediction of antibacterial activity can be based partly on the absolute concentrations of antibiotics and the ratios between the two compounds. In the present study, the concentrations of amoxicillin in all of the tissues tested exceeded the MICs for bacteria that are susceptible to the agent. Addition of clavulanic acid in vitro leads to a dramatic reduction in otherwise elevated MICs when the bacteria produce β-lactamases (1, 5, 25, 26). With the use of 200 mg of clavulanic acid, adequate levels in tissue (>2 μg/g) were not reached in all patients and this may explain the failure of prevention of postoperative infection with amoxicillin-clavulanic acid observed in some studies (reviewed in reference 24). Increasing the dose of clavulanic acid to 400 mg resulted in greater tissue penetration and allowed achievement of adequate concentrations in 72% of the samples tested. However, what constitutes an optimal antibiotic concentration in tissue is poorly understood and the problem may be even more complex for a combination (1). It is often defined as a concentration above the MIC for bacteria, but many examples of effective prophylaxis with antibiotic concentrations in tissue below the MIC and failures of prophylaxis with concentrations in tissue above the MIC exist (15, 20, 21). Antibiotic concentrations below the MIC do produce morphological damage in bacteria, thus decreasing the growth rate and favorably influencing the outcome of an infection (16, 23, 28). Many factors influence the control of postoperative infections: the discipline of the surgical team, meticulous surgical technique, proper preparation for surgery, and the status of the immune system of the patient, etc.

In conclusion, when clavulanic acid (200 mg) and amoxicillin (2,000 mg) are given for antibiotic prophylaxis in colorectal surgery, clavulanic acid may not reach concentrations in tissue high enough to be effective in vitro and MICs for potential pathogens may not be reduced from the resistant to the susceptible category. This regimen has been to be effective in clinical trials, but the postoperative infection rate remains around 12% after colorectal surgery. With the use of 400 mg of clavulanic acid, concentrations in tissues reached or exceeded the concentrations found to be effective in vitro in 89% of the samples tested. Such a regimen could be considered in selected patients with a high risk of postoperative infection.

ACKNOWLEDGMENT

This work was performed at the Anesthesia, Surgical, and Microbiology Departments, Sainte-Marguerite Hospital, Marseilles University Hospital System, Marseilles, France.

REFERENCES

1. **Appelbaum, P. C., M. R. Jacobs, S. K. Spangler, and S. Yamabe.** 1986. Comparative activity of β-lactamase inhibitors YTR 30, clavulanate, and sulbactam combined with β-lactams against β-lactamase-producing anaerobes. Antimicrob. Agents Chemother. **30:**789–791.
2. **Bartlett, S. P., and R. C. Burton.** 1980. Effects of prophylactic antibiotics on wound infection after elective colon and rectal surgery 1960 to 1980. Am. J. Surg. **145:**300–309.
3. **Baum, M. L., D. S. Anisch, T. C. Chalmers, M. S. Sacks, H. Smith, and R. M. Fagerstrom.** 1981. A survey of clinical trials of antibiotic prophylaxis in colon surgery. Evidence against further use of no treatment controls. N. Engl. J. Med. **305:**795–799.
4. **Bergamini, T. M., and M. C. Polk.** 1989. The importance of tissue antibiotic activity in the prevention of operative wound infection. J. Antimicrob. Chemother. **23:**301–313.
5. **Bourgault, A. M., and F. Lamothe.** 1986. *In vitro* activity of amoxycillin and ticarcillin in combination with clavulanic acid compared with that of new lactam agents against species of the *Bacteroides fragilis* group. J. Antimicrob. Chemother. **17:**593–603.
6. **Burke, J. F.** 1961. The effective period of preventive antibiotic action in experimental incisions and dermal lesions. Surgery **50:**161–168.
7. **Dandy, F., A. K. Chandhuri, and A. H. Azmi.** 1984. Augmentin tissue levels in skin, fat, muscle, gall bladder and gut, p. 237. *In* Abstracts of the Proceedings of the 4th Mediterranean Congress on Chemotherapy, Rhodes, 1984.
8. **Dipiro, J. T., R. P. F. Cheung, T. A. Bowden, and J. A. Mansberger.** 1986. Single dose systemic antibiotic prophylaxis of surgical wound infections. Am. J. Surg. **152:**552–559.
9. **Earnshaw, J. J., R. C. B. Slack, G. S. Makin, and B. R. Hopkinson.** 1987. Tissue and serum concentrations of amoxicillin and clavulanic acid in patients having reconstructive vascular surgery. J. Intern. Med. Res. **15:**205–211.
10. **Foulstone, M., and C. Reading.** 1982. Assay of amoxicillin and clavulanic acid, the components of Augmentin, in biological fluids with high-performance liquid chromatography. Antimicrob. Agents Chemother. **22:**753–762.
11. **Hall, C., F. Curran, D. W. Burdon, and M. R. B. Keighley.** 1989. A randomized trial to compare amoxycillin/clavulanate with metronidazole plus gentamicin in prophylaxis in elective colorectal surgery. J. Antimicrob. Chemother. **24**(Suppl. B)**:**195–208.
12. **Houang, E. T., N. Colley, and M. Chapman.** 1985. Penetration of sulbactam-ampicillin and clavulanic acid-amoxicillin into the pelvic peritoneum. Antimicrob. Agents Chemother. **28:**165–166.
13. **Kaiser, A. B.** 1986. Antimicrobial prophylaxis in surgery. N. Engl. J. Med. **315:**1129–1138.
14. **Kinzig, M., F. Sörgel, B. Brismar, and C. E. Nord.** 1992. Pharmacokinetics and tissue penetration of tazobactam and piperacillin in patients undergoing colorectal surgery. Antimicrob. Agents Chemother. **36:**1997–2004.
15. **Kosmidis, J., C. Stathakis, K. Mantopoulos, T. Pouriezi, B. Papathanassiou, and G. K. Daikos.** 1980. Clinical pharmacology of cefotaxime including penetration into bile, sputum, bone and cerebrospinal fluid. J. Antimicrob. Chemother. **6**(Suppl. A)**:**147–151.
16. **Lorian, V.** 1985. Low concentrations of antibiotics. J. Antimicrob. Chemother. **15**(Suppl. A)**:**15–26.
17. **Low, A. S., R. B. Taylor, and J. M. Gould.** 1989. Determination of clavulanic

acid by a sensitive HPLC method. J. Antimicrob. Chemother. **24**(Suppl. B)**:**83–86.
18. **Martin, C., C. Portet, D. Lambert, B. Bruguerolle, B. Sastre, P. De Micco, and F. Gouin.** 1992. Pharmacokinetics and tissue penetration of single-dose cefotetan used for antimicrobial prophylaxis in patients undergoing colorectal surgery. Antimicrob. Agents Chemother. **36:**1115–1118.
19. **Martin, C., B. Sastre, M. N. Mallet, B. Bruguerolle, J. P. Brun, P. De Micco, and F. Gouin.** 1991. Pharmacokinetics and tissue penetration of a single 1,000-milligram, intravenous dose of metronidazole for antibiotic prophylaxis of colorectal surgery. Antimicrob. Agents Chemother. **35:**2602–2605.
20. **Mutch, D., G. Richards, R. A. Drown, and D. S. Mudler.** 1982. Bioactive antibiotic levels in the human aorta. Surgery **92:**1068–1071.
21. **Pitt, M. A., R. B. Roberts, and W. D. Johnson.** 1973. Gentamicin levels in the human biliary tract. J. Infect. Dis. **127:**299–302.
22. **Stone, H. H.** 1984. Basic principles in the use of prophylactic antibiotics. J. Antimicrob. Chemother. **14**(Suppl. B)**:**33–37.
23. **Washington, J. A.** 1979. The effects and significance of subminimal inhibitory concentrations of antibiotics. Rev. Infect. Dis. **1:**781–786.
24. **Wilson, A. P. R., S. Shrimpton, and M. Jaderberg.** 1992. A meta-analysis of the use of amoxicillin-clavulanic acid in surgical prophylaxis. J. Hosp. Infect. **22**(Suppl. A)**:**9–21.
25. **Wise, R., J. M. Andrews, and K. A. Bedford.** 1978. In vitro study of clavulanic acid in combination with penicillin, amoxycillin, and carbenicillin. Antimicrob. Agents Chemother. **13:**389–393.
26. **Wise, R., J. M. Andrews, and K. A. Bedford.** 1980. Clavulanic acid and CP-45,899: a comparison of their in vitro activities in combination with penicillins. J. Antimicrob. Chemother. **6:**197–206.
27. **Wise, R., I. A. Donovan, J. Drumm, J. M. Andrews, and P. Stephens.** 1983. The penetration of amoxicillin/clavulanic acid into peritoneal fluid. J. Antimicrob. Chemother. **11:**57–60.
28. **Zack, O., and F. Kradoler.** 1979. Effects of subminimal inhibitory concentrations of antibiotics in experimental infections. Rev. Infect. Dis. **1:**862–879.