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Effect of Peritoneal Fluid pH on Outcome of Aminoglycoside Treatment of Intraabdominal Infections

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Netilmicin and clindamycin were administered to 47 patients with an intraabdominal infection who underwent emergency laparotomy. Thirty-one patients were cured, seven were improved, and therapy failed in nine patients despite the fact that all aerobic bacteria isolated from these patients were sensitive to netilmicin as determined by standard in vitro susceptibility tests. The pH of peritoneal and drainage fluid collected intraoperatively and during follow-up correlated with clinical outcome. Acidic pH was found in 21 of 33 (64%) specimens sampled from patients with therapeutic failure compared to 17 of 80 (21%) obtained from the categories "cured" and "improved" ($p < 0.001$). Netilmicin concentrations in serum or peritoneal/drainage fluid did not correlate with clinical outcome. Netilmicin levels were above the minimal inhibitory concentration of the pathogens in 59 of 64 (92%) drainage fluid specimens in which aerobic bacteria were isolated. Aerobic bacteria were isolated in 91% of drainage fluid specimens if the pH was less than 7.0, compared to 37% if pH was more than 7.0 ($p < 0.001$). Reduction of pH antagonized aminoglycoside activity in vitro against clinical isolates of *Escherichia coli*. Surgical reexploration should be considered in cases of deterioration following a laparotomy associated with detection of acidic drainage fluid.

Successful therapy of a variety of intraabdominal bacterial infections requires surgical intervention plus concomitant antibiotic treatment. An aminoglycoside combined with an antibiotic directed against anaerobes is considered a standard regimem. However, failure rates of 10% to 20% have been reported with this treatment with persistence of the intraabdominal infection caused by aerobic bacteria [1].

Low pH values often prevail in infected tissues or fluids. Mean pH values of 6.0 to 7.0 have been measured in abscesses [2–4]. In a previous investigation we determined pH in abscesses and in peritoneal and drainage fluid in the presence and absence of bacterial infection in patients undergoing abdominal surgery. The pH proved to be a reliable parameter to distinguish infected from noninfected peritoneal fluid [5]. Aminoglycoside antibiotics are known to be less active at acidic pH [6–10]. Standard in vitro susceptibility tests performed at neutral pH might therefore not reliably predict their activity in some clinical situations. We determined pH and netilmicin concentrations in peritoneal and drainage fluid of patients with intraabdominal infections and correlated these data with the therapeutic outcome and with the minimal inhibitory concentration (MIC) of the aerobic pathogens.

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Patients and Methods

Patients and Treatments

The study was performed at the Department of Surgery of the University Hospital of Zürich. Forty-seven patients (26 men, 21 women) with a mean age of 50.5 years (range 16–88) underwent emergency laparotomy and subsequent drainage by passive-type drains (Silastic Easy Flow®, Dow Corning, Valbonne, France). Patients with ascitic fluid due to hepatic failure or patients with malignant effusion were not included in the study. Table 1 lists the diagnoses of the enrolled patients. Infectious material was removed from the abdomen, and spillage of intestinal contents was stopped by resection or by suturing the perforation. All patients received netilmicin and clindamycin. Antibiotic therapy was started intraoperatively after cleaning out the abdomen and collecting specimens for bacteriologic analysis. Clindamycin was administered 600 mg tid and netilmicin 150 mg tid for the first three doses. Thereafter the dosage of netilmicin was adjusted to achieve serum concentrations of 4 to 8 mg/L as determined 2 hours after administration (peak level) and 2 mg/L or less before administration of the next dose (trough level) [11]. Both antibiotics were given in 100 ml of saline as 30-minute infusion. Patients were evaluated daily for signs of intraabdominal or wound infections.

Microbiologic Methods

Material for bacterial culture was obtained during surgery by aspiration of peritoneal fluid or pus and by collecting drainage fluid twice a week. Specimens were promptly processed for aerobic and anaerobic bacterial culture. The MICs of netilmicin were determined by standard microtiter method in Mueller-Hinton broth (BBL Microbiology Systems, Cockeysville, MD, USA) at inocula of 5×10^5 colony-forming units (CFU)/ml.

pH Measurement

The pH of peritoneal and drainage fluid was measured with a computerized pH meter (Gallenkamp pH Stick®, Zivy SA, 4104

Table 1. Diagnoses and outcome.

Site of infection	No. of patients	No. cured	No. improved	No. failed
Perforated appendicitis	17	13	1	3
Perforated sigmoid diverticulitis	12	6	3	3
Postoperative peritonitis				
Anastomotic leakage	7	2	2	3
Abscess following liver trauma	1	1		
Abscess following cesarean section	1	1		
Perforated gallbladder empyema	5	4	1	
Perforated colon (gunshot)	2	2		
Perforated salpingitis	1	1		
Perforated duodenal ulcer	1	1		
Total	47	31	7	9

Oberwil, Switzerland). Accuracy was determined by 100-fold measurements of reference buffer solutions at room temperature. Mean ± standard deviation of pH values were 3.98 ± 0.04 for reference solution of pH 4.0, 7.02 ± 0.02 for pH 7.0, and 9.02 ± 0.02 for pH 9.0.

Peritoneal and drainage fluids have been either clear or turbid effusions. The term peritoneal fluid was used to describe peritoneal effusion drawn with a syringe immediately after opening the abdomen. Drainage fluid was defined as peritoneal fluid sampled by a drain into a bag mounted on the lateral abdominal wall (Ureteroplast® bag, Biotrol Pharma, Paris, France). Portions of 1 ml of peritoneal or drainage fluid were obtained to determine the pH.

Netilmicin Assay

TDx-System®, a fluorescence polarization immunoassay of Abbott Laboratories (North Chicago, IL, U.S.A.) was used to determine netilmicin concentrations. Serum peak and trough concentrations and a sample of drainage fluid, obtained prior to administration of the next dose of netilmicin, were measured twice a week.

The TDx-Assay® is known to accurately determine aminoglycoside concentrations in serum [12]. However, no information was available on its accuracy with peritoneal and drainage fluid. Therefore netilmicin-free pooled drainage fluid specimens of 10 patients and pooled human serum samples of 5 volunteers were spiked with four clinically relevant concentrations of netilmicin and assayed in quintuplicate (laboratory standard powder of netilmicin was provided by Essex-Chemie AG, 6000 Luzern 6, Switzerland).

Definition of Outcome

Patients who recovered without signs of any infectious complication within 3 weeks after operation were considered "cured from infection." The classification "improved" was defined as clinical improvement with the development of a subcutaneous wound infection. Therapy was classified as "failure" if a patient had to be reoperated for an intraabdominal infection. None of the failures could be attributed to either surgical technique or advanced underlying disease.

Statistics

Statistical calculations were performed with StatView SE+Graphics™ on Macintosh (Abacus Concepts, Berkeley, CA, U.S.A.). Linear regression analysis, the chi-square test, and the Wilcoxon signed rank test were used. Box plots were used to describe variable distributions, displaying the 10th, 25th, 50th, 75th, and 90th percentile. The notches represent 95% confidence bands about the median.

Table 2. Netilmicin concentrations (mean ± SD).

Fluid	Successful/improved therapy No.	Successful/improved therapy Netilmicin (mg/L)	Therapy failure No.	Therapy failure Netilmicin (mg/L)
Serum				
2 Hours after administration	33	4.6 ± 1.7	21	6.6 ± 3.2
Trough concentration	32	1.7 ± 1.3	22	3.0 ± 2.5
Drainage fluid	109	3.2 ± 2.1	56	3.2 ± 1.8

Results

Diagnosis and Outcome

Table 1 lists the diagnoses and outcome of the 47 patients enrolled in the study. There were 31 patients cured and 7 patients improved. Therapy failed in 9 patients, who had to be reoperated due to purulent intraabdominal accumulation. Persistent aerobic bacteria were isolated from acidic drainage fluid of all nine patients despite high netilmicin concentrations achieved in serum and peritoneal fluid (Table 2). None of the failures could be attributed to surgical leakage. All aerobic microorganisms isolated from the patients in whom netilmicin therapy failed were sensitive to netilmicin in vitro. These patients were all reoperated owing to infectious complications. After laparotomy, antibiotic treatment was switched to either ceftazidime or piperacillin. Eight of the patients were ultimately cured; one died.

Measurements of pH in Drainage Fluid

The pH was measured in 135 specimens of drainage fluid recovered from 47 patients. At least two samples but not more than four were collected per patient. Figure 1 shows the distribution of pH measurements over time in function of clinical outcome of netilmicin-clindamycin therapy. Of 80 samples from patients considered "cured," 63 (79%) had a pH of more than 7.0, as did 21 of 22 samples (95%) from patients considered "improved" and only 12 of 33 samples (36%) from patients considered "failed" ($p < 0.001$). None of the patients with low pH in peritoneal fluid suffered from systemic acidosis, as confirmed by serial blood gas analyses.

TDx-System®

Excellent correlation of concentration (c) measurements of netilmicin in spiked serum and drainage fluid was obtained ($r^2 = 0.998$) ($c_{\text{drainage fluid}} = 0.146 + 1.042 \times c_{\text{serum}}$) (Fig. 2). These data could be confirmed using gentamicin.

Netilmicin Concentrations in Drainage Fluid

A total of 165 drainage fluid samples were analyzed for netilmicin concentration, and 108 peak and trough levels were

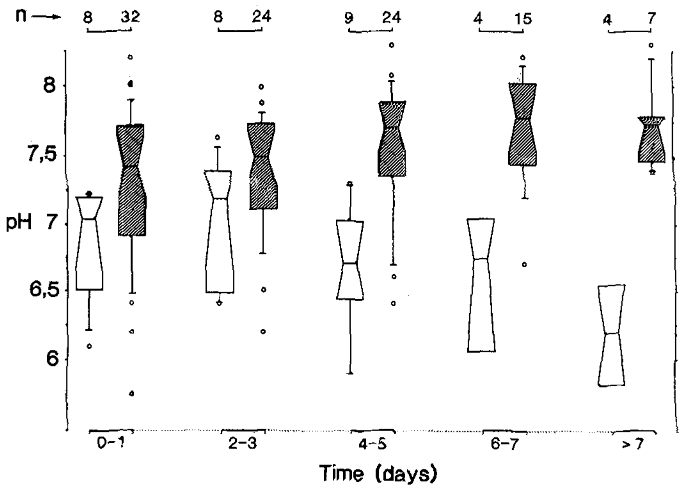

Fig. 1. Notched box plots show the pH of peritoneal or drainage fluid over time of patients in which netilmicin therapy failed (open boxes) and in patients who improved or were cured (hatched boxes). Chi-square test: $p < 0.001$).

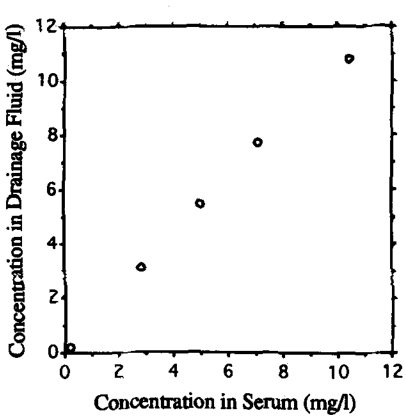

Fig. 2. Correlation of netilmicin measurement in spiked serum and drainage fluid specimens.

determined in serum. Table 2 summarizes the netilmicin concentrations in drainage fluid and serum from patients considered "cured," or "improved," and "failed." Surprisingly, significantly higher serum concentrations were observed in the failure group than in the "cured" or "improved" group ($p < 0.01$). Netilmicin concentrations achieved in the peritoneal fluid exceeded the MIC of the usually encountered aerobic pathogens.

Effect of pH on MIC

Figure 3 summarizes the in vitro effect of pH on the activity of three aminoglycosides against 13 *Escherichia coli* strains isolated from drainage fluid. The activity of gentamicin, netilmicin, and tobramycin was 100-, 10-, and 8-fold lower at pH 5.5 compared to in vitro tests performed at pH 8.5, respectively.

Bacterial Cultures in Drainage Fluid

Bacterial cultures were carried out in 106 samples of drainage fluid. Aerobic bacteria were isolated from 64 samples. Isolation of aerobic bacteria was associated with acidic pH of drainage fluid samples in 42 of the 64 culture-positive specimens (66%). In 42 of the 46 acidic specimens (91%), aerobic bacteria could be isolated compared to only 22 of the 60 alkaline specimens (37%) (Table 3). The median pH of the 42 acidic drainage fluid samples containing aerobic bacteria was 6.7. Netilmicin concentrations were above the MIC of the respective aerobic pathogens in 59 of the 64 samples. No association could be found between the isolation of aerobic bacteria and concentrations of netilmicin in drainage fluid (Table 4).

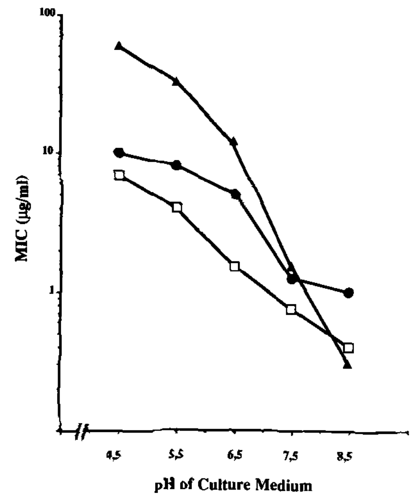

Fig. 3. Effect of pH on mean MIC of gentamicin (▲), netilmicin (□), and tobramycin (●) against 13 strains of *Escherichia coli* isolated from drainage fluid.

Table 3. Association of isolation of aerobic bacteria and acidic pH in 106 drainage fluid specimens.

pH	Aerobic bacteria isolated Yes (n = 64)	No (n = 42)
<7	42	4
>7	22	38

Aerobic bacteria were isolated in 42 of 46 (91%) drainage fluid specimens if the pH was <7.0, compared to 22 of 60 (37%) if the pH was >7.0 ($p < 0.001$).

Discussion

Acidic pH levels prevailing in infected peritoneal fluid antagonize the in vitro activity of aminoglycosides. So far, analysis of therapeutic failures of antibiotic treatment has not focused on milieu factors such as pH. Nine patients had to be reoperated for a purulent intraabdominal accumulation or an abscess. In all these patients aerobic pathogens persisted despite adequate netilmicin concentrations in serum as well as in peritoneal fluid. Poor outcome was attributed to antimicrobial failure rather than surgical technique, advanced disease, or other nonantibiotic factors. Furthermore, aerobic pathogens persisted in more than half of the cultured specimens despite the presence of supra-MIC concentrations of netilmicin in the drainage fluid. Both clinical failure and persistence of aerobic bacteria strongly correlated with acidic pH measurements in drainage fluid. In 89% of the drainage fluid specimens in which aerobic bacteria

Table 4. Lack of association between isolation of aerobic bacteria and concentration of netilmicin in 106 drainage fluid specimens.

Netilmicin concentration[a]	Aerobic bacteria isolated	
	Yes (n = 64)	No (n = 42)
>MIC[a]	59	36
<MIC	5	6

Netilmicin concentration was above the minimal inhibitory concentration (MIC) in 59 of 64 (92%) culture-positive specimens in contrast to 36 of 42 (86%) culture-negative specimens.

[a]Netilmicin concentration greater than or less than the minimal inhibitory concentration of the isolated aerobic bacteria.

were isolated, the netilmicin concentrations were above the MIC of the respective pathogens. Similarly, Richey and Schluepner observed gentamicin concentrations that exceeded the MIC of most aerobic pathogens encountered in patients with cirrhotic ascites [13]. The drainage fluid pH of patients in whom therapy failed was much lower than the pH of about 7.3 that prevailed during in vitro susceptibility tests. This discrepancy, in combination with the pronounced effect of pH on aminoglycoside activity, raises the question of whether modified in vitro test conditions should be considered for evaluating the most useful antibiotics for the treatment of abdominal infections [8, 10, 13].

The TDx-Assay® is well established for determining netilmicin concentrations in serum. However, little information is available so far on the accuracy of TDx-Assays® for determining netilmicin concentrations in peritoneal or drainage fluid. Analysis of netilmicin-spiked specimens of drainage fluid confirmed that reliable results can be achieved (Fig. 1).

Bacterial culture and analysis of pH of drainage fluid provides useful information for the management of patients after a laparotomy for intraabdominal infection. The measurement of pH is easy, quick, simple to perform and could be used as a bedside test. Other parameters such as polymorphonuclear cell count, glucose, and lactate might also be considered for diagnosis of an intraabdominal infection or during the postoperative follow-up [15, 16]. However, these parameters need laboratory facilities and are much more time-consuming.

Surgical reexploration should be considered in cases of deterioration following a laparotomy, associated with detection of acidic drainage fluid pH.

Acknowledgment

Bacterial cultures and sensitivity tests were performed at the Institute of Medical Microbiology, University of Zürich. The authors wish to thank Pietro Giovanoli, M.D., for helpful discussions and Mrs. C. König for her excellent technical assistance.

Résumé

Quarante-sept patients ayant eu une laparotomie en urgence pour infection intra-abdominale ont été traités par de la nétilmycine et la clindamycine. Parmi eux, 31 ont été guéris, sept se sont améliorés et neuf patients ont été considérés des échecs, et ceci malgré le fait que toutes les bactéries aérobies isolées se sont avérées être sensibles à la nétilmycine in vitro. Le pH des liquides intrapéritonéaux et des drainages, recueillis en per- et en post-opératoire, était corrélé avec l'évolution. En effet, un pH acide a été retrouvé dans 21 des 33 prélevements (64%) chez les patients considérés comme des échecs, comparés à 17 des 80 (21%) prélevements effectués chez les patients "guéris" ou "améliorés." Les taux de nétilmycine étaient supérieurs aux concentrations minimales inhibitrices des germes pathogènes chez 59 des 64 (92%) des prélèvements dans lesquels un germe aérobie a été retrouvé. Des bactéries aérobies ont été isolées dans 91% des prélèvements de drainage effectués lorsque le pH de ce liquide était < 7.0, comparés à 37% lorsque le pH était > 7.0 ($p < 0.001$). La réduction du pH avait une action antogoniste sur l'activité in vitro des aminosides contre les souches de E. coli. Une ré-exploration chirurgicale est indiquée lorsque l'on détecte un pH acide dans les liquides de drainage après une première laparotomie pour infection intra-abdominale.

Resumen

Se administró netilmicina y clindamicina a 47 pacientes con infección intraabdominal sometidos a laparotomía de urgencia. Se obtuvo curación en 31 casos; siete demostraron mejoría y hubo falla terapéutica en 9 a pesar de que la totalidad de las bacterias aeróbicas aislada en estos pacientes apareció sensible a la netilmicina según las pruebas estándar de sensibilidad in vitro. Se encontró correlación entre el pH del líquido peritoneal y de drenaje, recolectado intraoperatoriamente y en el curso de seguimiento, y el resultado clínico. Se halló pH acídico en 21 de 33 (64%) especímenes de pacientes con falla terapéutica en comparación con 17 de 80 (21%) obtenidos de los pacientes con curación o mejoría ($p < 0.001$). Las concentraciones de netilcimina en el suero o en el líquido peritoneal o de drenaje no exhibieron correlación con el resultado clínico. Los niveles de netilmicina se encontraron en concentraciones superiores al nivel mínimo inhibitorio de patógenos en 59 de 64 (92%) especímenes de líquido de drenaje en que se aislaron bacterias aeróbicas. Se aislaron bacterias aeróbicas en 91% de los especímenes de líquido de drenaje si el pH era <7.0, en comparación con 37% si el pH era >7.0 ($p < 0.001$). La reducción del pH antagonizó la actividad in vitro del aminoglicósido contra el E. coli. La exploración quirúrgica debe ser considerada en casos en que ocurra deterioro después de laparotomía con detección de líquido de drenaje acídico.

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Invited Commentary

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Operative management, when widely accepted at the beginning of the twentieth century, reduced the mortality of intraabdominal infection from 90% to 40% [1]. Later, antimicrobial therapy became available along with penicillins, cephalosporins, aminoglycosides, antianaerobic drugs, carbapenems, and quinolones. The proper use of all these powerful drugs was obscured for a long time by improper bacteriologic techniques.

The influence of the environment of infected tissue on the antimicrobial action of antibiotics is still poorly understood. We therefore welcome this important contribution that clarifies some aspects of the microenvironment of a surgical infection and its impact on aminoglycoside therapy. Hopefully, it will help to halt the misuse of aminoglycoside therapy for the treatment of peritonitis and other surgical infections.

Wound healing and host response, not only in intraabdominal infections but generally in any infected surgical wound, is a process accompanied by anaerobic metabolism and generally low pH [2]. On the other hand, it is well known that aminoglycosides are most active at alkaline pH, and even laboratory testing for sensitivity uses media with pH values above those of infected tissue. Nevertheless, the gold standard for antimicrobial therapy of intraabdominal infection has been a combination of an aminoglycoside with an antianaerobic drug such as clindamycin or later metronidazole [3]. With the introduction of better antimicrobial agents, it became slowly but progressively obvious that the aminoglycoside combination was inferior to any of the newer cephalosporins and carbapenems in combination with metronidazole. Unfortunately, the number of patients enrolled in prospective randomized comparative trials was never large enough to produce significant differences, although all studies showed a numerically worse outcome for the aminoglycoside combination.

The first paper showing a significant difference was published by Schoentag et al. in 1982 [4]. They clearly indicated that aminoglycosides are a bad choice for the treatment of intraabdominal infection. Despite this fact, patients continue to be treated with aminoglycoside therapy; high mortality rates in some series probably are due simply to the fact that tissue environments go unconsidered and aminoglycosides are consequently chosen as the first antibiotic choice. Aminoglycosides not only yield worse results than a third-generation cephalosporin combination with metronidazole but are uniquely distinguished by nephrotoxic and ototoxic side effects.

Hypovolemia due to fluid shifting into the peritoneal cavity, seen initially during the development of intraabdominal infection, reduces renal perfusion. Subsequent endotoxin spread potentiates the evolution of renal failure. If under these conditions tubulotoxic aminoglycosides are administered, the renal damage is perfect.

The work of Dr. Simmen's group is well known for their activity within the framework of the Study Group on Peritonitis of the Paul Ehrlich Society. It is a pleasure to see their work evolve to this important contribution, which represents an excellent piece of research and probably is going to be a reference for all of us. I am sure that this paper can dramatically help improve outcome of the sickest patients with whom a general surgeon has to deal.

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