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Bacteria: a Major Pathogenic Factor for Anastomotic Insufficiency

H. M. SCHARDEY,[1]* T. KAMPS,[1] H. G. RAU,[1] S. GATERMANN,[2] G. BARETTON,[3] AND F. W. SCHILDBERG[1]

Department of Surgery, University Hospital Grosshadern,[1] and Department of Pathology,[3] Ludwig-Maximilians-University, Munich, and Department of Microbiology, University of Lübeck, Lübeck,[2] Germany

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The aim of this study was to determine the influence of bacteria on the development of anastomotic insufficiency following gastrectomy in the rat. Fifty-seven male Wistar rats were randomly assigned to three groups and subjected to gastrectomy. Group I ($n = 20$) was orally inoculated with 10^9 *Pseudomonas aeroginosa* organisms on postoperative day 1. Group II ($n = 20$) served as the control group. Group III ($n = 17$) was decontaminated with 320 mg of tobramycin, 400 mg of polymyxin B, and 500 mg of vancomycin per liter of fluid administered from preoperative day 7 to postoperative day 10. Swabs from the oropharynx and rectum were cultured and analyzed daily for gram-positive and gram-negative bacteria. Surviving animals were sacrificed on postoperative day 10. All animals were autopsied immediately following death. Anastomotic insufficiency was defined as a histologically proven transmural defect at the suture line. Along with an effective reduction of pathogenic bacteria colonizing the oropharynx, the rate of anastomotic insufficiency could be reduced significantly, to 6% in decontaminated animals compared with 80% in controls ($P < 0.001$ by Fisher's exact test). Inoculation of group I animals with *P. aeruginosa* led to an increase of anastomotic insufficiency up to 95% and a significant increase in mortality ($P < 0.05$). We conclude that bacteria play a major role in the pathogenesis of anastomotic insufficiency following gastrectomy in the rat.

Anastomotic insufficiency is one of the major causes of morbidity and mortality following total gastrectomy. The pathogenesis of esophago-intestinal anastomotic insufficiency, however, is not completely understood. Impaired blood supply or local microcirculatory disturbances leading to necrosis (13), as well as foreign bodies (6, 7), have been thought to be the cause of anastomotic insufficiency for more than 100 years. Anastomotic insufficiency, however, is a septic disease. Exogenous or endogenous potentially pathogenic microorganisms colonizing the digestive tract may play a causative role in the pathogenesis in addition to microcirculatory disturbances. This idea led us to examine the influence of bacterial colonization on the incidence of anastomotic insufficiency.

By topical application of nonresorbable bactericidal antibiotics (16, 18), the colonizing microflora of the oropharynx and upper gastrointestinal tract can be easily manipulated. We therefore used deliberate colonization and topical decontamination with tobramycin, polymyxin and vancomycin to test the following hypotheses in an experimental study: (i) anastomotic insufficiency occurs as a result of bacterial infection, and (ii) anastomotic insufficiency can be prevented by preventing bacterial colonization.

MATERIALS AND METHODS

Fifty-seven male Wistar rats were randomly assigned to three groups. In group I (bacterial inoculation), all animals received one oral dose of 10^9 *Pseudomonas aeruginosa* organisms on the first postoperative day. *P. aeruginosa* was chosen because it is commonly causes nosocomial infections in humans (5, 8, 9). Group II animals served as the controls. Group III animals were decontaminated from preoperative day 7 to postoperative day 10 by addition of 320 mg of tobramycin, 400 mg of polymyxin B, and 500 mg of vancomycin per liter of fluid (administered orally).

All rats were operated on under sterile conditions with intraperitoneal pentobarbital anesthesia (50 mg/kg of body weight). The stomach was completely removed. The continuity of the intestinal tract was restored by an end-to-end esophagoduodenostomy. Anastomoses were performed by employing 6×0 PDS and a transmural, single-layer running suture technique. On postoperative day 10, surviving animals were sacrificed. All animals were autopsied. The anastomoses and the lungs were histopathologically examined.

The mechanical resistance of the anastomosis was determined by measuring the bursting pressure in situ. NaCl (0.9%) in a 50-ml syringe was pumped into the system at 2 ml/min with a perfusor pump (Braun; maximum producible pressure, 300 mm Hg [ca. 40,000 Pa]). The pressure changes were detected (Stadham ID23 pressure transducer) and recorded (Hellige monitor and writer).

Swabs for bacteriological analysis were collected daily from the oropharynx and rectum. Samples were immediately plated and incubated on MacConkey agar and blood agar and analyzed according to the guidelines of the German Society for Hygiene and Microbiology (4). Results were recorded as no growth or as low, moderate, or massive bacterial growth.

Tobramycin and vancomycin in the urine and serum samples from group III animals were analyzed by a fluorescence polarization immunoassay (11). The drug resistance of bacteria isolated from group III animals was determined by agar diffusion according to Deutsche Industrie Norm (4). The German National Research Council's guide for care and use of laboratory animals was followed.

Definitions. Anastomotic insufficiency was defined as a histologically proven transmural defect at the anastomotic suture line with a vital inflammatory reaction, e.g., exudation of fibrin and leukocytes.

Statistical methods. Means and standard deviations were computed, and the Mann-Whitney test, the Kruskall-Wallis test, Fisher's exact test, and the chi-square test were used. A value of $P < 0.05$ was considered to be significant.

* Corresponding author. Mailing address: Department of Surgery, University Hospital Grosshadern, Ludwig-Maximilians-University, Marchioninistr. 15, D-81377 Munich, Germany. Phone: 00-49-89-7095-2423. Fax: 00-49-89-7095-8893.

TABLE 1. Qualitative analysis

Species	% of samples positive				
	Oral cultures			Rectal cultures	
	Group I (n = 130)[a]	Group II (n = 165)	Group III (n = 166)	Group II	Group III
Escherichia coli	33.8	56.3	9.0	95.1	4.7
Proteus sp.	33.0	29.6	16.3	53.0	35.8
Pasteurella multocida	23.8	39.6	4.8		
Klebsiella sp.	3.0	4.2	0.6	0.6	1.3
P. aeruginosa	37.6				
Flavobacterium odoratum		0.6			
Streptococcus sp.	74.8	87.8	13.3	7.5	29.0
Staphylococcus sp.	13.0	53.9	5.4	0.6	8.1
S. aureus	0.8	1.3	4.2		0.6
Corynebacterium sp.	0.8		0.6		0.6

[a] n, number of samples.

TABLE 2. Semiquantitative analysis

Culture and strains	Group	% of samples[a]:			
		−	+	++	+++
Oral					
Gram negative	I (n = 130)[b]	27.3	31.2	31.2	10.5
	II (n = 165)	20.6	42.4	21.8	14.5
	III (n = 166)	71.6	20.4	4.8	2.4
Gram positive	I	16.1	32.2	36.9	13.1
	II	7.7	16.9	29.0	45.4
	III	77.1	17.4	3.6	1.2
Rectal					
Gram negative	II	0.6		8.3	91.0
	III	58.7	13.7	14.8	12.8
Gram positive	II	93.1	0.7	4.8	1.3
	III	66.2	29.6	8.7	5.4

[a] −, no bacterial growth; +, low bacterial growth; ++, moderate bacterial growth; +++, massive bacterial growth.
[b] n, number of samples.

RESULTS

Colonization rates. During the postoperative period, dynamic changes in the bacterial colonization pattern in the oropharynx were observed for all groups. In groups I and II there was an increase in gram-negative organisms and a transient decrease in gram-positive organisms. At the time of operation and during the entire observation period, the colonization rates in decontaminated animals stayed well below those in groups I and II.

Species of bacteria isolated. The spectra of bacteria species isolated from the oral cavity were similar for all groups (Table 1), with the exception of *P. aeruginosa*, which was artificially introduced into group I. Even though *P. aeruginosa* could be isolated from 95% of the animals in group I at one time or another during the monitoring, only 37.6% of oral samples were positive for *P. aeruginosa*. Decontamination caused a change in the composition of the oral microflora along with a marked reduction of most bacterial strains.

Mixed colonization with gram-negative and gram-positive bacteria was observed with similar frequencies in groups I (71%) and II (75%) but was a rare event in group III (11%); 12.1% of group I, 18.5% of group II, and 2.7% of group III animals showed the presence of more than one gram-negative strain or more than one gram-positive strain.

Semiquantitative analysis. In groups I and II the percentages of oral swabs yielding moderate or massive growth of gram-positive and gram-negative bacteria were higher than that in the decontaminated group (Table 2).

Influence of decontamination on rectal flora. Rectal colonization patterns also differed in the decontaminated and control groups, showing both semiquantitative and qualitative reductions of gram-negative bacteria and a shift towards gram-positive species (Tables 1 and 2).

Drug resistance. The development of drug resistance after 18 days of decontamination was not observed.

Bursting pressure. The bursting pressure of anastomoses in decontaminated animals (group III) was highest, at 247 ± 81) (mean ± standard deviation) mm Hg (ca. 32,900 ± 10,800 Pa). The bursting pressure in control animals (group II) was 178 ± 141 mm Hg (ca. 23,700 ± 18,800 Pa), and that in group I animals was 99 ± 97 mm Hg (ca. 13,200 ± 12,900 Pa). The bursting pressures in groups II and III did not differ significantly (P = 0.478; Mann-Whitney test), while the values for group I animals were significantly lower than those for both other groups (P = 0.0075; Kruskall-Wallis test).

Anastomotic insufficiency. Eighty percent of the specimens from the control group had an anastomotic insufficiency on histologic examination, whereas this was seen with only one animal of group III (6%). This reduction in the rate of anastomotic insufficiency was statistically highly significant (P < 0.001 by Fisher's exact test). Inoculation with *P. aeruginosa* in group I led to an anastomotic insufficiency rate of 95%, with only one animal left with an intact anastomosis (Table 3).

The only insufficiency in the decontaminated group occurred in an animal with a small transmural abscess. Free perforation with peritonitis was not observed. In the control group (group II), insufficiencies with peritonitis occurred in two animals (10%); both died by day 2. The other 13 animals with insufficiencies had transmural abscesses covered by granulation tissue with the beginning of a scarring reaction. In the *P. aeruginosa*-contaminated group (group I), five animals (25%) died from insufficiency with free perforation and peritonitis, and one died from secondary rupture of a very large transmural abscess followed by peritonitis on day 8. In all cases of peritonitis, *P. aeruginosa* could be recovered. One animal had a large transmural abscess covered by granulation tissue with the beginning of a scarring reaction, and the 12 remaining animals (60%) with anastomotic insufficiencies had extensive fistulas into the liver.

On histologic examination, all insufficient anastomoses from all groups presented with pus. Gram staining of the specimens demonstrated the presence of bacteria on all but two anastomoses. Both were from group II animals; one was insufficient

TABLE 3. Complications

Complication	% in:		
	Group I (n = 20)	Group II (n = 20)	Group III (n = 17)
Insufficiency	95	80	6[a]
Peritonitis	30	10	
Abscess	10	30	
Adhesions	80	50	6
Pneumonia	30	15	
Mortality	30	10	

[a] Significant difference (P < 0.001 by Fisher's exact test).

and presented with pus, and the other had healed by primary intention.

Other complications. Rates of peritonitis ($P < 0.05$), abscess ($P < 0.05$), adhesions ($P < 0.01$), pneumonia ($P < 0.05$), and mortality ($P < 0.05$) were reduced significantly under decontamination (Table 3).

Systemic antibiotic concentrations. Therapeutically active levels of aminoglycosides in serum could not be detected in any of the blood samples obtained.

DISCUSSION

Pathomechanism. Poor surgical technique either leaving gaps or causing an impaired blood supply to the anastomosis, resulting in necrosis at the suture line, is the generally accepted reason for anastomotic insufficiency. We propose that an additional factor may also be of major importance. The presence of bacteria which produce endo- or exotoxin may interfere with healing of the anastomosis in that they cause macrophage-mediated down regulation of fibroblast proliferation, as could be shown in vitro (2, 10). In addition, bacterial invasion and proliferation in necrotic tissue at the suture line may, in the absence of local defense mechanisms, cause direct infection, with intramural abscess formation resulting in anastomotic leakage. Although microcirculatory disturbances are the probable cause of necrosis (13), necrosis may promote local infection in the presence of bacteria. The severity of local infection associated with the release of bacterial toxins at the suture line may in turn determine the extent of necrosis and influence the occurrence of anastomotic leakage.

Colonization rates. One key to the initially proposed pathomechanism of infection-related anastomotic insufficiency is the postoperative susceptibility to colonization with pathogenic bacteria, which we could observe in both groups I and II. This has previously been observed after surgical trauma in rats (8) and is also consistent with clinical observations (20). Postoperative changes in the composition of the oropharyngeal microflora have been observed in connection with a decrease in cell surface fibronectin, possibly as a result of an increase in proteolytic activity of oropharyngeal secretions (9, 15).

Although we did not have direct access to the anastomosis during the postoperative period, we assumed that there was an increased colonization by pathogenic gram-negative bacteria at the suture line as well in group I and II animals because of the proximity of the anastomosis to the oropharynx.

Colonization prevention by decontamination. Decontamination in group III animals effectively reduced bacterial colonization of the intestinal tract during the entire postoperative period (Tables 1 and 2). Since we could prove that bacterial colonization of the oropharynx and rectum was reduced by decontamination, it may be assumed that bacterial counts at the anastomotic suture line were also reduced.

The antibiotic cocktail administered to group III animals in our study differed from the selective decontamination regimen used in clinical trials (16, 18) by the replacement of amphotericin B with vancomycin. Both regimens differ from that used for total gut decontamination (1, 14). Rats generally do not acquire yeast infections, an observation we also made during our preliminary trials. It was therefore unnecessary to prevent colonization by yeasts. However, invasion of necrosis at the suture line with subsequent infection by gram-positive cocci, especially *Staphylococcus aureus*, was very much within the realm of possibilities, and we considered it important to prevent colonization with these bacteria. We furthermore assumed a possibility of mixed infection with anaerobes and aerobes with the ability to cause abscess formation, as described by Onderdonk et al. (12). We added vancomycin in order to eliminate gram-positive organisms. We were aware that because of the addition of vancomycin, the colonization resistance of the gastrointestinal microflora, as proposed by van der Waaij et al. (19) for colonization resistance by aerobes, might be reduced. However, we did not observe any disadvantageous side effects in the outcome. The effectiveness of decontamination in colonization prevention differed for the individual strains (Table 1). Among gram-negative bacteria, the elimination of *Proteus* strains was least effective, possibly due to the primary resistance of members of this genus to polymyxin (5).

Anastomotic insufficiency. The diagnosis of anastomotic insufficiency was based on a histologically proven transmural defect at the suture line. Anastomotic insufficiency in the absence of infection was not observed, since pus and bacteria were present in the vast majority of insufficient anastomoses. This supports the results of morphologic studies on rabbit colon anastomoses by Schäfer et al. (13). The incidence of anastomotic insufficiency changed with the orophyngeal bacterial colonization rates. A reduction of oropharyngeal bacterial colonization (Tables 1 and 2) was paralleled by a significant reduction in anastomotic insufficiency ($P < 0.001$), from 80% in group II to 6% in group I (Table 3). Artificial oral inoculation with *P. aeruginosa* in group I led to an increase in the rate of anastomotic insufficiency. Although this increase was not statistically significant, the consequences of insufficiencies were very different, because they were associated with a significant elevation of mortality ($P < 0.05$). Morphologically, group I insufficiencies presented mostly as fistulas and not as intramural abscesses. Although semiquantitative analysis (Table 2) did not show an increase in the total number of gram-negative bacteria in group I, qualitative analysis did show a difference in the types of organisms isolated (Table 1). With decontamination neither intra-abdominal abscess nor peritonitis was observed, and along with a very low rate of septic complications there was no postoperative mortality in group III.

Bursting pressure measurements were useful in showing that wound healing in the presence of decontamination produced stable anastomoses.

In a clinical setting it has never been proven, to our knowledge, that antibiotics can significantly reduce the incidence of anastomotic insufficiency. Most trials conducted with intravenous antibiotic applications in colorectal surgery examined the question of septic complications in general (3). One reason for failure of intravenous antibiotics in the prevention of anastomotic leakage might be the inability to prevent bacterial invasion of necrosis, which, according to Schäfer et al., is the starting point of anastomotic insufficiency (13). Selective decontamination has also failed to prevent anastomotic dehiscence in patients with esophago-intestinal anastomoses following esophagectomy (17). In the randomized controlled clinical trial reported by Tetteroo et al. (17), examination of the influence of decontamination on the development of surgical complications was not the aim of the study. However, complications were equally distributed in control and treatment groups. The failure to prevent anastomotic leakage may be due to the facts that in several patients the antibiotics bypassed the anastomosis via a nasogastric tube or needle jejunostomy and that no topical medication was given to prevent colonization with gram-positive bacteria. Gram-positive bacteria accounted for 85% of infections in the decontaminated group, which had an overall infection rate of 32%. We wish to emphasize that in our study colonization with gram-positive bacteria was reduced by giving oral vancomycin

and that the antibiotics were in direct contact with the anastomotic suture line to prevent bacterial invasion of necroses.

We therefore conclude that bacteria indeed play a major role in the pathogenesis of anastomotic insufficiency. In order to find out whether these experimental results have clinical relevance, we carried out a prospective, nonrandomized pilot study with 40 patients, and on the basis of the results obtained, we conducted a prospective, randomized, double-blind multicenter trial with patients undergoing total gastrectomy for gastric cancer. The analysis was carried out on an intention-to-treat basis. Along with a significant reduction of colonizing gram-negative bacteria and *S. aureus*, the anastomotic insufficiency rate of the oesophage-jejunostomy could significantly be reduced as a result of decontamination compared with that of controls.

REFERENCES

1. **Altemeier, W. A., W. R. Culbertson, and R. P. Hummel.** 1968. Surgical considerations of endogenous infectious sources, types and methods of control. Surg. Clin. N. Am. **48**:227.
2. **Bankey, P., V. Fliegel, R. Singh, D. Knighton, and F. Cerra.** 1989. Hypoxia and endotoxin induce macrophage mediated suppression of fibroblast proliferation. J. Trauma **29**:972–980.
3. **Baum, M. I., D. S. Anish, T. C. Chalmers, H. S. Sackjs, H. Smith, Jr., 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. **Burkhardt, F. (ed.).** 1985. Verfahrensrichtlinien für mikrobiologische Diagnostik. Gustav Fischer, Stuttgart, Germany.
5. **Feeley, T. W., G. C. Du Moulin, J. Hedley-Whyte, L. S. Bushnell, J. P. Gilbert, and D. S. Finegold.** 1975. Aerosol polymyxin and pneumonia in seriously ill patients. N. Engl. J. Med. **293**:471–475.
6. **Gross, E., and F. W. Eigler.** 1989. Die nahtlose Kompressionsanastomose am distalen Colon und Rektum. Chirurg **60**:589–593.
7. **Halsted, W. S.** 1887. Circular suture of the intestine: an experimental study. Am. J. Med. Sci. **94**:436.
8. **Higuchi, J. H., and W. G. Johanson, Jr.** 1980. The relationship between adherence of Pseudomonas aeroginosa to upper respiratory cells in vitro and susceptibility to colonization in vivo. J. Lab. Clin. Med. **95**:698–705.
9. **Johanson, W. G., Jr.** 1984. Prevention of respiratory tract infection. Am. J. Med. **5A**:69–77.
10. **Metzger, Z. T. Hoffeld, and J. Oppenheim.** 1986. Suppression of fibroblast proliferation by activated macrophages: involvement of H_2O_2 and a non-prostaglandin E product of the cyclooxygenase pathway. Cell. Immunol. **100**:501–514.
11. **National Committee for Clinical Laboratory Standards.** 1984. User evaluation of precision performance of clinical chemistry devices, vol. 4, no. 8. National Committee for Clinical Laboratory Standards, Villanova, Pa.
12. **Onderdonk, A. B., J. G. Bartlett, T. Louie, N. Sullivan-Seigler, and S. L. Gorbach.** 1976. Microbial synergy in intra-abdominal abscess. Infect. Immun. **13**:22–26.
13. **Schäfer, K., H. Loeweneck, H. Stanka, R. Ernst, and V. Zumtobel.** 1990. Mikrozirkulationsstörungen bei Colonanastomosen und ihre Bedeutung für die Pathogenese der Nahtinsuffizienz. Langenbecks Arch. Chir. **375**:24–32.
14. **Schimpff, S. C.** 1980. Infection prevention during profound granulo-cytopenia. Ann. Intern. Med. **93**:358–360.
15. **Simpson, W. A., and E. H. Beachey.** 1983. Adherence of group A streptococci to fibronectin on oral epithelial cells. Infect. Immun. **39**:275–279.
16. **Stoutenbeek, C. P., H. K. F. van Seane, D. R. Miranda, and D. F. Zandstra.** 1984. The effect of selective decontamination of the digestive tract on colonisation and infection rate in multiple trauma patients. Intensive Care Med. **10**:185–192.
17. **Tetteroo, G. W. M., J. H. T. Wagenvoort, A. Castlein, H. W. Tilanus, C. Ince, and H. A. Bruining.** 1990. Selective decontamination to reduce gramnegative colonisation and infection after oesophageal resection. Lancet **335**:704–707.
18. **Unertl, K., G. Ruckdeschel, H. K. Selbman, U. Jensen, H. Forst, F. P. Zenhart, and K. Peter.** 1987. Prevention of colonization and respiratory infections in long-term ventilated patients by local anti-microbial prophylaxis. Intensive Care Med. **13**:106–113.
19. **van der Waaij, D., J. M. Berghuis de Vries, and J. E. C. Lekkerkerk van der Wees.** 1969. Colonisation resistance of the digestive tract in conventional and antibiotic treated mice. J. Hyg. Camb. **405**:71–75.
20. **Woods, D. E., D. C. Straus, W. G. Johanson, Jr., and J. A. Bass.** 1981. Role of salivary protease activity in adherence of gramnegative bacilli to mammalian buccal epithelial cells in vivo. J. Clin. Invest. **68**:1435–1440.