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CLINICAL FORUM

Rigid bronchoscopy induces bacterial translocation: an experimental study in rats

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Rigid bronchoscopy induces bacterial translocation: an experimental study in rats. A. Nayci, S. Atis, D.U. Talas, G. Ersoz. ©ERS Journals Ltd 2003.

ABSTRACT: Bronchoscopy has the potential to propagate infections. Bacterial translocation was hypothesised to be the cause of infections observed following bronchoscopy and this study was designed to assess the risk of bacterial translocation following rigid bronchoscopy in rats.

A total of 30 rats were evaluated. The study group (n=15) underwent rigid bronchoscopy. Arterial blood gas analysis was performed in all rats. Blood and tissue cultures from the ileum, caecum, mesenteric lymph nodes, liver, spleen, mediastinal lymph nodes and lung were obtained 24 h following bronchoscopy.

Bacterial translocation to the mesenteric lymph nodes was found in seven of 15 rats (46.7%) that underwent bronchoscopy, compared with none of the controls. Of the seven positives, three rats (42.8%) also demonstrated other organ involvement, such as the liver and spleen. *Escherichia coli*, *Salmonella typhymirium*, *S. enteritidis* and *Pseudomonas* spp. were found as translocating bacteria. In the study group, pH and arterial oxygen tension were significantly lower and arterial carbon dioxide tension was higher, compared with controls.

This study shows that rigid bronchoscopy may induce bacterial translocation in rats. Further investigations aimed at understanding the clinical consequences of this phenomenon are warranted.

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Rigid bronchoscopy has remained the primary tool for diagnosis and treatment of disorders of the trachea and major bronchi. It is commonly performed for removal of tracheobronchial foreign bodies, management of massive haemoptysis, laser procedures, dilatation of tracheobronchial strictures and placement of airway stents [1]. However, bronchoscopy has the potential to induce infections. It may intuitively appear that the bronchoscopic instrument traverses the oropharyngeal area and carries the indigenous microorganisms to the distal tracheobronchial tree and possibly the pulmonary parenchyma. In addition, mucosal damage induced by the bronchoscope or the associated procedure may facilitate penetration of bacteria into the bloodstream [2–4]. Clinical studies have reported fever and/or bacteraemia, ranging from 6.5–15%, following bronchoscopy [3–6]. Moreover, pneumonia and sepsis have also been reported after bronchoscopy, especially in immunodeficient patients [7, 8]. Recently, PUGIN *et al.* [8] have shown that bronchoscopy induces a systemic inflammatory response syndrome and suggested an endotoxin-mediated mechanism for this. The authors stated that bronchoscopy can precipitate a translocation from infected airways into the lymphatics or bloodstream through an impaired alveolar-capillary barrier.

There is increasing evidence that translocation of bacteria from the gastrointestinal (GI) tract is one of the potential causes of systemic infections and systemic inflammatory response syndrome, associated with an undefined focus of infection [9–11]. Bacterial translocation is defined as the phenomenon by which live enteric bacteria and/or their endotoxins pass from the intact intestinal mucosa to extraintestinal sites, such as the mesenteric lymph node (MLN), liver, spleen and bloodstream [10, 11]. Conditions known to promote bacterial translocation include lipopolysaccharides, immunosuppression, operations, thermal injury and smoke inhalation [11–13]. To the best of the authors' knowledge, there is no study evaluating the association between bronchoscopy and bacterial translocation.

Accordingly, bacterial translocation was hypothesised to be one cause of any infection observed following bronchoscopy and this study was designed to assess the risk of developing bacterial translocation following rigid bronchoscopy in rats.

Methods

Animals

Wistar-Albino rats (n=30), weighing 200–250 g (Experimental Medical Research Laboratory, Erciyes University, Turkey) were used. All rats were housed in standard laboratory cages, five per cage, for $\geqslant$2 weeks before manipulation. Animals had free access to standard laboratory diet [14] and water and were subject to a 12 h light/dark cycle, relative humidity of 45–55% and temperature of 22–25°C. The rats had no clinical signs of respiratory tract or other organ infections before the study.

Experimental protocol

This study protocol was approved by the Ethics Committee of Mersin University, Turkey. The rats were divided into two

groups; the study group (n=15), undergoing rigid bronchoscopy, and the controls (n=15). Arterial blood gas analyses from the femoral artery were measured (OPTI Critical Care Analyser; Roche, Indianapolis, IN, USA) at the end of bronchoscopy. The rats were killed 24 h after bronchoscopy. Blood and tissue samples from the mediastinal lymph nodes, lung, MLN, liver, spleen, ileum and caecum were taken for bacterial cultures.

Bronchoscopy

After an intramuscular injection of 20 $mg \cdot kg^{-1}$ of ketamine hydrochloride (Ketalar®; Eczacibasi Warner Lambert, Istanbul, Turkey), a lateral neck incision was performed to confirm that the bronchoscopic equipment was in the trachea. Likewise, the control animals underwent an intramuscular injection of 20 $mg \cdot kg^{-1}$ of ketamine hydrochloride and a lateral neck incision. An angiocath (14 G, 2×50 mm; Braun, Melsungen, Germany) was used as the rigid bronchoscope. The tip of the angiocath was rasped and smoothened. Four holes on each side were opened in the distal one-third of the canula (fig. 1). Bronchoscopy lasted ~30 min in each rat. Tracheobronchial secretions were aspirated. Bronchial lavage (1 cc) was performed during bronchoscopy.

Microbiological analysis

Before bronchoscopy, oropharyngeal specimens were inoculated onto 5% blood agar and eosin-methylene blue (EMB) agar plates for identification of oropharyngeal flora. Bronchial lavage fluid samples were cultured by inoculation onto 5% blood agar and EMB agar plates.

Testing for bacterial translocation

Rats were anaesthetised with intramuscular 50 $mg \cdot kg^{-1}$ ketamine hydrochloride 24 h after bronchoscopy and using sterile techniques the chest cavity was opened and animals were killed by cardiac blood aspiration. The control group also underwent the same procedure. Blood samples (100 μL×2) were incubated aerobically for 48 h at 37°C in 5 mL of thioglycollate broth and plated on 5% blood agar plates. The chest wall was retracted with sterile forceps, and the lung, heart and the mediastinal lymph node complex were removed. After this procedure, the abdominal cavity was entered and the viscera were swabbed with a sterile cotton stick, which was inoculated onto 5% blood agar plates. The liver, spleen and MLN were obtained, and all organs were divided into small pieces. The pieces of tissue were weighed separately and placed in a sterile grinding tube. The samples were homogenised with 1 mL of thioglycollate broth using sterile ground-glass stoppers. After mechanical grinding (Pottere S; Biolab, Melsungen, Germany), 500 μL of homogenate was transferred into a tube containing 4.5 mL of 0.9% NaCl and used to perform four serial dilutions. From this dilution, 100 μL aliquots were plated onto two different plates, including 5% blood agar and EMB agar. Finally, the terminal ileum and caecum were excised and placed in a tube of thioglycollate broth. All agar plates and thioglycollate broth tubes were incubated aerobically in 5% CO_2 for 24 h at 37°C. Gram-negative enterics were identified by biochemical profiles, and *Enterococcus* spp. were identified by esculin hydrolysis in the presence of bile, and grown in broth containing 6.5% NaCl. Quantitative culture results were determined by the number of colony-forming units (cfu) per gram of tissue, calculated from the formula from [15]:

$$\frac{\text{number of cfu} \times \text{reciprocal of dilution} \times 10}{\text{weight of tissue}} \quad (1)$$

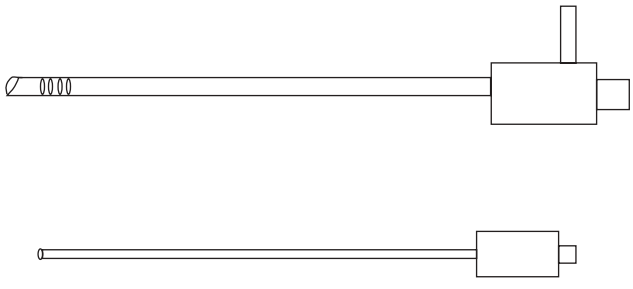

Fig. 1. – Bronchoscopic equipment and aspiration canule.

Statistical analysis

Bacterial concentrations were represented as the logarithm of geometric mean±SEM based only on organs in which bacteria were cultured.

The Z test was used to compare the rate of bacterial translocation between two groups. The Mann-Whitney U-test was used for comparison of arterial blood gas values between the two groups. A $p<0.05$ was considered statistically significant.

Results

All animals survived the bronchoscopic procedure. Predominantly, coagulase negative staphylococci (CNS), and *Pseudomonas* spp. were grown in the oropharyngeal swab. The bronchial lavage fluid of the animals was generally contaminated with the same bacteria of the oropharynx. The intestines of the animals were colonised with Gram-negative bacteria, predominantly *Escherichia coli*, *Salmonella typhymirium*, *S. enteritidis* and *Pseudomonas* spp. (table 1).

In the control animals, the oropharengeal and intestinal flora were identical to the study group. No bacteria were isolated from the blood or tissue cultures including MLN, liver, spleen, mediastinal lymph nodes and lung in the controls. No cases of bacterial translocation were present in the controls. In the study group, positive MLN cultures were observed in seven out of 15 rats (46.7%), indicating bacterial translocation ($p=0.011$). Of the seven positives, three rats (42.8%) also demonstrated other organ involvement, such as liver and/or spleen (table 1). Bacterial concentrations are presented as logarithm of geometric mean±SEM based only on organs in which bacteria were cultured (table 2). *E. coli*, *Pseudomonas* spp., *S. typhymirium* and *S. enteritidis* were found as the translocating bacteria. Blood cultures for both groups revealed no bacterial growth. In two out of 15 rats (13.3%), *Pseudomonas* spp. was isolated in the oropharengeal, bronchial lavage and lung cultures, indicating a bronchopulmonary origin.

The arterial blood gas parameters of the groups are shown in table 3. In the study group, pH and arterial oxygen tension (Pa,O_2) were significantly lower ($p=0.001$ and $p<0.001$, respectively) and arterial carbon dioxide tension (Pa,CO_2) was higher ($p=0.002$) following bronchoscopy, compared with the controls.

Discussion

The present study shows that rigid bronchoscopy induces bacterial translocation in rats. In recent years, the translocation

Table 1. – Bacteria in the blood and tissue cultures in the study group

Oro-Pharynx	Bronchial lavage	Ileum	Cecum	MLN	Spleen	Liver	Lung	Mediast LN	Blood
Lactobacil	CNS	*E. coli*	*E. coli*	*E. coli* 7×10^4					
CNS	*Pseud.* spp.	*E. coli*	*E. coli*		*Pseud.* spp. 6×10^3	*Pseud.* spp. 1×10^4	*Pseud.* spp. 2×10^6		
Pseud. spp.	*Pseud.* spp.	*Citrobacter* spp.	*E. coli*						
Pseud. spp. CNS	*Pseud.* spp. CNS	*Pseud.* spp.	*E. coli*						
Pseud. spp. CNS	*Pseud.* spp. CNS	*E. coli*	*E. coli*	*E. coli* 1.2×10^6	*E. coli* 1.4×10^5	*E. coli* 5×10^3		*E. coli* 2×10^6	
Pseud. spp. CNS	*Pseud.* spp.	*E. coli*	*E. coli*						
Pseud. spp.	*Pseud.* spp.	*E. coli* *S. typhym.*	*E. coli*						
CNS	*Pseud.* spp. CNS	*E. coli* *S. typhym.*	*E. coli*	*S. typhym.* 2×10^4					
Pseud. spp. *H. parainf.*	*Pseud.* spp.	*S. typhym.* *Pseud.* spp.	*S. typhym.* *Pseud.* spp. *Enterococcus* spp.	*Pseud.* spp. 1×10^6	*Pseud.* spp. 3×10^6			*Enterococcus* spp.	
CNS	*E. tarda*	*S. enteritidis*	*E. coli*	*S. enteritidis* 3×10^3					
CNS *Klebsiella* spp.	*Pseud.* spp.	*E. coli* *E. coli* *S. typhym.*	*E. coli* *E. coli*	*S. typhym.* 3×10^4					
Pseud. spp.	*Pseud.* spp. CNS	*E. coli*	*E. coli*				*Pseud.* spp. 8×10^3		
Klebsiella spp.	*E. tarda*	*E. coli* *S. enteritidis*	*E. coli*	*S. enteritidis* 9×10^4		*S. enteritidis* 3×10^4			

MLN: Mesenteric lymph node; Mediast LN: Mediastinal lymph node; *Lactobacil: Lactobacillus*; CNS: Coagulase negative Staphylococcus; *E. coli: Escherichia coli*; *Pseud. spp: Pseudomonas species*; *Citrobacter spp: Citrobacter species*; *H. parainf.: Haemophilus parainfluenza*; *S. enteritidis*: *Salmonella enteritidis*; *S. typhym.*: *Salmonella typhymirium*; *E. tarda: Edwersiella tarda.*

of bacteria from the GI tract has been increasingly recognised as one of the potential causes of systemic infections and systemic inflammatory response syndrome with an undefined focus of infection [9–11]. The first organ encountered by the organism or endotoxin undergoing translocation is the MLN. Subsequently, extension to the liver, spleen and the general circulation may occur. The host immune system is particularly effective in promoting the spread of translocating bacteria from the MLN to other organs [16]. The most direct method to show bacterial translocation is by culture of MLN. Peripheral blood culture containing bacteria of intestinal origin is an indirect marker [10, 16]. Positive culture of MLN is a sensitive indicator of bacterial infection, even if other measurements, including blood cultures and clinical infection symptoms, are negative [10, 16]. In the current study, bacterial translocation was confirmed by positive cultures of MLN in seven out of 15 rats (46.7%). No bacteria were grown in blood samples. Generally, the incidence of MLN involvement is higher compared with blood samples in the cases of bacterial translocation [9, 10]. Furthermore, most bacteraemic episodes after bronchoscopy are transient [3, 4]. Of three out of seven rats (42.9%) with positive MLN, the same bacteria were isolated in the liver and/or spleen. This indicates a spread of the translocating bacteria from the MLN to other extra-intestinal sites.

In two out of 15 rats (13.3%), *Pseudomonas* spp. was isolated from the lung. This could possibly demonstrate a bronchopulmonary origin for *Pseudomonas* spp. inasmuch as the oropharengeal and bronchial lavage was positive for this bacterium. Bronchoscopic instruments can transmit the

Table 2. – Bacterial translocation (BTL) to extra-intestinal organs and blood

	Study group		Control group	
	BTL positive	Bacterial concentration	BTL positive	Bacterial concentration
Mesenteric lymph node	7 (46.7)	$3.5\pm1.9\times10^5$	0 (0)	
Spleen	2 (13.3)	$1.6\pm1.4\times10^6$	0 (0)	
Liver	2 (13.3)	$1.75\pm1.2\times10^4$	0 (0)	
Lung	0 (0)		0 (0)	
Blood	0 (0)		0 (0)	

BTL data are presented as n (%); bacterial concentrations are presented as logarithm of geometric mean±SEM based only on organs in which bacteria were cultured. n=15.

Table 3. – Arterial blood gas parameters of the study and control groups

Parameter	Study group	Control group	p-value
pH	7.26±0.02	7.36±0.01	0.001
P_{a,O_2}	79.9±3.1	96.2±2.1	<0.001
P_{a,CO_2}	48.8±2.1	39.4±1.2	0.002
HCO_3-	22.1±0.98	23.5±0.7	>0.05

Data are presented as mean±SEM unless otherwise stated. P_{a,O_2}: arterial oxygen tension; P_{a,CO_2}: arterial carbon dioxide tension; HCO_3-: bicarbonate.

oropharyngeal flora to the distal tracheobronchial tree and possibly to the lung [2–4].

Most previous investigators have attributed the main role in bacterial translocation to aerobic and facultative Gram-negative bacteria [10, 16, 17]. Yet, some other studies have also shown the high translocation rate of anaerobes [11, 18]. The various species of indigenous bacteria do not all translocate at the same rate from the GI tract. Gram-negative facultative anaerobic Enterobacteriaceae, such as *E. coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* translocate at a greater rate from the GI tract to the MLN than other bacteria comprising the indigenous gastrointestinal flora [9, 10, 16]. It has been shown that *S. typhymirium* could be carried from the intestinal lumen across the intestinal barrier [19]. Similarly, *E. coli*, *S. typhymirium, S. enteritidis* and *Pseudomonas* spp. were found as translocating bacteria in the current study.

The translocation of bacteria from the intestine into the blood has been shown to initiate a systemic inflammatory response and lung injury in rats [20]. The frequency of bacterial translocation in humans is lower than that those observed in animal models [10]. There is some debate on the clinical importance of bacterial translocation in humans. Some authors believe that bacterial translocation may be a normal event necessary to allow the gut associated lymphoid tissue to generate immunocompetent cells [21]. However, the majority of studies indicate that bacterial translocation is a major contributor to systemic inflammatory response syndrome and multisystem organ failure in humans [9, 10, 16].

Physiopathological events causing bacterial translocation are not yet fully elucidated, but three major mechanisms have been proposed to explain this process: 1) bacterial overgrowth; 2) impairment of host immune defences; and 3) increased permeability of the intestinal mucosal barrier [10, 11, 16]. It is likely more than one mechanism is operating to promote bacterial translocation. The intestinal mucosa is very sensitive to acidosis and hypoxia. Even a short duration of hypoperfusion led to mucosal damage and increased permeability [22]. This may induce translocation of bacteria or endotoxins to systemic circulation and organs [23]. Rigid bronchoscopic procedures have been shown to induce alterations in arterial blood gases including acidosis, hypoxaemia, and hypercarbia [24, 25]. Likewise, the current study found a decrease in pH and Pa,O_2 and an increase in Pa,CO_2 values following bronchoscopy in rats. Although the interpretation of these results is probably more complicated, alterations of blood gases might have interfered with mesenteric blood flow. The physiopathological events leading to bacterial translocation were not beyond the scope of this study and, at present, the authors presume that all these events can explain bacterial translocation following bronchoscopy. However, further work needs to be performed looking specifically at this issue.

To conclude, the study demonstrates that rigid bronchoscopy induces bacterial translocation in rats. The results indicate that bacterial translocation may be one of the causes inducing bacteraemia, fever, or systemic infections that have been observed following bronchoscopy. However, further investigations aimed at understanding the clinical consequences of this phenomenon are warranted.

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