A study of interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) serum levels in rats subjected to fecal peritonitis and treated with intraperitoneal ropivacaine[1]

Avaliação dos níveis séricos de interleucina 6 (IL-6) e fator de necrose tumoral (TNF-α) em ratos submetidos a peritonite fecal e tratado com ropivacaína intraperitoneal

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ABSTRACT

PURPOSE: The objective of this study was to assess the cytokine serum levels of IL-6 and TNF-α in rats subjected to fecal peritonitis and treated with peritoneal lavage with 0.2% ropivacaine by peritoneal lavage.

METHODS: We subjected 16 Wistar rats to laparotomy 6 hours after the induction of fecal peritonitis with autogenous stool and subsequently divided the rats randomly into 4 groups: I-control, no treatment; II- drying of the abdominal cavity; III- lavage of the abdominal cavity with 3 mL of 0.9% normal saline and drying; IV- lavage of the abdominal cavity with 3 mL of 0.2% ropivacaine and drying. Six hours following the laparotomy, the animals underwent cardiac puncture, and 1 mL of blood was collected for cytokine assessment before the animals were euthanized.

RESULTS: The lavage with ropivacaine resulted in smaller TNF-α levels compared with those observed in the other treatment groups ($p < 0.05$). Regarding IL-6, the ropivacaine group showed lower cytokine levels than those observed in groups I and II, but there was no significant difference ($p > 0.05$) between groups III and IV.

CONCLUSION: Peritoneal lavage with 0.2% ropivacaine was shown to reduce plasma levels of IL-6 and TNF-α in the treatment of fecal peritonitis in rats.

Key words: Peritonitis. Anesthesia. Sepsis. IL-6. TNF-α.

RESUMO:

OBJETIVO: O objetivo do presente estudo foi avaliar as dosagens séricas das citocinas Il-6 e TNF-α em ratos submetidos à peritonite fecal e tratados com lavagem peritoneal com ropivacaína a 0,2%.

MÉTODOS: Utilizaram-se 16 ratos Wistar, submetidos à laparotomia 6 horas após a indução de peritonite fecal com fezes autógenas, distribuídos aleatoriamente em 4 grupos: I- Controle, nenhum tratamento; II- Enxugamento da cavidade abdominal; III- Lavagem da cavidade abdominal com 3 ml de solução salina 0,9% e enxugamento; IV- Lavagem da cavidade abdominal com 3 ml de ropivacaína a 0,2% e enxugamento. Seis horas após a laparotomia os animais foram submetidos à punção cardíaca com retirada de 1 mL de sangue

para a dosagem das citocinas e, a seguir, eutanasiados.

RESULTADOS: A lavagem com ropivacaína apresentou valores de TNF-α menores do que os observados com os outros tratamentos (p<0,05). Em relação aos valores da IL-6, o grupo da ropivacaína apresentou valores menores do que os observados com os grupos I e II, mas não houve diferença estatística (p>0,05) em relação ao grupo III.

CONCLUSÃO: A lavagem peritoneal com ropivacaína a 0,2% no tratamento da peritonite fecal em ratos demonstrou reduzir os níveis plasmáticos de IL-6 e do TNF-α.

Descritores: Peritonite. Anestesia. Sepse. IL-6. TNFα.

Introduction

The mortality and incidence of sepsis have increased in recent years. In the United States, an annual incidence of 750,000 patients with sepsis is estimated with a mortality rate of 28.6%, which represents a cost of 16.7 billion USD in health care[1]. Peritonitis is one of the most important causes of sepsis and death in surgery and intensive care units.

In peritonitis, sepsis occurs when an intra-abdominal focus of infection triggers a systemic inflammatory response. This response is characterized by the activation of several physiological systems (complement, coagulation, fibrinolysis and kinin) and cell populations (endothelial cells, leukocytes, monocytes, macrophages and mast cells), as well as the release of chemical mediators (oxygen free radicals, histamine, eicosanoids, clotting factors and cytokines).[2,3]

The classical treatment for peritonitis is the mechanical removal of contaminants, restoration of anatomic integrity and systemic administration of antimicrobial agents. The indiscriminate use of antibiotics has contributed to the development of resistance in several strains of microorganisms. In 1946, only 5% of staphylococci strains isolated from hospitals in the U.S.A. were resistant to penicillin. In 1949, 1950 and 1959, penicillin resistance was reported to be 29, 50 and 80%, respectively. In Brazil, currently over 80% of *Staphylococcus aureus* strains isolated from hospitalized patients and approximately 70% of the staphylococci strains isolated from patients in the community are penicillin-resistant.[4].

The increasing incidence of bacterial resistance associated with the difficulty in the development of new antibiotics motivates research into alternative techniques for the treatment of peritonitis. Several studies have proposed modulation of the inflammatory response aimed at increasing survival and reducing mortality in sepsis. In this context, several publications have suggested a wide range of anti-inflammatory actions of local anesthetics through their effects on immune cells, as well as platelets, erythrocytes and microorganisms themselves.[5] In fact, these agents have been used in the treatment of various conditions associated with inflammatory processes, such as interstitial cystitis, ulcerative proctitis, arthritis, herpes infections and burns.[6]

The anti-inflammatory mechanism of action of local anesthetics is not fully understood but seems to involve a reversible interaction with membrane proteins and lipids, as well as regulation of cellular metabolic activity, migration, phagocytosis and exocytosis. Based on these data, we have investigated the interleukin 6 (IL-6) and tumor necrosis factor alpha (TNFα) serum levels in rats subjected to fecal peritonitis by autogenous stool and treated with abdominal cavity lavage containing 0.2% ropivacaine.

Methods

After approval by the Research Ethics Committee from the Universidade Federal de Minas Gerais [*Federal University of Minas Gerais*] (UFMG) School of Medicine according to protocol # 028/09 (COEP-CETEA), 16 male Wistar rats (280-320 g) from the EMESCAN animal colony underwent surgery. The animals were randomly distributed into 4 groups of 4 animals each. These animals were kept under constant environmental conditions and acclimatized for 7 days before the start of the experiment. Rats were housed in cages with 5 animals each, fed a proper diet (Nuvital ®) and provided water *ad libitum*.

The sample size determination was based on a previous study,[7] which showed a difference of 40±15 pg/mL in IL-6 levels between the rats that underwent laparoscopy and the rats that underwent laparoscopic ligature following cecum puncture. Considering these data, four animals were necessary in each group to obtain a power of 95% and a type I error of 0.05.

The animals were anesthetized by an injection of S(+) ketamine hydrochloride (Cristalia®, São Paulo, Brazil) in the anterior muscle of the right thigh at a dose of 10 mg/kg of body weight.[8] Rats were also subjected to an abdominal puncture with a 16G Teflon catheter in the left lower quadrant of the abdomen. Next, an autogenous suspension of newly defecated feces was injected into the abdominal cavity, which was prepared with 2 g of feces diluted in 17 mL of saline. Before injection, the suspension was filtered through gauze to allow its free passage through the

interior of the needle. From this suspension, 10 mL/kg of body weight was injected in the abdominal cavity.[8]

Six hours after the induction of peritonitis, rats were anesthetized with a mixture of xylazine hydrochloride at a dose of 10 mg/kg (Lab. König. SA®, Argentina) and S(+) ketamine hydrochloride at 50 mg/kg (Cristalia®, São Paulo, Brazil). The animals were subsequently subjected to median laparotomy with an incision approximately 2 cm in length, and examination of the abdominal cavity.

The animals were divided into the following groups:

Group I- (n = 4) control, no treatment; Group II- (n = 4) smooth drying of the contents in the abdominal cavity with dry gauze; Group III- (n = 4) lavage of the abdominal cavity with 3 mL of 0.9% saline followed by drying and Group IV- (n = 4) lavage of the abdominal cavity with 3 mL of 0.2% ropivacaine and drying. In groups III and IV, after drying of the abdominal cavity with dry gauze, saline (group III) or 0.2% ropivacaine (group IV) was injected and maintained in the cavity for three minutes. During this period, the solution was carefully distributed among the abdominal viscera to allow greater contact with the peritoneum. After this procedure, the peritoneal fluid was wiped gently with dry gauze to remove as much liquid as possible. The abdominal wall was sutured in a single plane with a simple running suture using mono nylon (4-0).

Six hours after surgery, blood samples were collected (1 mL) from each animal in all groups by cardiac puncture with a 25G x 5.5 needle to assess the levels of IL-6 and TNF-α. After blood collection, all animals were euthanized intraperitoneally with pentobarbital (Cristalia®, São Paulo, Brazil) at a dose of 50 mg/kg of body weight. Cytokine assessment was performed using the CBA (Cytometric Bead Array) method according to previously described methodology.[9]

To compare the measurements of IL-6 and TNF-α, we applied the non-parametric Mann-Whitney test because the normality hypothesis was rejected. A p-value <0.05 was considered to be statistically significant. Statistical analysis was performed using the SPSS 19.0 software.

Results

TABLE 1 shows that the concentrations of TNF-α were lower (P <0.05) in the ropivacaine group compared with those of other groups.

TABLE 1 - TNF-α descriptive statistics and Mann-Whitney test results (*p values*) between groups

Groups	Median (pg/mL)	Max – Min (pg/mL)	*p* Values	
			Saline	Ropivacaine
Control	441.44	512.44 – 370.85	0.56	0.021
Drying	459.50	563.89 – 350.32	0.38	0.021
Saline	327.65	515.58 – 157.99	-	0.021
Ropivacaine	32.45	60.92 – 24.77	0.021	-

Conversely, we found that IL-6 concentrations were lower in the ropivacaine group compared with the control and drying groups (P <0.05). Although the IL-6 concentrations were lower in the ropivacaine group compared with the saline group, this difference was not statistically significant (P> 0.05)(Table 2).

TABLE 2 – IL-6 descriptive statistics and Mann-Whitney test results (*p values*) between groups

Groups	Median (pg/mL)	Max – Min (pg/mL)	*p* Value	
			Saline	Ropivacaine
Control	22,344.07	26450.78 -11618.65	0.08	0.021
Drying	14,791.69	15083.15-13807.35	0.073	0.021
Saline	6,993.76	14301.19-1717.45	-	1.0
Ropivacaine	3,220.87	4563.92-2329.52	1.0	-

Discussion

Previous studies have shown reduced mortality in rats subjected to fecal peritonitis that underwent lavage of the peritoneum with lidocaine[10] and bupivacaine.[11] Ropivacaine, despite having milder anti-inflammatory and anti-microbial activities compared with other anesthetics,[12] blunted the increase in plasma levels of TNF-α compared with other groups in our study. Conversely, although the levels of IL-6 were lower in the ropivacaine group compared with the other groups, this difference was not statistically significant compared to the control group that received saline. These results corroborate reported data suggesting the efficacy of using local anesthetics to reduce inflammation in experimental models of septic peritonitis.

Local anesthetics appear to act at various stages of the inflammatory cascade. Several studies have shown a reversible and

dose-dependent reduction in leukocyte adhesion to the vascular wall following anesthetic treatment.[13,14] Leukocyte migration also appears to be affected by local anesthetics, probably due to actions on the cytoskeleton or decreases in the release of chemotactic agents by leukocytes.[15]

Local anesthetics also produce a dose-dependent and reversible inhibition of granulocyte phagocytosis. A previous study has shown that intravenous administration of lidocaine at doses recommended for anti-arrhythmic treatment significantly reduced the phagocytic activity of leukocytes in the synovial fluid of joints with synovitis.[16] Experiments with ropivacaine, however, showed modest effects on the granulocytes phagocytic activity, in contrast to the results obtained with other local anesthetics.[12] The most plausible mechanism to explain the inhibition of phagocytic activity is a decrease in the surface receptor expression of leukocytes[17] and inhibition of actomyosin filament activity.[18]

Although the efficacy of peritoneal lavage is controversial in the medical literature,[19,20] a previous study conducted by the same author showed a significant reduction in mortality when local anesthetics were used in the peritoneum.[10] Local anesthetics increased survival in several studies using experimental models with mice[21] and dogs[22], even when administered systemically.

Besides their immunomodulatory effects, local anesthetics have proven antimicrobial activity. Other studies have shown that the antimicrobial potency of local anesthetics is primarily related to concentration and, to a lesser extent, chemical structure. Thus, anesthetics may be effective against most bacteria at sufficiently high concentrations.[23]

The precise mechanism of antibacterial activity is still unclear but may be related to the interaction of local anesthetics with the bacterial cell wall or cell surface macromolecules. Electrostatic interactions between cationic local anesthetics and anionic membrane components could induce functional changes in the cell membrane therefore reducing the membrane fluidity.[24]

In this study, peritoneal lavage with saline was not effective for decreasing IL-6 and TNF-α levels compared with the control and drying groups. Although not statistically significant, the of IL-6 measurements had lower p-values than those found for TNF-α. This finding is probably due to the half-life of the studied cytokines and the time of sample collection.

Other studies have shown that TNF-α has a peak plasma concentration of 2 hours followed by a rapid decline (18.2 min. half-life), and IL-6 has a biphasic peak concentration at 6 and 74 hours after exposure to endotoxin.[25]. Because the collection was performed 12 hours after the induction of peritonitis, TNF-α concentrations were probably decreasing, which caused the treatment groups to exhibit similar results.

The lack of statistically significant differences in the IL-6 results determined for the ropivacaine and saline groups was due to the confidence-interval values for the ropivacaine group, which were included in the saline-group interval.

Conclusion

Peritoneal lavage with 0.2% ropivacaine in the treatment of fecal peritonitis induced with autogenous stool in rats reduced the plasma levels of IL-6 and TNF-α-related to the inflammatory response and sepsis.

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