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Original

Rectal pre-treatment with ozonized oxygen (O_3) aggravates clinic status in septic rats treated with amoxicillin/clavulanate

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ABSTRACT

Introduction: Despite the advanced antibiotic therapies, sepsis continues being a clinical entity with high morbidity and mortality. The ozone/oxygen mixture (O_3/O_2) has been reported to exhibit positive effects on immunity. The aim of our study was to analyze whether (O_3/O_2) combined with amoxicillin/clavulanate has any influence on the morbidity and mortality of septic rats.
Methods: We used 48 Sprague-Dawley rats randomly allocated to 6 groups ($n = 8$): healthy (C), septic (I), healthy + ozone therapy (O_3), septic + amoxicillin/clavulanate (AMC), septic + amoxicillin/clavulanate + ozone therapy (AMC/O_3) and septic + ozone therapy (I/O_3). O_3/O_2 was administered rectally at increasing O_3 concentrations during 10 days prior to the onset of sepsis model (intraperitoneally injection of fecal material) or saline administration in healthy control rats. Later (post-inoculation), 3 days per week, O_3 was also administered. Vital signs were recorded, and microbiological, hematological and histopathological studies were performed.
Results: The number of surviving animal/total was higher in AMC (8/8) than in AMC/O_3 (4/8) $p = 0.077$. The percentage of surviving animals with pneumonia was higher in AMC/O_3 than in AMC (100% *vs* 37.5%). In dead animals, AMC/O_3 rats had a significantly higher percentage of lesions: Cardiac lesions, pulmonary hemorrhages and pleuritis (100%) and serositis/peritonitis (75%). Only *Escherichia coli* (2 different biotypes) was isolated from blood and/or peritoneal fluid from all infected groups. A significant decrease in the percentage of band neutrophils from the surviviors belonging to AMC/O_3 *vs* AMC was observed ($p < 0.05$).
Conclusion: Rectal pre-treatment with O_3/O_2 aggravates clinic status in septic rats treated with amoxicillin/clavulanate.

El pretratamiento rectal con ozono (O_3) empeora el estado clínico de ratas sépticas tratadas con amoxicilina/clavulánico

Palabras clave:
Ozono
Rata
Amoxicilina/clavulánico
Sepsis

RESUMEN

Introducción: A pesar de los avances en terapia antibiótica, la sepsis sigue siendo una entidad clínica con alta morbimortalidad. Se ha publicado que la mezcla ozono/oxígeno (O_3/O_2) presenta efectos beneficiosos sobre el sistema inmunológico. El objetivo de este estudio es analizar si (O_3/O_2) combinado con amoxicilina/clavulánico tiene efectos en la morbimortalidad de ratas sépticas.

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Eacherichia coli
Pulmón
Corazón
Leucograma

Métodos: Utilizamos 48 ratas Sprague-Dawley distribuidas aleatoriamente en 6 grupos ($n = 8$): sanas (C), sépticas (I), sanas + ozonoterapia (O_3), sépticas + amoxicilina/clavulánico (AMC), sépticas + amoxicilina/clavulánico + ozonoterapia (AMC/O_3) y sépticas + ozonoterapia (I/O_3). (O_3/O_2) se administró por vía rectal a concentraciones crecientes de O_3 los 10 días previos a la instauración del modelo de sepsis (inyección intraperitoneal de material fecal) o de la administración de solución salina, en las ratas control. Posteriormente (postinoculación) se continuó administrando (O_3/O_2), 3 días por semana. Registramos los signos vitales y realizamos estudios microbiológicos, histopatológicos y hematológicos.
Resultados: El número de supervivientes/total fue mayor en AMC (8/8) que en AMC/O_3 (4/8), $p = 0{,}077$. El porcentaje de supervivientes con neumonía fue mayor en AMC/O_3 que en AMC (100% *vs* 37,5%). Entre los fallecidos, AMC/O_3 tenía un porcentaje mayor de lesiones: cardiacas, hemorragias pulmonares y pleuritis (100%) y serositis/peritonitis (75%). A partir de la sangre y/o líquido peritoneal de los grupos infectados se aislaron exclusivamente *Escherichia coli* (2 biotipos diferentes). Observamos una disminución significativa en el porcentaje de neutrófilos en banda en las supervivientes pertenecientes a AMC/O_3 *vs* AMC ($p < 0{,}05$).
Conclusión: El tratamiento rectal previo con (O_3/O_2) agrava el estado clínico en ratas sépticas tratadas con amoxicilina/clavulánico.

Introduction

Despite the huge advanced antibiotic therapies, supportive treatments and technological facilities, sepsis continues to be a clinical entity with high morbidity and mortality.[1] The pathophysiology of sepsis involves complex interactions between host organs and the invading pathogen. The lung is the organ which is affected initially, and sepsis leads to acute lung injury.[2] Sepsis and particularly septic shock induce the production of large amounts of free radicals in a non-regulated mode associated with high-oxidative potential damage.[3] Several generating sources of free radicals have been detected in sepsis and septic shock, demonstrating the presence of oxidative stress, early production of reactive oxygen species (ROS), and development of multiple system organ dysfunction.[4–8]

The ozone/oxygen mixture has been reported to exhibit several effects on the immune system, such as the modulation of phagocytic activity.[9] This gas may stimulate host defenses against microbes, mainly by oxidative reactions[10] enhancing pro-inflammatory cytokine release.[11,12] It has been reported to be effective in osteomyelitis, peritonitis or in vascular disorders.[12] Unfortunately, these studies have been usually performed without adequate control groups, not fulfilling standards of evidence based medicine.[13] Thus, currently ozone treatment is not recognized in traditional medicine and it is subsumed under complementary (alternative) medicine.[14] However, the interest in ozone as a therapeutic or prophylactic agent has been renewed since the reports that it is a bio-molecule, produced by neutrophils as part of adaptive immunity,[15,16] thus being the most important host defense against bacterial pathogens.

Whether ozone pre-treatment is beneficial in peritonitis is unclear, since there are contradictory reports of its immunological effects.[17] Recent studies[18,19] have shown that ozone therapy reduced tissue oxidative stress, regulated the systemic inflammatory response, and abated cellular infiltration to the lung. Those studies support the use of the ozone therapy as adjuvant therapy to antibiotherapy in protecting the lung against septic injury. However, an aggravating effect of ozone pre-treatment on the systemic inflammatory response in a sepsis rat model and a decreased survival has been also reported.[20] These contradictory findings reinforce the need of studies testing the efficacy of ozone therapy on septic animals, to clarify its mode of action. The aim of our study was to analyze whether the rectal pre-conditioning with ozonized oxygen combined with amoxicillin/clavulanate (AMC) treatment, has any influence on the morbidity/mortality of septic rats.

Materials and methods

This study was approved by the Ethic Committee of the Hospital Universitario de Gran Canaria Dr. Negrín, and was performed in compliance with standard operating and quality procedures following published guidelines (OECD Principles on Good Laboratory Practice [as revised in 1997], Council Directive of 22 September 2010 on the protection of animals used for experimental and other scientific purposes [2010/63/UE]).

Rats and housing

We used 54 female Sprague-Dawley rats weighing 270–320 g (Charles River Laboratories, Barcelona, Spain). Food (R.02-E Standard Diet, Prolabor, Barcelona, Spain) and tap water were available *ad libitum*. Ambient temperature was $21 \pm 1\,^{\circ}C$ and relative humidity was $55 \pm 5\%$ with an air change rate of 15 times/h. Routine microbiological monitoring, revealed no evidence of infection or parasitosis with common murine or rats pathogens.

Experimental design. Induction of sepsis

For induction of peritoneal sepsis we used the model referred by Zamora et al.[21] Briefly, sepsis was induced by intraperitoneal injection of a mixture 1:1, fecal material (0.65 g/kg) and Ringer lactate solution. Fecal material was collected from the caecum of sacrificed donor rats (same strain, weight and age). Post inoculation analgesia consisted of 0.05 mg/kg buprenorphine given subcutaneously twice daily (Buprex®, Mundipharm, Limburg, Germany) during the first 3 days post inoculation. After that, animals received food and water *ad libitum*. In accordance with the recommendations of some authors,[22,23] animals were assessed every 12 h (from 6 h post inoculation) for several vital signs (Table 1). At the end of the trial (10 days post inoculation), survivors were anesthetized

Table 1
Scoring of rodent protection test.

Vital signs	
1. Ruffled fur	6. Ataxia
2. Weight loss	7. Tremor
3. Ocular discharge	8. Hypothermia
4. Lethargy	9. Cyanosis
5. Hunched posture	
Conditions	**Suggested action**
5 + 6 (or 7 or 8 or 9)	Euthanasia

Source: Adapted from Acred P. et al. Lab Anim (1994). 28(1):13–18.