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Activity of Ertapenem and Ceftriaxone in the eradication of *Salmonella* in a model of experimental peritonitis in mice

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Since the beginning of the 1990s, the prevalence of isolates of non-typhoidal *Salmonella* species resistant to antimicrobial agents, including those commonly used for the treatment of *Salmonella* infections such as fluoroquinolones, trimethoprim-sulfamethoxazole or β -lactams, has increased substantially. Infections caused by multidrug-resistant strains of non-typhoidal *Salmonella* are now frequently encountered.

In order to consider an appropriate role of Ertapenem in *Salmonella*-due intraabdominal infections, in the present study we compare the efficacy of Ertapenem *versus* that of Ceftriaxone in a mouse peritonitis model.

Bacteriological eradication from blood, liver and mesenteric lymph nodes was observed after 5 and 7 days of treatment in all infected mice receiving ceftriaxone.

Although both antimicrobial agents –Ertapenem and Ceftriaxone– were observed to be effective in reducing mortality in inoculated mice, our data suggests a reduced efficacy of Ertapenem in the bacteriological eradication of *Salmonella enterica* serotype Typhimurium in a mouse peritonitis model.

Key words: Ceftriaxone. Ertapenem. Bacteriological eradication. Experimental peritonitis.

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Actividad de ertapenem y ceftriaxona en la erradicación de *Salmonella* en un modelo experimental de peritonitis en ratones

Desde la década de 1990, la prevalencia de aislamientos de diferentes especies de *Salmonella* no-typhi resistentes a antimicrobianos, incluidos los utilizados más frecuentemente en el tratamiento de infecciones por

Salmonella como fluorquinolonas, SXT o β -lactámicos, se ha incrementado notablemente. Las infecciones causadas por cepas multirresistentes son en estos momentos frecuentemente descritas.

Con el fin de considerar el papel de ertapenem en el tratamiento de infecciones intraabdominales causadas por *Salmonella* se diseña el presente estudio que compara la eficacia de ertapenem frente a ceftriaxona en un modelo experimental de peritonitis en ratones.

La erradicación bacteriológica en sangre, hígado y ganglios mesentéricos fue observada a los 5 y 7 días de tratamiento en todos los ratones infectados que recibieron ceftriaxona.

Aunque ambos antimicrobianos, ertapenem y ceftriaxona, demostraron ser eficaces reduciendo la mortalidad de los ratones inoculados, los datos obtenidos sugieren que ertapenem posee una eficacia reducida en la erradicación bacteriológica de *Salmonella* no-typhi en este modelo experimental.

Palabras clave: Ceftriaxona. Ertapenem. Erradicación bacteriológica. Peritonitis experimental.

INTRODUCTION

Salmonellosis is a frequent worldwide cause of food-borne gastroenteritis. Although enterocolitis is often a self-limited disease, in prolonged diarrhoea, severe clinical infections, neonates, immunocompromised patients or extra-intestinal infectious *Salmonella*-due complications treatment is mandatory.

Since the beginning of the 1990s, the prevalence of isolates of non-typhoidal *Salmonella* (NTS) species resistant to antimicrobial agents, including those commonly used for the treatment of *Salmonella* infections such as fluoroquinolones, trimethoprim-sulfamethoxazole or β -lactams, has increased substantially.¹ Infections caused by multidrug-resistant strains of NTS are now frequently encountered.²

Resistance to β -lactams includes third-generation cephalosporins, usually due to the production of extended-

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spectrum β -lactamases (ESBLs), leaving very limited options for treatment in serious extra-intestinal infections.^{3,4} The only betalactams currently available against these strains are the carbapenems.

Recently, a novel carbapenem –Ertapenem– has been approved for human therapy.⁵ It exhibits broad activity against most *Enterobacteriaceae*, including *Salmonella* strains. Ertapenem is indicated for the treatment of moderate-to-severe intra-abdominal infections, including peritonitis, diverticulitis, appendicitis and intra-abdominal abscesses.⁶ Its prolonged half-life and high protein binding capacity allows once-daily dosing, similar to Ceftriaxone.

In order to consider an appropriate role of Ertapenem in *Salmonella*-due intraabdominal infections, in the present study we compare the efficacy of Ertapenem versus that of Ceftriaxone in a mouse peritonitis model.⁷

MATERIALS AND METHODS

Antimicrobial agents

Treatment with subcutaneous injections (0.2 ml) of Ceftriaxone (Roche Laboratories, Neuilly-sur-Seine, France) or Ertapenem (Merck Research Laboratory, Rahway, N.J.) started at 2 hours post- inoculation. Ceftriaxone was administered at doses of 50 mg/kg and 100 mg/kg.

The dose ranges studied for Ertapenem were 15 mg/kg/12h, 30 mg/kg/day, 50 mg/kg/12h, 50 mg/kg/day, and 100 mg/kg/day. Ceftriaxone was dosed according to a previously published murine model⁸ and Ertapenem according to a neutropenic mouse model.⁹

Strains

A clinical isolate of *Salmonella enterica* serotype Typhimurium isolated from the peritoneal fluid of a patient with intraabdominal abscess was used. The strain was susceptible to Ceftriaxone (MIC E-test: 0.047 μ g/ml), Ertapenem (MIC E-test 0.016 μ g/ml) and non-ESBL-producer.^{10,11}

Mouse peritoneal model

Five-week-old male mice (OF1) with a mean weight of 25–30 g were included in this study.

The animals were housed in accordance with EU regulations concerning the use of animals for experimental purposes and other scientific aims. For handling, the norms concerning the handling of animals stipulated in 86/609/CEE were followed and the animals were provided with food and water *ad libitum*.

A suspension of each test isolate was prepared from a fresh subculture that had been incubated for less than 20 h and diluted to achieve an inoculum of 10^7 CFU/ml. Seven groups of mice (twenty mice per group) were inoculated intraperitoneally with 0.1 ml of a bacterial suspension (infective dose 5×10^7). Final inoculum concentrations were confirmed with serial dilution.

Twenty infected non-treated mice were used as controls. Death and survival were monitored at 24h. Ten mice in each treated group were sacrificed over 5 and ten over 7 days post-treatment.

The efficacy of both antibiotics was measured considering bacterial counts of *Salmonella enterica* serotype Typhimurium in lymph nodes, liver and blood.

Sample processing

The experimental protocols were approved by the Committee for Animal Care and Use of the University of Salamanca and were implemented under International Rules and the European Council Agreements concerning applied animal experimentation (Directive 86/609/EEC).

At the end of the experimental period, with the animals under general anaesthesia, heart blood was obtained by puncture and samples of liver and mesenteric lymph nodes were obtained under sterile conditions. Culture processing was performed immediately after sample collection.

Once samples had been obtained for culture, they were placed in sterile bags for later homogenization in a Stomacher® (Seward Medical, London, UK). Dilution at different concentrations was carried out at Trypticase broth before high-intensity homogenisation for 8 min in Stomacher 80®.

Once the samples had been homogenised, 100, 500 and 100 μ l were cultured on three blood-agar plates under aerobic conditions at 37 °C over 48 hours. Gram-staining was performed on all positive samples. The number of colonies per gram of tissue was calculated, and the results of the three culture plates were used to obtain the weighted mean.

RESULTS

Mortality at 24h in the non-treated group was 100%.

Bacteriological eradication from blood, liver and mesenteric lymph nodes was observed after 5 and 7 days of treatment in all infected mice receiving Ceftriaxone (20 animals received a dose of 50 mg/kg/day and a further 20 were administered a dose of 100 mg/kg/day; in each group 10 animals were sacrificed at 5 days after the start of treatment and the rest after 7 days of treatment).

In only 4 of the 100 Ertapenem-treated mice was *Salmonella enterica* serotype Typhimurium eradicated from peritoneal lymph nodes; in two samples of mesenteric lymph nodes from a mouse treated with 50 mg/kg/12 h (one sacrificed at 5 days and the other at 7) and in another two from a mouse treated with 100 mg/kg/day (one sacrificed at 5 days and the other at 7). In these four cases, the blood and liver cultures were also negative.

The growth *Salmonella enterica* serotype Typhimurium was detected in pure culture from 26 animals (26% of the animals treated with Ertapenem); in the blood of 48 mice, and in mesenteric lymph nodes in 96.

No differences were observed with respect to the dose received.

The results are shown in table 1.

DISCUSSION

Salmonella infections can manifest themselves as acute abdominal problems and may demand emergency surgery. Some examples are: salmonella-related intestinal perforations, gallbladder involvement, salpingitis, and peritonitis. Mesenteric lymphadenitis associated with *Salmonella* mimics acute appendicitis and it is often difficult to establish a timely and opportune diagnosis. Because of the difficult diagnostic process, a significant number of patients with *Salmonella* infections develop acute abdomen and undergo needless operations.

The aim of the present study was to determine the role of Ertapenem in *Salmonella*-due intraabdominal infection, comparing the efficacy of the antimicrobial agent with that

of Ceftriaxone, one of the efficacious antibiotics for these clinical pictures.^{12,13} This was to determine whether it might offer a valid therapeutic alternative in an experimental model of peritonitis. The rationale for this was based on the reported increased resistance to Ceftriaxone.^{14,15}

The results obtained reveal the low efficacy of Ertapenem in the eradication of *Salmonella enterica* serotype Typhimurium. In contrast, and as expected, the results obtained with Ceftriaxone confirmed its therapeutic efficacy.¹⁶

Salmonella is a gram-negative bacillus that behaves as a facultative intracellular pathogen.¹⁷ Its habitat is the gastrointestinal tract of animals and humans: it is never present as normal microbiota. Owing to its invasive capacity and its ability to survive challenge by phagocytes it is associated with many infectious diseases.

Salmonella has 5 pathogenicity islands. Several genes of pathogenicity island 1 (SPI-1) are involved in the invasion and apoptosis of macrophages. The genes located in SPI-2 and SPI-3 regulate the survival and replication of the bacterium in the intracellular compartments of phagocytes and epithelial cells. SPI-4 is thought to participate in adaptation to intracellular environments, and SPI-5 encodes factors involved in the fluid secretion and inflammatory reaction of the intestinal mucosa.

Salmonella typhimurium is an intracellular pathogen capable of proliferating within the vacuolar compartments of non-phagocytic eukaryotic cells.¹⁸⁻²⁰

Ceftriaxone is able to penetrate cells,^{21,22} like Ertapenem, which is active against intraphagocytic *Staphylococcus aureus* but not against *Listeria monocytogenes*.²³ Ertapenem will probably be ineffective against intraphagocytic forms

Table 1

Bacteriological eradication of *Salmonella enterica* serotype Typhimurium in mice treated with ceftriaxone and ertapenem

	N.º	Exitus 24 h	Bacteriological eradication After day 5 posttreatment (n.º 10)	Bacteriological eradication After day 7 posttreatment (n.º 10)
Control	20	20	–	–
Ceftriaxone 50 mg/kg/day	20	0	10	10
Ceftriaxone 100 mg/kg/day	20	0	10	10
Ertapenem 15 mg/kg/12h	20	0	0	0
Ertapenem 30 mg/kg/day	20	0	0	0
Ertapenem 50 mg/kg/12h	20	0	1	1
Ertapenem 50 mg/kg/day	20	0	0	0
Ertapenem 100 mg/kg/day	20	0	1	1

of *L. monocytogenes* for reasons that remain to be discovered. Conversely, Ertapenem could offer an alternative to ampicillin and meropenem against intraphagocytic *S. aureus* since its longer half-life may allow high concentrations to be maintained for longer times.²³

It remains for the clinical meaning of this lack of eradication to be explained and whether the result can be extrapolated to human beings.

CONCLUSIONS

Although both antimicrobial agents –Ertapenem and Ceftriaxone– were observed to be effective in reducing mortality in inoculated mice, our data suggests a reduced efficacy of Ertapenem in the bacteriological eradication of *Salmonella enterica* serotype Typhimurium in a mouse peritonitis model.

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