

Effects of boric acid-linked ampicillin on the rat intra-abdominal sepsis model

Ufuk Oguz Idiz^{1*}, Erhan Aysan², Deniz Firat³, Cilem Ercan², Selami Demirci⁴ and Fikrettin Sahin³

¹Department of General Surgery, Sisli Etfal Training and Research Hospital, Istanbul, Turkey

²Department of General Surgery, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey

³Department of General Surgery, Bursa Sevkett Yilmaz Training and Research Hospital, Istanbul, Turkey

⁴Department of Genetics and Bioengineering, Yeditepe University, Istanbul, Turkey

Abstract: The aim of this study was to determine efficiency of a new molecule that was obtained by linking boric acid with ampicillin in treating intra-abdominal infection. Following intraperitoneal *E. coli* injection to twenty-one female Wistar albino rats, group 1 was administered boron-linked ampicillin, group 2 was administered only ampicillin and group 3 was injected intraperitoneally with physiological serum. IL-6, and a white blood cell analysis was performed from the blood before and on the seventh day of treatment. No statistically significant difference in blood WBC levels after treatment was found among the groups. There was no statistically significant difference in the IL-6 values of group 2 and group 3 before and after the treatment ($p=0.195$ and 0.193 , respectively); however, the reduction in the serum IL-6 values of group 1 was statistically significant ($p=0.003$). Boric acid-linked ampicillin is a more effective intra-abdominal infection treatment compared with ampicillin alone.

Keywords: Boric acid, sepsis, treatment, ampicillin, intra-abdominal.

INTRODUCTION

Intra-abdominal infection (IAI) and associated peritonitis are quite frequent and diverse (Schein, 2004; Singh *et al.*, 2011; De Waele, 2010). In addition, sepsis and the mortality rate are very high (Brocco, 2012). Although the mortality rate is 30% in primary IE, it is approximately 50% in recurrent cases (Marshall and Innes, 2003). At first, local peritonitis develops into IE and its eradication is often facilitated by local inflammatory mechanisms during this stage of the pathological process. The systemic inflammatory response occurs based on the sum of the factors associated with the number of pathogens, its virulence, the presence of toxin and the presence and quality of the response by the host's immune system (Bone, 1992; Garrido *et al.*, 2004; Dellinger 2003; Farthmann and Schoffel 1998). The conventional treatment for IE-associated peritonitis is the mechanical removal of the content, which causes contamination (surgical drainage), in addition to the re-establishment of anatomical integrity and antibiotherapy (Brocco, 2012). As with other infections, culturing is very important for determining which antibiotics are to be administered for treating IE-associated peritonitis. However, there are some associated issues because polymicrobial factors are generally involved in IE-associated peritonitis (Evans *et al.*, 2001).

As one of the trace elements, boron does not appear naturally in its elemental form; instead, it exists most

notably as boric acid and boronax (Duydu *et al.*, 2012; Turkez *et al.*, 2012). In addition to its traditional use in the health care system, boron is widely used in industrial, agricultural and cosmetic applications (Murray, 1998). Boric acid is fairly rapidly absorbed after its administration and appears to be rapidly distributed throughout the body via passive diffusion (Murray, 1998). However the metabolic effects and pathways of boron in humans and animals are not completely understood (Aysan *et al.*, 2011; Kucukurt *et al.*, 2015; Armstrong *et al.*, 2001; Nielsen *et al.*, 1987)

Due to the rapid increase in antibiotic resistance, new antibiotics are being investigated and used in the treatment of peritonitis (Tavares, 2000; Batai *et al.*, 1999). For the present study, we created a new compound by linking a boron atom to an ampicillin molecule for the added benefit of the rapid and easy passage of boron through cell membranes due to its high biocompatibility and small atomic radius. We then assessed the antibacterial efficiency of this new compound on the intra-abdominal sepsis model we constructed in rats.

METHODS AND MATERIALS

This study was performed at the Experimental Animal Production and Research Laboratory of Bezmialem Vakif University and was approved by the local Animals Ethics Committee. All protocols were in accordance with the regulations governing the care and use of laboratory animals in the Declaration of Helsinki.

*Corresponding author: e-mail: oguzidiz@yahoo.com

Twenty-one female Wistar albino rats (mean weight 285 g; mean age 4 months) were divided into three equal groups. According to a power analysis using 0.05 accuracy and a power of 0.95, seven rats were assigned to each of the groups. Throughout the experiment, the rats were kept alive in standard cages produced for mice and rats; the bases and sides of the cages were made of plastic, and the tops were covered with iron wire netting. The rats were fed a pellet-type fabricated feed specially produced for small experimental animals, and the room they were kept in was under a 12-hour light/12-hours dark cycle.

The boric acid-linked ampicillin molecule was obtained by adding 64 mg boric acid (Boric Acid Granular®, Eti Mining Enterprises, Kutahya, Turkey) into a 4-ml solution containing 1g ampicillin (Ampisina® 1gr Flakon, Mustafa Nevzat Pharmaceuticals, Istanbul, Turkey) and 3 ml 0.9% NaCl; the resulting solution was stored at +4°C.

After all rats were administered general anesthesia with xylazine (Rompun® Bayer Co.; 5mg/kg body weight), 1 ml blood was obtained from each subject through intracardiac entry and centrifuged. The plasma was stored at -80 °C. Fresh feces that were obtained from the rats 24 hours later was brought to a microbiology laboratory and diluted in 0.9% NaCl to contain a suspension of a hundred million *E. coli* per 1ml. Within the same day, 1ml of the fecal suspension was injected into intraperitoneal space of the rats percutaneously via the right lower quadrant of the abdomen.

Twenty-four hours after the intraperitoneal *E. coli* injection, antibiotics were administered as follows: group 1 was treated with 50mg/kg boric acid-ampicillin solution, group 2 with ampicillin alone and group 3 with 0.9% NaCl. All three groups were administered the same dosage for each treatment at the same time of day intraperitoneally for five days.

All the rats were administered general anesthesia seven days after intraperitoneal *E. coli* injection. The rats were then sacrificed after obtaining 3ml of blood through intracardiac entry. The blood samples were centrifuged

and the plasma was obtained. The plasma and blood that was acquired at the beginning of the study were analyzed for white blood cell (WBC) and interleukin-6 (IL-6) levels. Serum IL-6 levels were tested using enzyme-linked immunosorbent assay (ELISA) kits (Invitrogen, catalog no: KRC0061, North America for IL-6). To determine IL-6 levels, the serum samples of the rats were diluted to a ratio 1:2 in a standard diluent buffer. Incubation buffer, standards and samples were added to the appropriate microtiter wells in the plate. After incubation for 2 hours at 37°C, biotin-conjugate solution was added to each well and the plate was incubated for 1 hour and 30 minutes at room temperature (RT). Then, streptavidin HRP working solution was added to all wells, and the plate was incubated for 30 minutes at RT. After incubation with stabilized chromogen for 30 minutes at RT, stop solution was added to each well. Finally, the absorbance of each well was determined using a microplate reader (Chromate Manager 4300, Palm City/USA).

STATISTICAL ANALYSIS

The statistical analyses were performed using IBM SPSS Ver. 21.0. In addition to descriptive statistical methods (mean and standard deviation), we used the paired t-test for in-group variable comparisons and the Kruskal Wallis test for comparing inter-group variables. The results were evaluated at the $p < 0.05$ significance level.

RESULTS

Mortality was observed only in two rats in the control group during the study and, occurred during intracardiac blood withdrawal at the beginning of the study. As a result, two new subjects were added to this group and the study was continued with an equal number of subjects in each group.

The IL-6 levels of the intracardiac blood from the subjects were assessed before and after treatment. No significant difference was observed among the groups before ($p = 0.361$) and after treatment ($p = 0.363$). However, the serum

Table 1: Showing the mean IL-6 levels of groups before and after treatment. (SD: Standard derivation)

	IL-6 Levels Before Treatment (pg/ml)	IL-6 Levels After Treatment (pg/ml)	P Value
Group 1 (Mean±SD)	45.51±9.8	23.71±12.07	0.003
Group 2 (Mean±SD)	40.73±6.8	33.20±13.65	0.195
Group 3 (Mean±SD)	42.06±7.4	32.79±16.73	0.193

Table 2: Comparison of mean WBC levels between the groups before and after the treatment. (SD: Standard derivation)

	Group 1	Group 2	Group 3	P Value
Mean±SD WBC ($10^3/\text{mm}^3$) before treatment	7.75±1.27	6.90±1.19	6.60±0.93	0.312
Mean±SD WBC ($10^3/\text{mm}^3$) after treatment	6.34±1.74	5.36±0.91	5.11±1.30	0.322

IL-6 levels of the boric acid-linked ampicillin group (group 1) before treatment decreased significantly after treatment ($p < 0.05$, table 1). No statistically significant difference was observed when the blood WBC levels were compared before ($p = 0.312$) and after treatment ($p = 0.322$) (table 2).

DISCUSSION

Because the mortality and morbidity of IE-associated peritonitis remains high with the available treatment approaches, the search for new treatment alternatives is expected. Hermann *et al.*, who intended to benefit from the immunomodulator effect of volatile anesthetics in organ failure, assessed the effect of a water-soluble metabolite of sevofluran, hexafluoroisopropanol, on abdominal sepsis that was generated after cecal ligation and puncture in rats. The 1-week survival values were compared between the IV saline-administered group and hexafluoroisopropanol-administered group in their study. The survival percentage of the hexafluoroisopropanol-administered group was statistically higher compared with the saline-administered group (77% vs. 17%, $p = 0.037$) (Herrmann *et al.*, 2013). In another study, the efficiency of an anti-inflammatory substance hydroalcoholic extract (HEPs) was studied in an peritonitis model that was constructed in rats by intraperitoneal liposaccharide injection. The levels of inflammatory cells, TNF- α and IL-1 β were significantly reduced in rats using HEPs during the liposaccharides-associated acute inflammatory response (Borges *et al.*, 2013). Brocco *et al.* assessed the efficiency of Ropivacaine on IE treatment by, injecting a fecal solution into the abdominal cavity of 16 Wistar albino rats and evaluating IL-6 and TNF- α level, which were determined to be reduced in the Ropivacaine treated group at a statistically significant level (Brocco *et al.*, 2012).

In the study by Cao *et al.*, HMGB1 nuclear protein was elevated in the peritoneal dialysis fluid of cases of peritoneal dialysis-associated peritonitis. It was observed that the HMGB1 levels correlated with WBC, IL-6 and TNF- α levels, with HMGB1 levels decreasing in the peritoneal fluid after antibiotherapy. Cao *et al.* demonstrated that the HMGB1 inhibitor glycyrrhizin provided protection against peritoneal inflammation and dysfunction in peritonitis due to liposaccharides (Cao *et al.*, 2013).

In a study by Hernandez *et al.*, it was determined that boron-based antibacterial molecules inhibited the resistance of Gram (-) bacteria, especially *E. coli* and *Pseudomonas* (Hernandez *et al.*, 2013). In the study by Livermore *et al.*, it was demonstrated that the combination of biapenem and boronate β -lactamase inhibitor superior over class-A carbapenems against Enterobacteriaceae (Livermore and Mushtaq, 2013).

Boron is immediately to the left of the carbon in the periodic table; as the boron atom is very similar to the carbon atom, many carbon-based molecules have the same properties as boron-based molecules. The most important difference is the boron has a smaller atomic structure, and boron has accordingly been used for approximately 30 years in boron neutron capture therapy (Altieri *et al.*, 2008). Boron neutron-capture therapy applications have been used in brain tumors; however, clinically successful outcomes could not be obtained (Farr *et al.*, 1954; Asbury *et al.*, 1972). The reason was determined to be the inability of boron reach the deeper tissues 3-4 cm in the brain, its ability to diffuse due to its small molecular weight and its inability to accumulate at a particular location within tumors (Choi *et al.*, 1989; Archanbeau *et al.*, 1964). Given that boron is a small atom that easily enters and diffuses into the cell, we based this study on the idea that boron will also facilitate the entry and diffusion of an antibiotic molecule that forms a solution with boron.

Due to its chemical structure, boron displays stable linkage to molecules containing a halogen group. When different antibiotics were examined, we observed that antibiotics in the group possessing this molecular structure are not an appropriate option for IE treatment. Thus, we decided to utilize the β -lactam group, which is closest to this molecular structure and is an effective antibiotic group. Linking boron to the cefoperazone (Cefobid ® 1 gr. Flakon, New York, USA) molecule was examined. This molecule was not successful due to its complex three-dimensional structure, its non-reactive cyclic molecular site content and the occurrence of breakages in its molecular structure during the linkage process. Although amine and methyl groups are not as reactive as halogens, we decided to employ ampicillin due to its more reactive linking percentage compared with the cyclic structures in cefoperazone and used ampicillin in this study after molecular linkage with boron.

The proinflammatory cytokine IL-6 is a highly effective marker of the response to antibiotics treatment (Brocco, 2012; Cao *et al.* 2013; Hernandez *et al.*, 2013).

Accordingly, we used serum IL-6 and blood WBC values as assessment parameters in this study.

No statistically significant difference among the groups was observed in the number of WBCs. However, the serum IL-6 levels of the group treated with boron-linked ampicillin were significantly reduced compared with the group treated with ampicillin alone.

In conclusion, our results support our hypothesis and demonstrate that linking boric acid to the ampicillin molecule is more effective in IE treatment compared with ampicillin alone. Benefiting from its biocompatibility and

small molecular diameter, testing the efficiency of this new molecule in treating other site infections and in other combinations of antibiotics may increase the efficiency of antibiotherapies.

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