

EFFICIENCY OF TAZOBACTAM/PIPERACILLIN IN LETHAL PERITONITIS IS ENHANCED AFTER PRECONDITIONING OF RATS WITH O₃/O₂-PNEUMOPERITONEUM

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ABSTRACT—Insufflation of ozonized oxygen into the peritoneum (O₃/O₂-pneumoperitoneum [O₃/O₂-PP]) of rats reduced the lethality of peritonitis. We evaluated the prophylactic effect of O₃/O₂-PP combined with tazobactam/piperacillin (TZP) in polymicrobial lethal peritonitis. Wistar rats were conditioned by daily repeated insufflation of ozone for 5 days, and hematologic parameters were determined. Sepsis was induced by i.p. injection of cecal material derived from donor rats. Simultaneously, TZP was applied at a single dosage of 65 mg/kg or at two dosage schedules of 65 mg/kg each at an interval of 1 h. The conditioning effect of O₃/O₂-PP on the number of blood cells was measured before inoculation of bacteria. The mRNA levels of proinflammatory cytokine IL-1 β and TNF- α were determined at 4 h post infection in spleen and liver by semiquantitative *in situ* hybridization analysis. Preconditioning of rats by O₃/O₂-PP enhanced the number of blood leukocytes and granulocytes and increased the survival rate of septic rats up to 33%. The combination of O₃/O₂-PP and TZP further enhanced the survival rate up to 93%. This effect was accompanied by a reduced amount of IL-1 β and TNF- α mRNA in spleen and liver. In contrast, in non-infected animals the combination of O₃/O₂-PP and TZP enhanced IL-1 β and TNF- α mRNA in the spleen and IL-1 β mRNA in liver when compared with TZP- and sham-treated controls. The preconditioning effect of O₃/O₂-PP seems to support the biological effectiveness of TZP by altering the immune status before and during the onset of sepsis. The combined therapy could be a simple, preoperative intervention for abdominal surgery to reduce postoperative morbidity and mortality.

KEYWORDS—Antibiotic therapy, cytokines, mortality, oxidative stress, sepsis

INTRODUCTION

Modern antibiotics are without any doubt the most important cornerstones in the treatment of mild and severe infectious diseases. But, when they are given in high dosages in severe clinical situations, and when long-term application is necessary, adverse effects and antibacterial resistances may develop with increased antibiotic consumption (1–3).

Tazocin is an injectable antibiotic mixture consisting of the semisynthetic antibiotic piperacillin-sodium (PIP) and the β -lactamase inhibitor tazobactam-sodium (TZB). It is generally considered safe, and only in very rare cases can high doses cause pseudomembranous colitis, significant thrombocytopenia, leukocytopenia, bone marrow suppression (4–9), or hemolytic anemia (10). One clinical use of TZP is the treatment of intraabdominal infections (11, 12). However, TZP could interact with other drugs given before or simultaneously. This interference could be beneficial or detrimental in the course of infection and inflammation. The clearance of tazobactam can be prolonged by substances such as probenecid (13) and thus might increase adverse effects.

In the rat model of lethal sepsis, positive as well as negative effects of antibiotics with distinct influence on the outcome of a severe abdominal infection have been shown (14, 15). In the search for new experimental strategies, hyperbaric oxygen therapy (HBO) was found to be a beneficial adjuvant therapy for different infectious diseases (16, 17). This oxygen therapy by inhalation was also shown to be beneficial in peritonitis, endotoxic shock, or multiple organ failure (18, 19), and it was found to prevent bacterial translocation after mechanical obstruction or thermal injury (20). In a lethal peritonitis study in rats we have recently shown that an oxidative preconditioning use as a new therapeutic approach (O₃/O₂-pneumoperitoneum, O₃/O₂-PP) enhanced the survival rate up to 33% (21). Recently, ozone has been described as an endogenously produced gas (22), suggesting a physiological role of this gas *in vivo*. On the basis of the oxidant properties of ozone and based on the possibility that lipid oxidation products (23, 24) may exert antiinflammatory properties that are potent enough to protect animals from LPS-induced tissue damage (25), we postulate that ozone may attenuate the susceptibility to septic shock.

The aim of our study was to analyze whether the preconditioning with ozonized oxygen has any influence on the survival/mortality of septic rats when combined with an intramuscular (i.m.) injection of TZP given in a bolus-like low concentration comparable to that used in children (26). To comprehend a possible biological mechanism of TZP in

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combination with insufflation of ozonized oxygen into the peritoneum, we measured the number of blood cells before and after O₃/O₂-PP. Additionally, the expression of proinflammatory cytokines (IL-1 β and TNF- α) in spleen and liver early (4 h) after induction of a lethal bacteremia were analyzed.

MATERIAL AND METHODS

Animals

For mortality analysis, adult male Wistar rats (180–200 g) were obtained from the National Center for Laboratory Animals Production (Havana, Cuba). The Health Certificate No. RG.CC.O2.95 showed that the animals were free from *Salmonella* sp., *Streptobacillus moniliformis*, *Streptococcus- β -pyogenes*, *Bordetella bronchiseptica*, *Corynebacterium kutscheri*, *Citrobacter freundii*, *Streptococcus pneumoniae*, and *Shigella* sp. For hematologic, microbiologic, and histologic analysis, healthy (as given by FELASA recommendation) adult male Wistar rats (209–215 g) were purchased from Harlan (Borchen, Germany). All animals were kept in rooms with standardized air conditioning: 20–22°C, 50%–57% humidity, and a 12-h artificial day/night rhythm. The rats had free access to standard diet food pellets delivered either by The Division de Alimentation y Nutricion CENPALAB (No. FF.CC.01.95; Havana, Cuba) or by Altromin (No. 1320; Lage, Germany). Water was available *ad libitum*. Survival analysis performed in Cuba was in accordance with the European Union Guidelines for animal experimentation and was approved by the Institutional Animal Care Committees (ARCA No. 015). Hematologic and histologic experiments performed in Germany were in accordance to the guidelines of FELASA and were approved by the RP Giessen (Az: 17a-19c20-15[1]) according to the Germany Animal Protection Law.

Experimental design and treatment of rats

For mortality analysis ozonized oxygen was generated by an Ozomed machine (Ozomed, Ozone Research Center, Havana, Cuba). In the case of animal experiments, which were performed for microbiological and histological analysis, an Ozonosan gas processor (PTN 60, Dr. Hänslers GmbH, Iffezheim, Germany) was used. Ozone concentrations were monitored by an Ozonosan photometer (Dr. Hänslers GmbH, Iffezheim, Germany). The gas mixture containing 5% ozone and 95% medical oxygen (O₃/O₂) by volume was immediately insufflated with a standardized volume of 80 mL/kg rat by injection (needle size 21 g) into the right lower abdomen of the rats. The O₃/O₂ treatment was repeated daily for 5 days.

Fresh fecal material was obtained from the cecum of male Wistar rats. The mean spectrum of cecal organisms was (in colony-forming units [CFU]/g): *E. coli* (1×10^2 – 1×10^7), *Bacteroides distasonis* (0.5×10^7 – 1×10^8), *Prevotella oralis* (0.5×10^7 – 1×10^8), *Proteus mirabilis* (1×10^5 – 1×10^7) and *Enterococcus faecalis* (1×10^6 – 1×10^7) with highest frequencies and *Streptococcus* sp. (1×10^7), *Staphylococcus aureus* (1×10^4), *Bacillus* sp. (1×10^1) and *Micrococcus* (3×10^1) with lesser frequency. The infection with cecal material started 24 h after the last gas insufflation. Simultaneously to the infection, TZP in a combination of TZP and PIP in a fixed ratio of 1:8 dissolved in saline (Wyeth, Muenster, Germany) was injected i.m. at an empirical dosage of 65 mg/kg body weight (b.w.). This dose is low in comparison to the recommended high doses in humans based on a body-surface-area basis (mg/m²) (27). A second group received an additional injection of 65 mg/kg at 1 h after infection resulting in a total amount of TZP of 130 mg/kg b.w. A sham group (n = 15) was pretreated with daily injections of 80 mL/kg filtered room air or pure oxygen of 80 mL/kg b.w., infected with cecal material and received an equal amount of saline instead of the antibiotic. Animals (n = 12) that were pretreated with daily injections of the O₃/O₂ gas mixture but that received no injection of cecal material were used as controls for pure ozone effects. Untreated animals (n = 20) that received no injection of any gas, no infection with cecal material, and no saline injection were used as the basal control. For histologic analysis 4 h after infection, rats were first narcotized with forene by inhalation followed by a lethal intracardial injection of T61 (Hoechst Roussel Veterinary, Germany). For mortality analysis rats were observed for 5 days.

Hematologic Parameters

To measure basic hematology and differential blood counts, we obtained 50 μ L EDTA blood O₃/O₂ for 5 days by puncturing the retroorbital sinus under forene anesthesia. For the hematologic investigations we used an autoanalyzer (Vet abc™ Animal Blood Counter, ABX Diagnostics, Göttingen, Germany), which has been carefully validated for the analysis of rat blood. For basic hematology we determined the hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). For differential blood count the number of red blood cells (RBC) and white blood cells (WBC), which were further differentiated into lymphocytes,

monocytes, granulocytes, and platelets, were measured. Data were expressed as mean values \pm SEM. Significant differences were determined using the Student *t* test followed by ANOVA (n = 6).

In situ hybridization

In situ hybridization was performed for ³⁵S-labeled IL-1 β and TNF- α mRNA templates on 14- μ m-thick cryostat sections as previously described in detail (21). In short, pGEM-T vectors (Promega, Mannheim, Germany) containing rat specific cDNA fragments for IL-1 β or TNF- α were linearized with the appropriate restriction enzymes, and ³⁵S-labeled sense and antisense ribonucleotide probes were generated by *in vitro* transcription using SP6 or T7 polymerases (Boehringer Mannheim, Germany) as appropriate in the presence of [³⁵S]UTP (Amersham Life Science, Germany). All labeled cRNAs were purified over Micro Bio-Spin® chromatography columns (Bio Rad, Germany) and diluted in hybridization buffer (100 mM Tris, pH 7.5, 600 mM NaCl, 1 mM EDTA, 0.5 mg/mL t-RNA, 0.1 mg/mL sonicated salmon sperm DNA, \times 1 Denhardt's, 10% dextrane sulfate, 50% formamide) to 50,000 cpm/ μ L. Autoradiograms were taken by exposing the sections to an autoradiography film (Hyperfilm- β max, Amersham, Dreieich, Germany) for 1–3 days. For control of the specificity of the *in situ* hybridization signals sense ³⁵S-labeled cRNAs for IL-1 β and TNF- α were used and showed no signals in any of the experimental groups. The *in situ* hybridization experiments were performed twice. For semi-quantitative image analysis the x-ray films were digitized, and the mean relative optical density (ROD) was measured by using the MCID image analysis system. Data were expressed as mean values \pm SEM from s = 12 measurements per group. Significant differences were determined using the student *t* test followed by ANOVA.

RESULTS

Effect of TZP in combination with O₃/O₂-PP preconditioning on the survival rates of septic rats

To test if a low dose of TZP used in a single (65 mg/kg) or repeated (2 \times 65 mg/kg) application can protect adult Wistar rats from a lethal peritonitis, the survival rate was determined for an observation period of 5 days. A single dose of TZP given at the same time point when sepsis was initiated did not influence the mortality rate and mean time of death within the first 24 h (Fig. 1A), whereas two doses of TZP prolonged the mean time of death up to 48 h. Nevertheless, all animals died within 72 h (Fig. 1A). To analyze if the weak effect of the low dose of TZP on the survival time can be enhanced by a prevention therapy with ozonized oxygen, which was found to enhance survival up to 33% when given alone (Fig. 1A), we applied TZP in animals that were preconditioned by the repeated insufflation of ozonized oxygen into the peritoneum (O₃/O₂-PP). We found a dramatic effect of TZP in the O₃/O₂-PP-preconditioned experimental group. The survival rate was enhanced by TZP up to 78% when it was given as a single dose and was further enhanced up to 93% when two doses of TZP were given (Fig. 1B). Body weight analysis of the surviving animals showed that O₃/O₂-PP-preconditioned and TZP-treated rats had a slightly decreased body weight $-3.6\% \pm 3.9\%$ after the 5 days of post infection. In contrast, non-infected control rats showed a normal weight gain development of $+11.7\% \pm 1.6\%$. The specific effect of ozonized oxygen in combination with TZP (130 mg/kg) was shown in comparison to the effects of pure oxygen and atmospheric air (Fig. 1B). In TZP-treated animals that received a preconditioning with atmospheric air, all animals died within 24 h of post infection. In TZP-treated animals preconditioned with pure oxygen, the time of death was delayed, but all animals died within 120 h after initiation of sepsis, too.

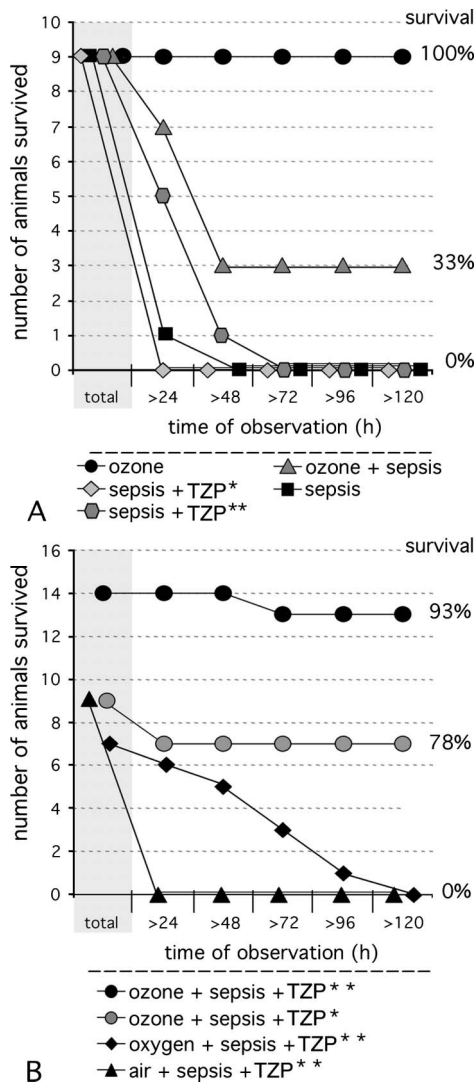


FIG. 1. Effect of TZP and ozone preconditioning on the survival of rats infected with a lethal dosage of polymicrobial bacteria. The survival rate of rats within 24-h intervals up to the end of observation at 120 h is shown. The number of animals per group is given under "total" in the diagrams. (A) Sole effects of ozone preconditioning (ozone + sepsis) and of TZP given at a single dose parallel to the inoculation of the bacteria (sepsis + TZP*) or given at two doses at 0 h and 1 h after infection (sepsis + TZP**) of rats are compared to septic rats (sepsis) that received no gas pretreatment or antibiotic challenge. Ozone-preconditioned rats that received no bacterial inoculation (ozone), were used as a control group. (B) Effects of a combination therapy of ozone preconditioning and single (*) or double (**) TZP treatment (ozone + sepsis + TZP) on the survival rate are shown. Pure oxygen (oxygen + sepsis + TZP) or filtered room air (air + sepsis + TZP) were used as gas controls.

Influence of O₃/O₂-PP preconditioning on hematologic parameters

Obviously, O₃/O₂-PP preconditioning protects rats from a lethal outcome of infection with polymicrobial cecal material. To prove the hypothesis that O₃/O₂-PP preconditioning mediates its beneficial effect by altering the immune status, we measured various hematologic parameters. We found a significant increase in the number of white blood cells (WBC) after a repetitive preconditioning of rats by daily application of O₃/O₂ (Fig. 2B). Analysis of the cellular types of the WBC revealed lymphocytes (Fig. 2C) and granulocytes (Fig. 2D) as the most likely cell types that contribute to these changes rather than

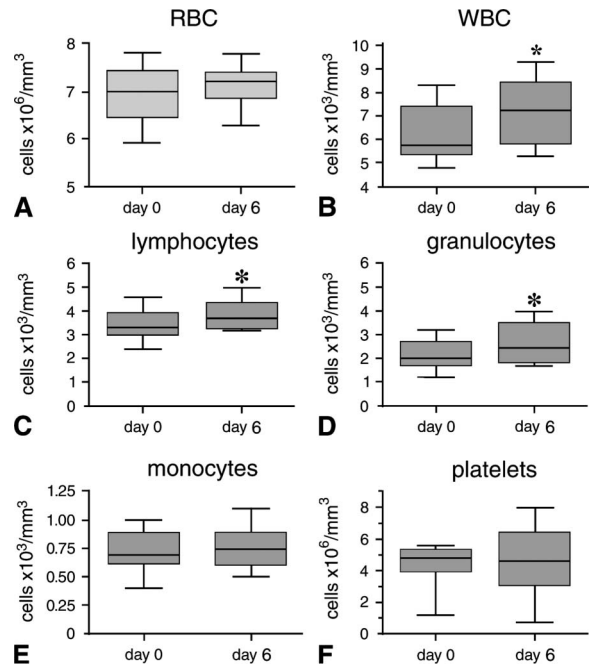


FIG. 2. Effect of O₃/O₂-PP on the number of red blood cells (A) and white blood cells (B) in the blood. White blood cells were further differentiated into lymphocytes (C), granulocytes (D), monocytes (E), and platelets (F). The number of cells was determined at day 0, directly before the first O₃/O₂ insufflation into the peritoneum and at day 6, 24 h after the last gas insufflation. Data were expressed as mean values ± SEM. Asterisks indicate statistically significant differences (*P < 0.05) in the number of a given cell population between day 0 and day 6. Significant differences were determined using the Student *t* test followed by one-way ANOVA test (n = 6). Abbreviations: RBC, red blood cells; WBC, white blood cells.

monocytes. This indicates a preconditioning effect of the oxidative gas mixture on both the innate immune system represented by granulocytes and on the adaptive immune system represented by lymphocytes. The hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were unaffected by the oxidative preconditioning (data not shown).

Analysis of cytokine mRNA expression levels in O₃/O₂-PP-preconditioned TZP-treated rats

We further found that the pronounced effect on the number of lymphocytes and granulocytes is based on changes in the bacterially induced expression of proinflammatory cytokines. For this, we measured the mRNA expression of IL-1β and TNF-α mRNA in the spleen and liver at an early time point (4 h) after inoculation of the cecal material. Semiquantitative image analysis of x-ray autoradiograms derived from *in situ* hybridization analysis with ³⁵S-labeled cRNAs for IL-1β and TNF-α revealed a low basal expression of IL-1β mRNA and TNF-α mRNA in the spleen of normal non-infected rats that was not affected by the treatment with TZP (Fig. 3). Application of TZP in combination with O₃/O₂-PP significantly enhanced the amount of IL-1β and TNF-α mRNA in the spleen (Fig. 3) relative to sham-treated non-infected controls. In the liver the expression of IL-1β mRNA but not of TNF-α mRNA was influenced by TZP in combination with O₃/O₂-PP

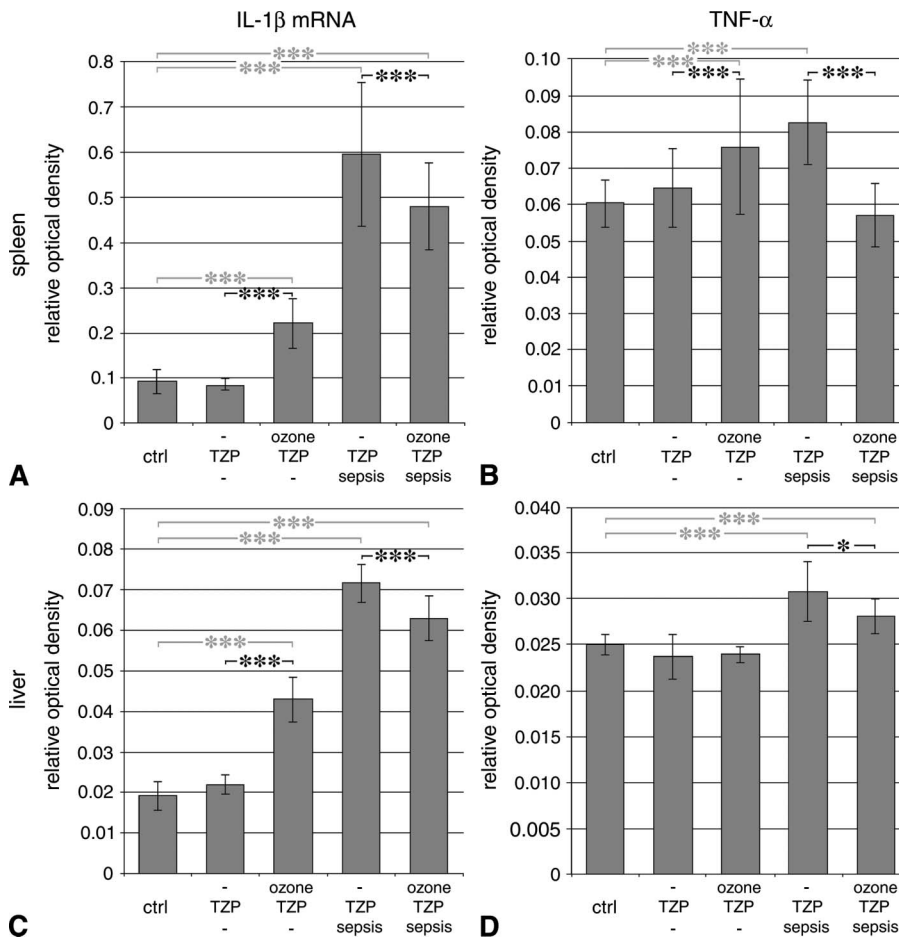


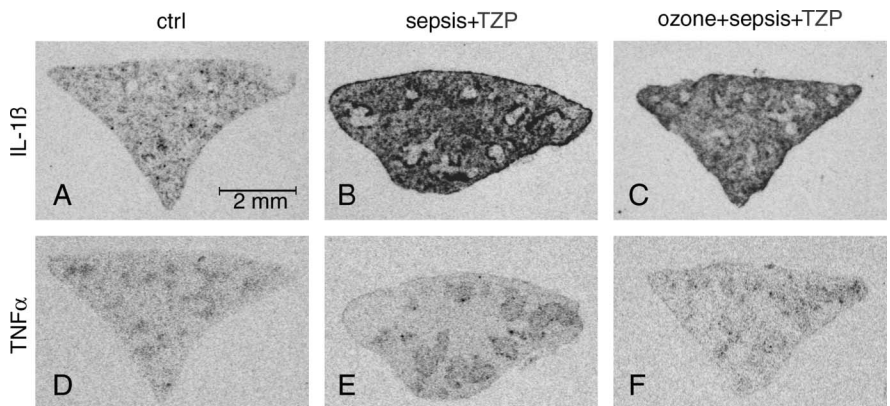
FIG. 3. Semiquantitative analysis of autoradiograms derived from *in situ* hybridization experiments with ³⁵S-labeled cRNA templates for IL-1β (A, C) and TNFα (B, D) mRNA. Cytokine mRNA levels were measured in spleen (A, B) and liver (C, D) of normal sham-injected rat (ctrl), of rats that received a single injection of TZP in combination with (ozone) or without (–) prior treatment with O₃/O₂-PP, or of septic rats (sepsis) that received an i.p. injection of cecal material and in parallel TZP with or without prior treatment with O₃/O₂-PP. Semiquantitative image analysis was performed by using the MCID image analysis system. Data are expressed as the mean relative optical density (ROD) ± standard variations calculated from s = 12 measurements per group. Asterisks indicate statistically significant differences between the marked groups (*P < 0.05; **P < 0.01; ***P < 0.001) calculated by the Student *t* test followed by one-way ANOVA test.

(Fig. 3). Under septic conditions TZP strongly enhanced the expression of IL-1β and TNF-α mRNA in spleen and liver at 4 h after inoculation of cecal material. Interestingly, O₃/O₂-PP preconditioning in combination with TZP significantly attenuated the increase of IL-1β and TNF-α mRNA in spleen and liver. The effect of O₃/O₂-PP preconditioning in TZP-treated infected animals was not a result of different expression patterns within each organ as seen by *in situ* hybridization analysis. In the stimulated spleen, IL-1β and TNF-α mRNA were enhanced in cells preferentially located within the marginal zone of the periarteriolar lymphatic sheaths (Fig. 4). The enhancement was independent of the application of O₃/O₂-PP preconditioning

(Fig. 3). Furthermore, expression patterns of IL-1β and TNF-α in the stimulated liver of TZP-treated animals were unaffected by O₃/O₂-PP preconditioning (Fig. 5). However, the expression of IL-1β mRNA in spleen and liver was much stronger and seen in many more cells than that of TNF-α mRNA.

In conclusion, TZP alone did not influence the basal cytokine expression levels and exhibited no beneficial effect on sepsis-induced mortality. In combination with an O₃/O₂-PP preconditioning, a low dose of TZP modulated the IL-1β and TNF-α mRNA levels in spleen and liver by enhancing the basal and attenuating the sepsis-induced cytokine levels and exhibited excellent effects on the survival rate.

FIG. 4. Autoradiographic detection of IL-1β mRNA (A–C) and TNF-α mRNA (D–F) in rat spleen by *in situ* hybridization analysis. Autoradiograms of representative spleen slices from the experimental groups of sham-injected animals (A, D) or of non-ozone-treated (B, E) and ozone-treated animals (C, F) that received TZP in parallel to the injection of cecal material at 4 h after injection are shown.



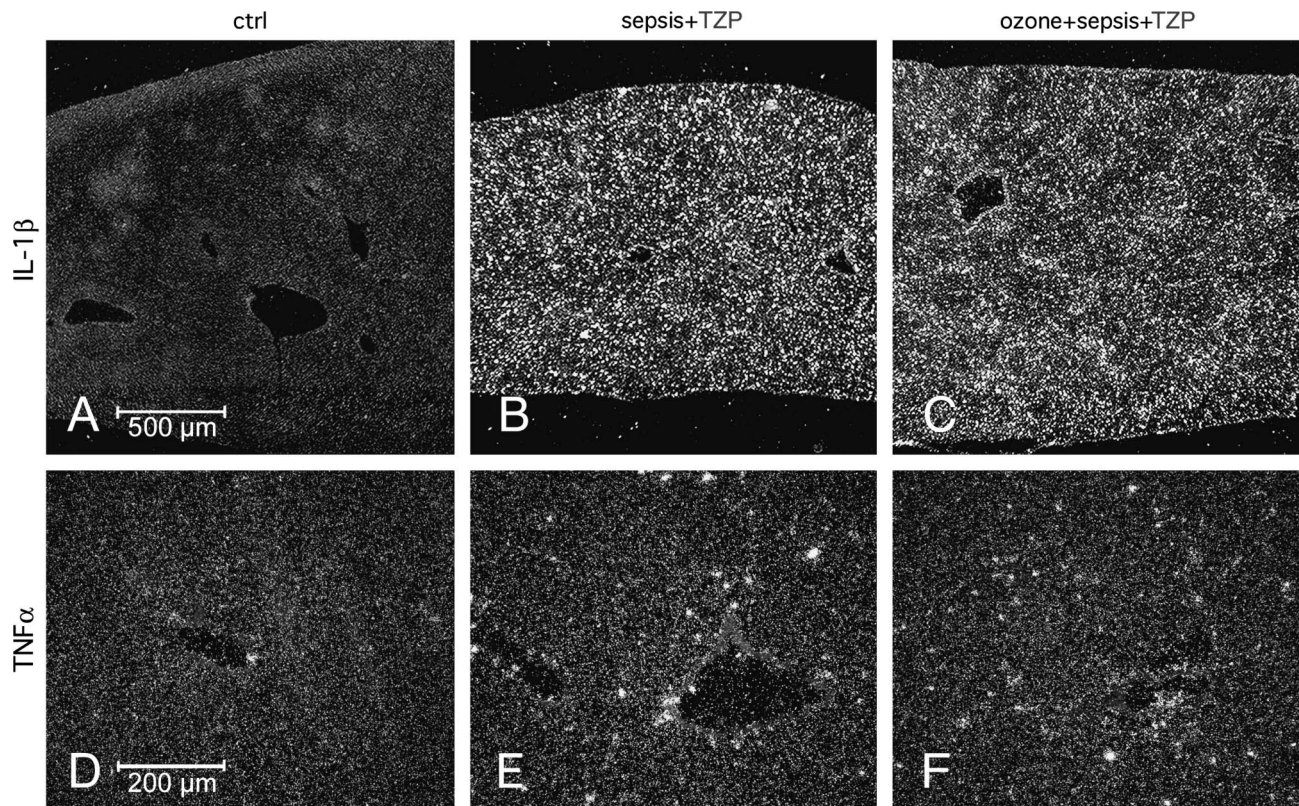


FIG. 5. Dark-field autoradiograms of emulsion-coated liver slices showed no basal expression of IL-1 β mRNA (A) or TNF- α mRNA (B) in a healthy control animal and a strong induction of IL-1 β mRNA in septic animals that received a repeated injection of TZP and were either pretreated with ozone (B) or not (C). Expression of TNF- α mRNA was also induced in septic animals (E, F), although in a smaller number of cells. Scale bars are shown (A, D).

DISCUSSION

The excellent activity of TZP against a broad spectrum of bacteria that are present in our inoculum derived from the cecum of a donor rat has been shown in *in vitro* studies (28). However, in humans the failure rate of TZP in the therapy of intraabdominal infections was reported to be more than 34%, and many other antibiotics also show severe failure rates in clinical trials (29). From this point of view and the search for novel combination therapies to overcome this phenomenon, we established a rat model of lethal polymicrobial sepsis in which a low dosage of TZP was used to mimic the therapeutic failure in man. We applied a subtherapeutic concentration of 130 mg TZP per kg b.w., which resulted in a slight delay in time of death but was insufficient to prevent a lethal septic outcome. We addressed the question whether oxidative preconditioning of rats by ozone, which has been shown to be beneficial on the outcome of the polymicrobial infection of the abdomen used in this study (21), could be an appropriate strategy to enhance the therapeutic potency of TZP in man. Insufflation of an O₃/O₂ gas mixture into the peritoneum alone enhanced the survival of rats up to 33% in comparison to 0% survival in nontreated septic rats. The combination of preconditioning by O₃/O₂-PP and the usage of TZP at the onset of sepsis further increased the survival rate in the rat model of polymicrobial sepsis up to 93%. Therefore, preconditioning of patients with intraperitoneal insufflation of O₃/O₂ gas mixture during their time of preparation for severe abdominal surgery could reduce the risk

of a postsurgical sepsis, especially when used in combination with a β -lactamase inhibitor combination TZP.

The mechanisms that led to the beneficial effects of O₃/O₂ preconditioning are still unknown. Numerous mediators such as microbial signal molecules, cell adhesion molecules, coagulation factors, complement activation products, and cytokines have been thought to play a central role in the pathophysiology of sepsis (30).

One explanation for the beneficial effect of O₃/O₂-PP could be that the repeated insufflations of O₃/O₂ gas mixture may cause induction of adhesive tissue in the peritoneal cavity, which immediately affects the entry of inoculated bacteria into the circulation by immobilizing/encapsulating/binding of bacteria/bacterial products. In this case, the observed reduced inflammatory response to the polymicrobial induced sepsis in the O₃/O₂-pretreated group would be the result of less bacteria/bacterial products getting into the circulation. If this were the case, in organ samples obtained on day 6, before bacterial challenge, or 4 h after bacterial inoculation, formation of adhesive tissue should be seen by macro or micro pathologic examinations. In fact, we have never found any formations of adhesions in the abdomen by macroscopic and microscopic analysis in our O₃/O₂-PP-treated animals after short- or long-term observations (31). Additionally, electron microscopic analyses of O₃/O₂-treated animals revealed no morphologic changes in the peritoneum (32, 33). Therefore, an increased induction of adhesive tissue or formation of adhesive tissue by pretreatment with oxygen/ozone in the abdominal cavity seems

unlikely to be the pivotal mechanism for enhanced survival in our rat model.

Because we have found a significant increase in the number of circulating leukocytes and granulocytes in the blood after O_3/O_2 -PP preconditioning, an altered alertness of the immune system seems likely. This might be the result of the observed enhanced cytokine expression levels *in vivo* followed by the repeated application of O_3/O_2 -PP. The combination of both TZP and O_3/O_2 -PP, but not only one of these, without sepsis (21) affected the basal cytokine levels. Interestingly, after bacterial inoculation into the peritoneum, the amount of IL-1 β or TNF- α mRNA in spleen and liver was reduced rather than further increased when compared with spleen and liver of rats that were not preconditioned with O_3/O_2 . Both effects of enhanced cytokine expression after O_3/O_2 -PP and reduced cytokine levels during the early stage of bacterial infection could be explained by the presence of oxidative modified lipids. Oxidized phospholipids such as 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (PAPC) were found to induce the expression of several inflammatory genes *in vivo* and *in vitro* (34). In connection with this finding, the presence of oxidized phospholipids could explain the observed significantly increased amount of IL-1 β in spleen and liver and of TNF- α in the spleen of normal noninfected rats treated with O_3/O_2 -PP and TZP. Furthermore, under inflammatory conditions, oxidized PAPC can inhibit inflammation and protect mice from lethal endotoxic shock (25). This protective effect of oxidized PAPC was mediated by blocking the interaction of LPS with LPS-binding protein and CD14 but not by the interference with TN- α -induced or IL-1 β -induced NF- κ B-mediated up-regulation of inflammatory genes (25). The presence of oxidized phospholipids during the onset of a bacterial infection could therefore explain the reduced cytokine mRNA expression of IL-1 β and TNF- α in the spleen and liver in our rat model. According to our observed changes in the gene expression level, a reduced amount of TNF- α serum level in LPS-stimulated mice was described (35, 36), supporting that the effect of ozone is not limited on the mRNA level and blocked by posttranscriptional elements (37). Data showing that decreased plasma and peritoneal fluid levels of TNF- α and IL-6 after antibiotic treatment (38) or immune suppression by dehydroepiandrosterone (DHEA) (39) and a reduced systemic inflammatory response (40) in the murine model of cecal ligation and puncture (CLP) are associated with increased survival rates further supports our observation that the O_3/O_2 -PP-mediated reduction in splenic and liver IL-1 β and TNF- α mRNA reflects an O_3/O_2 -PP-dependent inhibition of an early immune response that led to the enhanced survival rate.

Furthermore, besides the presence of oxidative products, which reduce proinflammatory cytokine production during sepsis, antioxidative substances such as pyrrolidine dithiocarbamate (PDTC) and phenyl *N*-*tert*-butyl nitron (PBN), when given after induction of microbial peritonitis, led to enhanced production of antiinflammatory cytokine IL-10 and reduced mortality (41).

The physiological cellular balance between reduction and oxidation (redox) has been shown to be a critical factor for adequate immune responses, which are disturbed under septic conditions (42). Because reactive oxygen has an essential

physiological role in the host defense (43), we hypothesize that preconditioning with ozone might interfere with the endogenous redox system by enhancing the basal status of the reduction systems. As a consequence, the oxidative stress that appears during a severe sepsis might be attenuated, and an overwhelming immune response might be limited, by lower levels of free radicals. Jacobs et al. have shown that increased serum levels of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels in septic rats correlated well with an enhanced survival (44). Both of these primary endogenous antioxidants were significantly enhanced by TZP treatment, and thus, additive effects between ozone-preconditioned reduction systems and enhanced SOD and GSH levels might contribute to the increased survival rate. This could also be a possible mechanism in our peritonitis model, but we did not determine the endogenous redox status. Furthermore, the beneficial effects of ozone oxidative preconditioning on levels of superoxide dismutase (SOD), catalase (CAT), and glutathione on the generation of nitric oxide in a rat model of hepatic ischemia-reperfusion supports the influence of this oxidative stressor on the cellular redox balance (45).

In summary, a preconditioning effect by O_3/O_2 insufflation into the abdomen seems to support the biological effectiveness of TZP by altering the immune status before and during the onset of a polymicrobial sepsis. This combination therapy could be a simple, nonexpensive, perioperative intervention for abdominal surgery to reduce postoperative morbidity and mortality at least in combination with a β -lactamase antibiotic.

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