Journal of Surgical Research **149,** 69–75 (2008)
doi:10.1016/j.jss.2008.01.020

The Novel NF-κB Inhibitor, Dehydroxymethylepoxyquinomicin, Prevents Local and Remote Organ Injury Following Intestinal Ischemia/Reperfusion in Rats

Tomomi Suzuki, M.D.,* Kenichiro Yamashita, M.D.,† Wataru Jomen, M.D.,* Shinya Ueki, M.D.,* Takeshi Aoyagi, M.D.,* Moto Fukai, M.D.,* Hiroyuki Furukawa, M.D.,* Kazuo Umezawa, Ph.D.,‡ Michitaka Ozaki, M.D., Ph.D.,†,[1] and Satoru Todo, M.D.*

**Department of Surgery; †Department of Molecular Surgery, Hokkaido University School of Medicine, Sapporo, Hokkaido, Japan; and ‡Department of Applied Chemistry, Keio University, Yokohama, Japan*

Submitted for publication June 25, 2007

Background. **Nuclear factor-κB regulates the expression of several genes involved in inflammation, the immune response, apoptosis, cell survival, and proliferation. Many of these same genes are activated during ischemia/reperfusion (I/R) injury. Here, we examined the anti-inflammatory efficacy of a newly developed nuclear factor-κB inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ), in the intestinal I/R injury model of rats.**

Materials and methods. **Intestinal ischemia was induced by occluding the superior mesenteric artery for 60 min. The experimental animals were divided into two groups: untreated group, control; treated group, DHMEQ-treated (20 mg/kg). DHMEQ were administered intraperitoneally at 60 min prior to clamping and 5 min prior to reperfusion. Animal survival rates, intestinal tissue blood flow, serum levels of tumor necrosis factor-alpha, and interleukin-6, and the histopathology of both the intestine and the lung were analyzed.**

Results. **The DHMEQ-treated animals exhibited higher values of intestinal tissue blood flow and suppression of tumor necrosis factor-alpha and interleukin-6 production, resulting in marked prolongation of their survival times. Histopathological findings obtained by examining tissues from control animals revealed severe intestinal mucosal damage and disruption of the lung alveolar architecture accompanied by hemorrhage and marked neutrophilic infiltration. These findings were significantly ameliorated in DHMEQ-treated animals.**

Conclusion. **DHMEQ effectively prevented both intestine and lung injuries in rat intestinal I/R models. This agent may possess a good potency for clinical application in various pathological settings including intestinal I/R and/or inflammatory acute lung injury.**

Key Words: **NF-kB inhibitor; DHMEQ; ischemia-reperfusion; small bowel transplantation; ARDS.**

INTRODUCTION

Intestinal ischemia/reperfusion (I/R) leads to severe inflammatory responses in both local and remote organs, particularly in lung. Patients with intestinal intramucosal acidosis experience an increased incidence of multiple organ failure associated with a high mortality rate [1–3]. Although the detailed mechanisms of intestinal I/R injury still remain to be elucidated, various kinds of pro-inflammatory cells or mediators such as neutrophils, reactive oxygen metabolites, platelet-activating factors, and cytokines have been implicated thus far [4–6].

Nuclear factor-κB (NF-κB) is a well-known and one of the most studied transcription factors and is a member of the Rel family of proteins. Very typically, NF-κB exists as a heterodimer of p50 and p65 subunits. In quiescent cells, it exists as a latent cytoplasmic complex bound to its inhibitor protein (IκB). Following the phosphorylation or degradation of IκB, NF-κB translocates to the nucleus and induces the expression of various genes that are critical for cell survival, inflammation, and immunity [7, 8]. NF-κB is also activated in the intestine by a number of pro-inflammatory stimuli, including sepsis, cytokines, and oxidative stress [9–11].

[1] To whom correspondence and reprint requests should be addressed at Department of Molecular Surgery, Hokkaido University School of Medicine, N-15, W-7, Kita-ku, Sapporo, Hokkaido, 060-8638 Japan. E-mail: mozaki@m07.itscom.net.

0022-4804/08 $34.00

Dehydroxymethylepoxyquinomicin (DHMEQ), a newly developed low-molecular-weight NF-κB inhibitor, is a 5-dehydroxymethyl derivative of the antibiotic epoxyquinomicin C [12]. DHMEQ has been found to inhibit tumor necrosis factor-alpha (TNF-α)-induced NF-κB activation by suppressing nuclear translocation but not IκB-phosphorylation or degradation [13]. Recently, the antitumor effects of DHMEQ on breast [14], thyroid [15], and prostate [16] cancers as well as its anti-inflammatory and immunosuppressive effect on mice models [17, 18] have been reported.

In this study, we applied rat intestinal I/R model to examine the anti-inflammatory efficacy of DHMEQ on injury of both the intestines and the lungs.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats weighing 250–300 g were purchased from SLC Inc. (Shizuoka, Japan) and were provided free access to commercial chow and water. The experiment was approved by the Animal Facility of the Hokkaido University and was conducted in accordance with the guidelines proposed by the Institutional Animal Care and Use Committee.

Reagents

DHMEQ was synthesized according to the previously described method, and the purity of this drug used in this study was over 99% [12]. It was dissolved in dimethyl sulfoxide, adjusted to 50 mg/mL, and stored at −80°C as a stock solution until use. The stock solution was dissolved in 0.5% carboxymethylcellulose and was administered intraperitoneally (i.p.) to the rats. For the control vehicle, 0.5% carboxymethylcellulose with an appropriate amount of dimethyl sulfoxide was used. With reference to our recent study using mice cardiac allograft transplant models, the dose of DHMEQ at 20 mg/kg was chosen in this I/R model [18].

I/R Model

The rats were anesthetized with isoflurane, and median laparotomy was performed. The superior mesenteric artery (SMA) was isolated, and ischemia was induced by completely occluding the SMA for 60 min using a microvascular clamp [19]. During the ischemic time, the abdominal incision was temporarily closed to prevent hypothermia. The experimental animals were divided into the two following groups: untreated group, control vehicle; treated group, DHMEQ-treated (20 mg/kg). Both the control vehicle and the DHMEQ were administered 60 min prior to clamping and 5 min prior to reperfusion. Animals that performed the sham operation, i.e., without intestinal ischemia, served as negative controls ($n = 4$). The number of animals in the survival study and the other studies were eight and six in each group, respectively.

Animal Survival

The effect of DHMEQ on animal survival was compared between untreated and treated groups. The SMA was clamped for 60 min as described above. Subsequently, the abdominal incision was closed, and the animals were returned to their cages where food and water were available *ad labium*. The animals were kept under observation for 72 h after intestinal reperfusion.

Intestinal Tissue Blood Flow (ITBF)

ITBF was measured using a laser Doppler flowmeter (Omega Flow Type 2; Omega Flow Company Ltd., Tokyo, Japan) at the following time points: prior to the induction of ischemia; at 5 and 60 min after the onset of ischemia; and 5, 60, and 180 min after reperfusion. ITBF was expressed as a percentage of its pre-ischemic value ($n = 6$, each).

Cytokine Assays

Duplicate measurements of the serum TNF-α and interleukin (IL)-6 levels were performed 60 and 180 min after reperfusion by enzyme-linked immunosorbent assay using a cytokine assay kit (R&D Systems, Minneapolis, MN).

Histological Study

Small intestine and lung tissues were fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin-eosin. The severity of the I/R injury in both the jejunum and the ileum was assessed semi-quantitatively according to the microscopic criteria for grading of intestinal tissue injury described by Park [20]; the grading was as follows: normal mucosal villi, 0; subepithelial spaces at the villi tips, 1; wider subepithelial spaces, 2; epithelial lifting along the sides of the villi, 3; denuded villi, 4; loss of villus tissue, 5; crypt layer infarction, 6; transmucosal infarction, 7; and transmural infarction, 8. The lung tissue was graded using a score of 0 to 12 (0 to 4 for intra-alveolar edema, intra-alveolar hemorrhage, and neutrophilic infiltration; absent, 0; mild, 1; moderate, 2; severe, 3; overwhelming, 4) as described by Gloor [21].

Statistical Analysis

Animal survival time was plotted by the Kaplan-Meier method, and the log-rank test was used for comparison. Data were expressed as the mean ± SD, and statistical analysis was performed by one-way analysis of variance (Fisher's protected least significance difference post-hoc test). P values <0.05 were considered statistically significant.

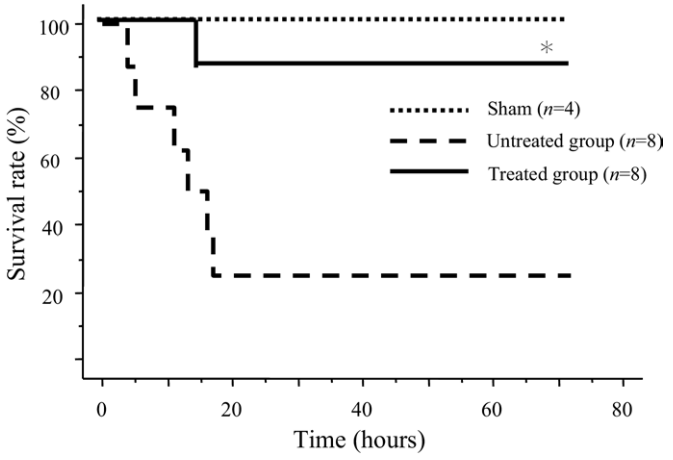

FIG. 1. The animal survival rates of untreated (control) and treated groups (DHMEQ-treated) rats. A significant improvement was observed in the survival rate of the treated group animals treated with DHMEQ. *P values <0.05 *versus* untreated group ($n = 8$, each).