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# The role of free radical scavenger on survival rate after endotoxemia in bile duct ligated rat.

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## Abstract

**Background: Free radicals are involved in pathophysiology directly and indirectly in sepsis or endotoxemia. Obstructive jaundice may promote bacterial overgrowth with resultant endotoxemia or sepsis. The purposes of the studies are to test the protective effect of free radical scavenger in obstructive jaundice.**
**Materials and methods: Male Sprague-Dawley rats were randomized to three groups. Group I underwent common bile duct ligation and simultaneously be treated with edaravone (free radical scavenger). Group II underwent common bile duct ligation and simultaneously be treated with normal saline (vehicle). Group III underwent sham operation and simultaneously be treated with edaravone. Lipopolysaccharide (LPS) were injected intraperitoneally the afternoon of third postoperative day. The survival rates after endotoxin challenge were recorded.**
**Results: After endotoxin challenge, the 72 hour survival rates were 33.3%, 8.3% and 58.3% for group 1, 2 and 3 respectively after observation for 72 hours.**
**Conclusion: Edaravone may improve the survival rates in bile duct ligated rats after endotoxin challenge.**

**Keywords:** Free radical, Endotoxin, Bile duct ligation, Rat, Edaravone.

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## Introduction

Free radicals are involved in pathophysiology directly and indirectly in sepsis or endotoxemia [1]. As it is well known, not only free radical itself but also lipid peroxidative metabolites may cause cytotoxicity on endothelial cells and hepatocytes. Moreover, free radical induces proinflammatory cytokine and chemokine productions by tissue macrophages immediately, which subsequently attracts inflammatory cells in to the liver [2,3] therefore, free radicals play a critical role in endotoxemia and hence therapies against oxidants could be useful in inflammation or sepsis [4,5]. Antioxidants such as diphenyleneiodonium sulfate and allopurinol have been reported to scavenge free radicals [6-9]. These chemicals, however, may not be appropriate for clinical use due to their toxicity and instability. 3-methyl-1-phenyl-2-pyrazolin-5-one (edaravone; EDA) is a potent and novel scavenger of free radicals inhibiting not only hydroxyl radicals but also iron-induced peroxidative injuries [10,11]. Edaravone has been reported to have protective effects on both hemispheric embolization and transient cerebral ischemia in rats [12]. Furthermore, it has been reported that edaravone have protective effects against brain damage after ischemia-reperfusion by scavenging hydroxyl radical clinically. These effects were predominantly thought of as a result of protection of endothelial cells and neurons against lipid peroxidation. There is limited knowledge about the role of free radical scavenger in obstructive jaundice. The free radical scavenger may play an important role in the pathophysiologic changes induced by obstructive jaundice. Therefore, the purposes of the studies are to test the protective effect of free radical scavenger in obstructive jaundice.

## Materials and Methods

Male Sprague-Dawley rats, weighing 280-300 g were randomized to three groups (n=12 in each group).

**Group I (OBED)** underwent common bile duct ligation and simultaneously be treated with Edaravone (free radical scavenger). The first dose of Edaravone (3 mg/kg) was injected intraperitoneally after the bile duct ligation. Subsequent doses of Edaravone (3 mg/kg bid, intraperitoneally) were given on

1st and second postoperative days. The last dose was given in the morning of third postoperative day.

**Group II (OBNS)** underwent common bile duct ligation and simultaneously be treated with normal saline (vehicle). The first dose of same amount of normal saline was injected intraperitoneally after the bile duct ligation. Subsequent doses of normal saline (the same amount of normal saline bid, intraperitoneally) were given on 1st and second postoperative days. The last dose was given in the morning of third postoperative day.

**Group III. (SHED)** underwent sham operation and simultaneously be treated with Edaravone. The first dose of Edaravone was injected intraperitoneally after the bile duct ligation. Subsequent doses of Edaravone (the same amount bid, intraperitoneally) were given on 1st and second postoperative days. The last dose was given in the morning of third postoperative day.

### *Endotoxin challenge*

Lipopolysaccharide (LPS) was injected intraperitoneally (15 mg/kg) at the afternoon of third postoperative day. Animals were observed for 72 hours before sacrifice. The survival rates after endotoxin challenge were recorded.

## Results

After endotoxin challenge, the 72 hour survival rates were 58.3%, 33.3% and 8.3% for SHED, OBED and OBNS groups respectively after observation for 72 hours (Table 1).

*Table 1. Survival Rates After Endotoxin Challenge.*

| Group | Duration | |
|---|---|---|
| | 24 hours | 72 hours |
| SHED | 7/12 (58.3%) | 7/12 (58.3%) |
| OBED | 4/12 (33.3%) | 4/12 (33.3%) |
| OBNS | 1/12 (8.3%) | 1/12 (8.3%) |

SHED: Sham operation with Edaravone
OBED: Bile duct ligation with Edaravone
OBNS: Bile duct ligation with normal saline

## Discussion

Despite improvements in operative technique and the development of potent, broad-spectrum antibiotics, biliary tract surgery in patients with obstructive jaundice is still associated with high morbidity and mortality rates [13,14]. Although the responsible pathophysiological mechanisms are not fully understood, it is generally thought that the removal of bile from the gastrointestinal tract promotes bacterial overgrowth and increased translocation of endotoxin LPS to the liver, thus serving to inhibit hepatic macrophage (Kupffer cell) function in these patients [15,16]. In support of this proposed pathogenic mechanism are reports that oral bile salt replacement can significantly reduce the incidence of postoperative renal dysfunction and endotoxemia in jaundiced patients [17,18] presumably due to the inhibition of intestinal bacterial overgrowth, bacterial translocation, and absorption of endotoxin [19-22]. However, obstruction of the common bile duct has other effects on the host in addition to promoting bacterial translocation, including a direct cholestatic injury to liver cells [23,24]. In fact, some authors have claimed that biliary tract obstruction is a more important factor in the development of endotoxemia and endotoxin-related death in rodent models of obstructive jaundice than the absence of gastrointestinal bile salts [25].

In our study a small amount of endotoxin was administered to animals in order to create an infectious situation that might happen in perioperative period. The 72-hour time point was chosen in this model in order to study the acute biliary obstruction that is usually encountered in clinical conditions. After endotoxin challenge, the 72 hour survival rates were 58.3%, 33.3% and 8.3% for SHED (sham operation with edaravone), OBED (bile duct ligation with edaravone) and OBNS (bile duct ligation with normal saline) groups respectively after observation for 72 hours (Table 1). LPS challenge in sham operation rat created a situation which similar to sepsis that may lead to the lethality of rat. With the protective effect of edaravone, the survival rate in SHED group, therefore could be as high as 58.3% furthermore, it seems that edaravone also may improve the survival rates in bile duct ligated rats after endotoxin challenge. The real mechanism for this protective effect of edaravone is not fully understood. The following two facts might offer some explanation:

- Not only free radical itself but also lipid peroxidative metabolites may cause cytotoxicity on endothelial cells and hepatocytes [1].
- Free radical induces proinflammatory cytokine and chemokine productions by tissue macrophages immediately, which subsequently attracts inflammatory cells in to the liver [2,3].

In summary, edaravone may improve the survival rates in bile duct ligated rats after endotoxin challenge. These findings are interesting and deserve further evaluation. Hence, if confirmed in clinical trial, the administration of edaravone may provide a rational adjuvant strategy for the treatment of patients with obstructive jaundice in early stage and may be expected to reduce the incidence of perioperative mortality and morbidity in obstructive jaundice. Nevertheless, the importance of earlier restoration of biliary flow cannot be overemphasized.

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