ORIGINAL ARTICLE

The Therapeutic Efficacy of Edaravone in Extensively Burned Rats

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Background: Extensive burn injury leads to production of free radicals subsequent to massive fluid resuscitation, which in turn increases the risk of acute lung injury. Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a novel free radical scavenger, is clinically effective in improving the prognosis after cerebral infarction. However, the effect of edaravone against extensive burn injury has not been tested.

Objected: To evaluate whether edaravone can reduce free radical precursors in a 30% burn model in rats.

Design: Prospective, randomized controlled experiment.

Setting: Animal basic science laboratory.

Subjects: Male Wistar rats weighing 200 to 220 g.

Main Outcome Measures: All rats (n=10) were given a 30% full-thickness burn according to the Walker and Mason method. Immediately after the burn, edaravone was injected into the rats (n=5) intraperitoneally at a dose of 9 mg/kg. One hour after burn injury, blood and tissue samples were collected to analyze free radical changes of serum and tissue malondialdehyde (MDA) and xanthine oxidase (XOD) and lung white blood cells.

Results: Statistical significance was found between nontreatment and edaravone treatment relative to serum MDA (mean±SD, 2.50±0.54 vs 1.74±0.29 nmol/mL), serum XOD (mean±SD, 5.04±1.67 vs 2.26±0.83 U/L), tissue MDA (mean±SD, 1268.7±289.9 vs 569.1±135.9 nmol/mg protein), tissue XOD (mean±SD, 256.3±58.1 vs 50.96±19.60 mU/g tissue), lung white blood cells (mean±SD, 3088±1144 vs 1542±575 mU/g tissue), and lung XOD (mean±SD, 428.3±210.5 vs 81.8±36.0 nmol/mg protein).

Conclusions: Edaravone treatment induces significant reduction of free radical precursors and their metabolites compared with controls in burn rats. This suggests that edaravone could be helpful in the clinical treatment of large burns.

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PRODUCTION OF FREE RADICALS, such as superoxide and peroxynitrite, in the early phase of an extensive burn exacerbates many aspects of the injury process, such as an increase in microvascular permeability and the production of inflammatory mediators. The aggressive fluid replacement needed to stabilize burn patients in this period appears to exacerbate the lipid peroxidation that leads to the production of free radicals.[1,2] Free radical release subsequently exacerbates inflammatory cytokine production and the influx of activated neutrophils into the lungs. Therefore, several antioxidant therapies have been tried to improve critical condition in extensive burns.

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is approved for the treatment of acute cerebral infarction. The protective effect of edaravone against brain damage after ischemia-reperfusion injury is mediated by its ability to scavenge hydroxyl radicals (OH^-).[3,4] Edaravone was shown to inhibit postischemic increases in OH^- production and tissue injury in the penumbral or recirculated area in rat cerebral ischemia models.[3] In addition, clinical use of edaravone is well established and has led to excellent outcomes in cerebral infarction.[5-7]

Although edaravone possesses potent free radical scavenging ability, the effect of edaravone in severe burns is not known. The purpose of this study was to investigate the effect of edaravone against free radical formation in extensively burned rats.

METHODS

MATERIALS

Male Wistar rats (body weight, 200-220 g; Saitama Animal Provider, Saitama, Japan) were used in this experiment. Ten rats were assigned to 2 groups; a nontreatment group (n=5) and an edaravone treatment group (n=5). The experiments were conducted and animals were

cared for in accordance with the law for protection and management of animals in Japan. The protocols were approved by the Experimental Animal Committee at Kyorin University.

ANIMAL MODEL OF BURN INJURY

Rats were anesthetized using the intraperitoneal injection of pentobarbital sodium (35 mg/kg). Next the rats were given a 30% full-thickness burn on the dorsum using a modification of the procedure published by Walker and Mason.[8,9] Immediately after the burn, edaravone (Mitsubishi Pharma Corporation, Osaka, Japan) was injected intraperitoneally at a dose of 9 mg/kg into the rats in the edaravone treatment group. Five minutes after the burn, saline solution (22.0-26.4 mL/kg) was injected into the peritoneal cavity in both treatment groups to resuscitate a loss of volume. One hour after burn injury, all of the rats were euthanized, and tissue samples were collected for the analysis of free radical changes.

SERUM MALONDIALDEHYDE AND XANTHINE OXIDASE, TISSUE MALONDIALDEHYDE AND XANTHINE OXIDASE, AND LUNG XANTHINE OXIDASE AND WHITE BLOOD CELLS

Blood samples were collected by cardiac puncture. Hematocrit was measured using standard techniques. Serum levels of malondialdehyde (MDA) and xanthine oxidase (XOD) were measured by chromogen 2,2′-azino-di-(3-ethylbenzthiazoline)-6 sulfonic acid (ABTS).[10]

Burned skin was collected for the measurement of XOD and MDA. The skin was treated with formalin and then homogenized. Tissue MDA and XOD were measured on the homogenates using chromogen ABTS.[10]

The whole lung was excised and treated with formalin. The treated lung was then used to measure WBCs and XOD using chromogen ABTS.[10]

The measurements of serum and tissue MDA and XOD and lung MDA were analyzed using the *t* test. Lung WBCs were analyzed using the Welch test because of unequal variance.

RESULTS

SERUM MDA AND XOD, TISSUE MDA AND XOD, AND LUNG XOD AND WBC

Edaravone treatment of burn rats significantly reduced serum MDA and XOD levels below that of nontreated burn controls, inducing 30% and 55% drops, respectively, in the levels of these chemicals (**Table 1**).

Edaravone treatment had a similar effect on MDA and XOD levels in burned skin to that in the serum. It significantly reduced MDA and XOD levels in burned skin below that of nontreated burn controls, as shown in **Table 2**. The reduction in skin XOD levels was especially striking: edaravone treatment induced a 5-fold reduction in the levels of this metabolite.

In the lung, we measured the effect of edavarone treatment on the levels of XOD and on the infiltration of WBCs. It was useful to estimate the infiltration of phagocytic cells because these produce abundant free radicals through their nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Dinauer[11] proposed that the NADPH oxidase of phagocytes is a multisubunit complex that generates superoxide (O_2^-) in a 1-electron reduction in O_2^- using electrons supplied by NADPH. The edaravone treatment group significantly reduced the lung levels of XOD and the infiltration of WBCs (**Table 3**).

Edaravone treatment had no effect on percentage of hematocrit and body weight compared with untreated controls (data not shown).

Table 1. Serum MDA and XOD Values for Treated Preparations

	Serum MDA, nmol/mL	Serum XOD, U/L
Nontreatment	2.50 ± 0.54*	5.04 ± 1.67†
Edaravone treatment	1.74 ± 0.29*	2.26 ± 0.83†

Abbreviations: MDA, malondialdehyde; XOD, xanthine oxidase.
*P=.02.
†P=.004.

Table 2. Tissue MDA and XOD Values for Treated Preparations

	Tissue MDA, nmol/mg Protein	Tissue XOD, mU/g Tissue
Nontreatment	1268.7 ± 289.9*	256.3 ± 58.1†
Edaravone treatment	569.1 ± 135.9*	50.96 ± 19.6†

Abbreviations: MDA, malondialdehyde; XOD, xanthine oxidase.
*P=.001.
†P<.001.

Table 3. Lung XOD and WBC Values for Preparations

	Lung XOD, nmol/mg Protein	Lung WBCs, mU/g Tissue
Nontreatment	428.3 ± 210.5*	3088 ± 1144†
Edaravone treatment	81.8 ± 36.0*	1542 ± 575†

Abbreviations: WBC, white blood cell; XOD, xanthine oxidase.
*P=.005.
†P=.03.

COMMENT

The purpose of this study was to investigate the ability of edaravone to reduce free radical formation in extensively burned rats. Our results indicate that edaravone treatment reduced the formation of free radical precursors and their metabolites, such as serum MDA and XOD, tissue XOD and MDA, and lung XOD and WBCs relative to untreated controls (Tables 1-3).

Many articles have reported that the use of edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) in cerebral infarction prevents the ischemia-induced formation of the penumbra. Edaravone treatment has protective effects against brain damage after ischemia-reperfusion injury through its ability to scavenge OH^-.[3,4] Clinically, use of this drug has led to excellent outcomes in cerebral infarction.[5-7] Moreover, as established by the seminal study of Kono et al,[12] edaravone inhibits both recruitment of inflammatory cells

and expression of inflammatory cytokine levels in the livers of rats challenged with lipopolysaccharide. In that study, edaravone treatment attenuated production of serum tumor necrosis factor α and interleukin (IL) 6 and decreased hepatic messenger RNA expression of tumor necrosis factor α, IL-6, interferon-γ, and IL-10. Saibara et al[13] demonstrated that edaravone treatment significantly improves the survival rate of mice given paraquat compared with untreated controls. Tomatsuri et al[14] evaluated the effect of edaravone treatment on small-intestine mucosal injury in a model of reperfusion injury. The levels of luminal protein, hemoglobin, thiobarbituric acid–reactive substances, and tissue-associated myeloperoxidase activity were all increased significantly by ischemic injury, but these increases were significantly inhibited by treatment with edaravone. In a recent study, Koh et al[15] reported that edaravone was useful in reducing pathophysiologic changes in burn rats. They evaluated insensible water loss and lung dry-wet ratio in rats subjected to a 30% total burn surface area treated with saline alone or saline containing 1.5 mg of edaravone. Edaravone treatment significantly reduced insensible water loss and improved lung dry-wet ratio in comparison with saline treatment alone. Our data indicate that edaravone treatment decreases several free radical precursors and their metabolites in burn rats. Further studies will be aimed at determining if edaravone can reduce overvolume resuscitation in the early state of severe burn and prevent acute respiratory distress syndrome, which is related to the formation of free radicals.

Several studies support the concept that therapeutically reducing free radical levels can reduce the fluid resuscitation needs in extensive burns. Matsuda et al[16-18] reported that the ratio of MDA in the lymph and plasma rose significantly throughout the postburn period; this burn-related increase in MDA levels was significantly attenuated by ascorbic acid therapy. Furthermore, this group showed that high-dose ascorbic acid therapy in adult guinea pigs reduced the total 24-hour fluid resuscitation needs from 4 to 1 mL/kg per percentage of burn while maintaining adequate cardiac output. Low-dose ascorbic acid (170 mg) significantly improved cardiac output compared with that measured in animals given fluid resuscitation alone, whereas higher doses of ascorbic acid (340 and 680 mg) improved cardiac output to a significantly greater extent. These authors also showed that ascorbic acid therapy diminished early postburn lipid peroxidation owing to its capacity to scavenge O_2^-, OH^-, and singlet oxygen ($^1O_2$).[18-22] Other authors have reported that oral antioxidant regimens (such as ascorbic acid, glutathione, and *N*-acetylcysteine) prevented burn sepsis–mediated mortality, changes in cellular energetics, and changes in tissue antioxidant levels.[23,24] In addition, several reports have shown that antioxidant therapies such as allopurinol,[25] polyethylene glycol catalase,[26] vitamin E,[27] α-tocopherol,[28,29] and superoxide dismutase[30] can inhibit postburn lipid peroxidation. Although these antioxidant therapies can be useful in preventing tissue and organ injury, appropriate clinical regimens are still unknown. In contrast, edaravone treatment to prevent brain damage after ischemia-reperfusion injury is well established clinically. Our observation demonstrates that edaravone remarkably reduces the production of free radical precursors and metabolites in burn rats. We suggest that edaravone treatment may prevent the lipid peroxidation that occurs after aggressive volume replacement in patients with severe burns.

The pharmaceutical action of edaravone is such that it inhibits both the water-soluble and lipid-soluble peroxyl radical–induced peroxidation systems, decreasing the production of OH^-, LOO (active species of the lipid peroxyradical), and $ONOO^-$ (peroxynitrite anion) but not O_2^-. In our experience, edaravone treatment decreased the postburn levels of XOD. This result suggests that XOD formation is downstream of free radical production (OH^-, LOO, and $ONOO^-$). Alternatively, there may be another pathway by which edaravone inhibits XOD formation.

A variety of parameters have been used to evaluate the effects of large burns on the lungs. The lung dry-wet ratio has been measured to estimate the extent of postburn lung injury.[15] However, we believe that the measurements of lung XOD and WBCs may be superior to lung dry-wet ratio in the acute lung injury induced by severe burns. The XOD is downstream of free radical action in the lung. Recent studies have demonstrated that several cytokines can upregulate xanthine dehydrogenase/XOD gene expression in the respiratory system. Dupont et al[31] first demonstrated transcriptional regulation of the enzyme, finding that interferon-γ selectively induced gene activation in pulmonary microvascular cells and in rat lungs in vivo. We believe that the reduction in XOD, which resulted from OH^- scavenged by edaravone, may decrease the influence of cytokine storms that induce acute lung injury.

In conclusion, we demonstrate that edaravone treatment in burn rats induces significant reduction in free radical precursors and their metabolites compared with untreated controls. Furthermore, we think that edaravone could be helpful for the clinical treatment of large burns.

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Announcement

The editorial staff of the *Archives of Surgery* would like to announce a new section titled The Residents' Corner. The main objectives of this section are to encourage residents to prepare peer-reviewed manuscripts, allow residents to critically evaluate manuscripts by their peers, and encourage residents to read and review manuscripts in surgical journals. At the time of submission to The Residents' Corner, the first author must be a resident in training. The submission must be accompanied by a letter or e-mail from the resident's program director verifying that the resident is in good standing. We encourage the submission of short articles limited to 1000 words and no more than 3 figures. A narrative abstract of no more than 135 words should be included. We will consider any appropriate short article, but envision the submission of interesting case reports, small case series, historical reviews, summaries of recent developments in surgery, and laboratory studies.

The review process will be supervised by the editorial staff of the *Archives of Surgery* and coordinated by two Resident Editors, Jayme Locke, MD, and Jordan Winter, MD. We will request peer reviews from other residents nationally and globally. When submitting articles for this section, please select The Residents' Corner as the manuscript type in our online peer review and submission system. We look forward to this exciting new section and to the training of our younger colleagues.

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Deputy Editor