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Preventive effects of a biscoclaurine alkaloid, cepharanthine, on endotoxin or tumor necrosis factor-α-induced septic shock symptoms: Involvement of from cell death in L929 cells and nitric oxide production in raw 264.7 cells

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Abstract

The preventive effects of cepharanthine, a biscoclaurine alkaloid isolated from *Stephania cepharantha* Hayata, on the lethality and cell death caused by endotoxin or tumor necrosis factor (TNF)-α-induced syndrome in septic shock were investigated. In these experiments, we estimated the survival of mice treated with a lethal dose of endotoxin (50 mg/kg, i.p.) or recombinant human (rh) TNF-α (10,000 units/mouse, i.v.) together with a sublethal dose (1 mg/kg, i.p.) of endotoxin. Cepharanthine clearly protected mice from endotoxin-induced and endotoxin/rhTNF-α-induced lethal shock. In *in vitro* experiments, cepharanthine (3 μg/ml) definitely inhibited cell death in mouse L929 fibroblast cells incubated with rhTNF-α (100 units/ml) at 37 °C for 24 h. On the other hand, non-apoptotic programmed death of cells was observed by fluorescence microscopy in rhTNF-α (100 units/ml)-treated L929 cells. In the 3-(4,5-Dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) assay after 48-h drug exposure, the cell proliferation of L929 cells was significantly increased by the addition of cepharanthine (1 and 3 μg/ml). It seems that the preventive effect of cepharanthine on rhTNF-α-induced cytotoxicity in fibroblast cells occurs through an increase of cell proliferation by the drug. In addition, cepharanthine suppressed nitric oxide (NO) production by endotoxin-stimulated Raw 264.7 mouse macrophage cells. These findings suggest that cepharanthine prevents lethality or cytotoxicity through suppression of endotoxin-induced NO in macrophages and that its effects are possibly mediated by the enhancement of the proliferation of fibroblast cells. Cepharanthine may therefore protect against some of the various disturbances caused by endotoxin through its ability to inhibit NO production in septic shock.

Keywords: Cepharanthine; Endotoxin; TNF-α; Septic shock; Cell death; Preventive effect; Nitric oxide (NO)

1. Introduction

Despite the remarkable progress in clinical medicine, sepsis and shock continue to be major clinical problems in intensive care units. Sepsis is the leading cause of death in critically ill patients in the USA; sepsis occurs

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in 750,000 people annually, and more than 210,000 of them die [1]. Endotoxin from gram-negative bacteria induce shock symptoms in humans and animals, a state characterized by fever, hypotension, intravascular coagulation and finally multi-organ failure. Therefore, investigators have recently turned their attention to the metabolic alterations that develop during gram-negative sepsis or endotoxic shock. Endotoxin is believed to be initially detoxified in the reticuloendothelial system (RES), particularly in liver Kupffer cells. On the other hand, macrophages stimulated by microorganisms or their toxins induce a variety of biologically active mediators known as cytokines, and tumor necrosis factor (TNF)-α is recognized to be an important mediator in the development of endotoxicity or septic shock [2]. TNF-α is considered to be a major early mediator in the systemic inflammatory response syndrome observed during gram-negative sepsis.

Cepharanthine, one of several biscoclaurine alkaloids, is extracted from *Stephania cepharantha* Hayata and its structure is shown in Fig. 1. It has been shown to have several pharmacological actions, including anti-inflammatory, anti-allergic and immunomodulatory activities *in vivo*. In addition, this drug has been reported to inhibit O_2^- production by activated neutrophils *in vitro* via stabilizing plasma membrane [3]. Biscoclaurine alkaloids also inhibit the activity of protein kinase C [4], which plays a role in TNF-α production by monocytes in endotoxemia [5,6]. A series of our studies demonstrated that oxidative stress caused by endotoxin can reduce the levels of scavengers or quenchers of free radicals [7,8]. In our previous studies, we suggested that the endotoxin-induced oxidative stress was regulated, at least in part, by Ca^{2+} mobilization [9,10], or selenium [11] and Zn^{2+} levels [12]. Interestingly, we also suggested that TNF-α-induced oxidative stress occurs as a result of bacterial or endotoxin translocation under conditions of RES function in various disease states [13]. Many studies have linked the production of nitric oxide (NO) to endotoxin-induced hypotension, vascular hyporesponsiveness and death, suggesting that excess production of NO plays an important role in the development of septic shock [14,15]. NO has been suggested to be an important regulator of many cellular functions in endotoxemic animals, and the NO radical functions efficiently as a mediator, messenger or regulator of cell function in various physiological systems and pathophysiological states. Therefore, based on the current information regarding responses to endotoxin, we designed the following experiments to investigate whether cepharanthine is involved in attenuation of the lethal effects or cell death and NO production resulting from the endotoxin or TNF-α-mediated syndrome in septic shock.

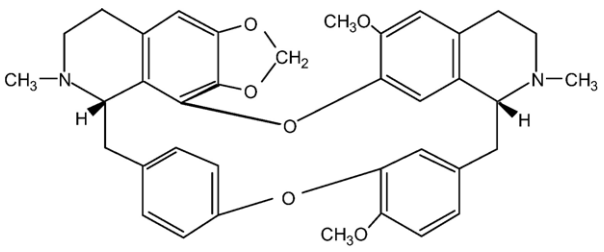

Fig. 1. Chemical structure of cepharanthine, a biscoclaurine alkaloid.

2. Materials and methods

2.1. Chemicals

Cepharanthine was kindly provided by Kaken Shoyaku Co., Ltd. (Tokyo, Japan). Recombinant human TNF-α (rhTNF-α) was generously provided by Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). The endotoxin content of this cytokine was shown to be less than 0.04 ng/mg proteins using a Limulus test kit (Seikagaku Co., Tokyo, Japan). *Salmonella typhimurium* lipopolysaccharide (endotoxin) was obtained from Difco Laboratories (Detroit, MI, USA). Bisbenzimide H33342 fluorochrome trihydrochloride (Hoechst 33342) was purchased from Nakalai Tesque (Kyoto, Japan). Propidium iodide (PI) was obtained from Sigma Chemical (St Louis, MO, USA).

2.2. Animals and treatment

Male ddY mice, 4 weeks old and weighing 18–20 g, were purchased from Japan SLC. Inc. (Hamamatsu, Japan) and maintained in the Tohoku Pharmaceutical University Experimental Animal Center. They were housed in an air-conditioned room at 23 ± 1 °C and humidity of 55 ± 5% with a 12-h light/dark cycle. During the experimental period, the animals were fed a commercial pellet diet (CE-2, Clea Japan, Inc., Tokyo, Japan) and allowed access to water *ab libitum*. Cepharanthine was orally administrated once a day for 3 days, and endotoxin was administrated 24 h after the final injection of the drug. Cepharanthine was also administrated intraperitoneally 30 min before injection of endotoxin (i.p.)/rhTNF-α (i.v.). Survival was observed for 24, 48 and 72 h after injection of endotoxin/rhTNF-α. The numerator represents the survivors and the denominator represents the number of mice treated.

2.3. Cell lines and cultures

The L929 mouse fibroblast cell line was obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). Raw 264.7 murine macrophage cell line was obtained from the American Type Culture Collection (Manassas, VA,