NITRIC OXIDE PRODUCTION IN MOUSE PERITONEAL MACROPHAGES ENHANCED WITH GLYCYYRHIZIN

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The enhancement of nitric oxide (NO) production in glycyrrhizin (GL)-induced macrophages (Mφ) in response to lipopolysaccharide (LPS) was investigated. NO production in GL-induced macrophage culture supernatants was stimulated in response to LPS (10 μg/ml) for 24- or 48- h cultures, and these levels were compared three times with the levels in saline-induced peritoneal exudate cell cultures. Furthermore, Mφ induced with proteose peptone (PP) containing GL could generate greater NO production than Mφ induced with PP alone. However, no stimulation of NO production was observed by addition of GL in the cultures of Mφ induced with thioglycollate or Bacillus Calmette Guerin. Moreover, GL-induced Mφ showed cytostasis against such tumor target cells as L 1210 and P 388 lymphoma cell lines. These observations indicate that GL can activate the Mφ in vivo system and stimulate NO production in response to LPS.

KEY WORDS glycyrrhizin; nitric oxide; macrophage; lipopolysaccharide; antitumor activity

Glycyrrhizin (GL), a component of Glycyrrhiza radix, has been extensively studied in relation to various biological activities. GL and its aglycone, glycyrrhetic acid, were found to suppress the action of tumor promoter in mouse skin.2,30

Recently, a striking relationship has been found between the stimulation of nitric oxide (NO) production by activated murine macrophages (Mφ) and antitumor activity.4 Mφ-derived NO mediated cytostasis action tumor cells and microbial pathogens. In this present work, we have found that Mφ induced with proteose peptone (PP) containing GL produced NO in response to LPS, and the Mφ could kill the tumor target cells.

Various sources of peritoneal exudate cells were collected 3-4 days after i.p. injection of GL (1 mg/mouse, dissolved in pyrogen free saline), 2 ml of thioglycollate (3 w/v %, Difco), PP (20 %) or BCG (1 mg/mouse, suspended in saline) into C3H/HeN mice. Adherent Mφ were prepared by plating 5-10 X 10^5 peritoneal exudate cells/ml culture medium in a 24-well plastic plate and incubating for 2 h in a humidified

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**Fig. 1.** GL and PP with GL-induced Mφ Can Produce NO Cultured with LPS

Data were expressed as the mean ± SD of quadruplicate cultures.

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CO\textsubscript{2} incubator. Nonadherent cells were then removed, and the remaining adherent cells were covered with fresh medium with or without of 10 µg/ml LPS. M\textsubscript{ϕ} culture supernatants were collected and mixed with equal volume of Griess reagent (1 % sulfanilamide and 0.1 % naphthylethylenediamine dihydrochloride in 2 % phosphoric acid) to determine the NO\textsubscript{2} concentrations. The absorbance at 550 nm was measured, and nitrite concentrations were determined using NaNO\textsubscript{2} as a standard.

When the M\textsubscript{ϕ} were cultured for 24 and/or 48 h, GL-induced M\textsubscript{ϕ} could produce much higher levels of NO\textsubscript{2} upon stimulation with LPS (10 µg/ml), but not resident M\textsubscript{ϕ} (Fig. 1). Recent studies demonstrated that thioglycollate- and BCG-induced M\textsubscript{ϕ} synthesized and released large amounts of NO in \textit{in vitro} culture supernatant,\textsuperscript{5,7} and these types of M\textsubscript{ϕ} showed cytotoxic action toward murine tumor target cells.

We next examined the dose-dependent NO production in culture stimulated with various doses of LPS in GL-induced M\textsubscript{ϕ}. A concentration-dependent NO increase in response to LPS and the time course of NO production in culture supernatants are shown in Fig.
2 A and B, respectively. There was no influence on NO production in cultures, when GL alone was exposed to BCG- and thioglycollate-elicited Mφ (data not shown) without LPS. Furthermore, we investigated whether the GL-induced Mφ facilitates cytotoxic action on the tumor target cells (P 388 and L 1210). As shown in Fig. 3 A and B, GL Mφ indicated cytostasis when Mφ was exposed with LPS.

Glycyrrhizin revealed not only antitumoridal activity, but also antiviral activity against varicella-zoster virus, herpes simplex virus and human immunodeficiency virus both in vivo and in vitro.5-11 However, the details of its mechanisms are still not clear. It has previously been shown that GL enhances interferon-γ (IFN-γ) production in human T-lymphocytes.12 IFN-γ enhanced the endotoxin-induced production of NO in murine macrophages.13,14 Recently, Kwon et al.15 have reported that the antitumor action of NO was attributed to inhibition of ribonucleotide reductase, a rate-limiting enzyme in DNA synthesis.16 The ribonucleotide reductase was susceptible to inhibition at a very low level of NO concentration.17 It is likely that this enzyme in microbes is also susceptible to macrophage-derived NO. More recent reports have suggested that NO production from Kupffer cell can prevent endotoxin-induced hepatic injury.17,18 Thus, our results strengthen the relationship between the stimulation of NO production and antitumor activity. The enhancing effect of GL on NO production in Mφ may participate in antiviral and hepatoprotective activities of GL.

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