Inhibitory effects of herbal medicines on nitric oxide production in macrophage and hepatoma cell lines induced by inflammatory stimuli

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(*Received May 17, 1996. Accepted July 3, 1996.*)

Abstract

Although nitric oxide (NO) is an important biological mediator, excessive NO production in inflammation is a causative factor of cellular injury and carcinogenesis. In this study, examination was made of the effects of traditional Chinese herbal medicines (Kampo medicines) on NO production induced by inflammatory stimuli in murine macrophage and hepatoma cell lines *in vitro*. Murine macrophage cell line RAW 264 cells were stimulated with lipopolysaccharide (LPS) and rat hepatoma cell line McA-RH 7777 cells, with TNF-α and IL-1β to induce NO production. Using these 2 experimental systems, Kampo medicines and crude drugs were investigated. Of 129 Kampo medicines, Oren-gedoku-to (黄連解毒湯, OGT) was shown to have the strongest activity for inhibiting NO production from LPS-stimulated macrophages as well as TNF-α/IL-1β-stimulated hepatoma cells. Of 4 crude drugs consisting of OGT, Oren (*Coptidis Rhizoma*) was mainly effective in cytokine-stimulated hepatoma cells, and Ogon (*Scutellariae Radix*) in LPS-stimulated RAW 264 cells. This indicates that contributory components to the inhibitory effects differ depending on cell type and/or type of stimuli. Our results thus suggest that a mixture of different crude drugs, i.e. Kampo medicines, is much more effective than a single crude drug for inhibiting excessive NO production in inflammatory diseases.

Key words nitric oxide, macrophages, hepatoma cells, Oren-gedoku-to (Huang-Lian-Jie-Du-Tang), 黄連解毒湯.

Abbreviations NO, nitric oxide ; NOS, nitric oxide synthase ; iNOS, inducible nitric oxide synthase ; PBS, phosphate buffered saline ; OGT, Oren-gedoku-to ; IL-1β, interleukin-1β : TNF-α, tumor necrosis factor-α.

Introduction

In inflammatory liver diseases such as viral hepatitis, Kupffer cells and infiltrating lymphocytes are activated to produce many cytokines, oxyradicals and nitric oxide (NO) radicals.[1,2] Hepatocytes also produce NO on stimulation with inflammatory cytokines such as interferon-γ (IFN-γ), interleukin 1β (IL-1β) and tumor necrosis factor-α (TNF-α).[2-5] NO causes deterioration of cellular functions by inhibiting enzymes involved in cell respiration and DNA synthesis.[6,7] NO has been shown to cause formation of carcinogenic N-nitroso compounds, deamination of DNA and to be mutagenic.[8-10] This indicates the possibility that chronic elevation of NO synthesis, which may occur in chronic viral hepatitis and liver cirrhosis, could deteriorate liver cell function and increase the risk of hepatocellular carcinoma.[11-13] The effective inhibition of NO production induced by inflammatory stimuli from hepatocytes, hepatoma cells and macrophages may thus prove beneficial for

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the prevention of liver cell carcinogenesis as well as damage to tissue components.

We thus established *in vitro* NO production models using rat hepatoma and murine macrophage cell lines in which NO production is induced on treatment with TNF-α / IL-1β or lipopolysaccharide (LPS). We examined traditional Chinese herbal medicines (Kampo medicines) for inhibitory effects on NO production induced by these inflammatory stimuli in macrophage and hepatoma cell lines *in vitro*. Of 129 Kampo medicines, Oren-gedoku-to (黄連解毒湯, OGT) had the strongest activity for inhibiting NO production from LPS-stimulated macrophages and TNF-α / IL-1β-stimulated hepatoma cells. The mechanisms for this and active components were also investigated.

Materials and Methods

Cell cultures : The rat hepatoma cell line McA-RH7777 was obtained from American Tissue Culture Collection and grown in phenol red-free RPMI1640 medium supplemented with 5 % fetal bovine serum (FBS), 100 u/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5 % CO_2/ 95 % air. Cells were plated at a density of 1×10^5/ml and preincubated for 20 hr before treatment with cytokines. NO production was induced by 1 ng/ml TNF-α (murine, recombinant ; GIBCO BRL) and 1 ng/ml IL-1β (murine, recombinant ; Sigma). The murine macrophage cell line RAW264 was obtained from Riken Cell Bank. The cells were plated at a density of 3×10^5/ ml and preincubated for 4 hr. RAW264 cells were stimulated to induce NO production with 1 μg/ml lipopolysaccharide (LPS, E. coli 0111 : B4, DIFCO). Incubation period was 48 hr for both cell lines unless otherwise indicated.

Crude drugs and Kampo medicines : Crude drugs and Kampo medicines were extracted by boiling water and the aqueous extracts thus obtained were spray-dried to prepare extract powder. Each extract powder was weighed and stock solution at 10 mg/ml was prepared in sterile distilled water. The stock solutions of aqueous extracts were used for subsequent experiments by being added to culture medium at desired concentrations. Spray-dried extract powders of crude drugs and Kampo medicines were prepared by Tsumura & Co. (Tokyo, Japan). Kampo medicines were prepared as an extract powder from mixture of crude drugs according to the traditional prescriptions. For example, Oren-gedoku-to (Huang-Lian-Jie-Du-Tang in Chinese) extract powder consists of crude ingredients extracted by boiling water from the following four medicinal plants mixed in the ratios in parenthesis ; *Scutellariae Radix* (3.0), *Coptidis Rhizoma* (2.0), *Gardeniae Fructus* (2.0) and *Phellodendri Cortex* (1.5).

Measurement of NO production and cell viability : The Griess assay, a spectrophotometric determination for nitrite, was conducted to quantify the nitrite levels in the conditioned media of McA-RH7777 or RAW264 cells treated with cytokines or LPS. Briefly, to 100 μl sample, an equal volume of Griess reagent (1 % Sulfanilamide, 0.1 % N-1-naphthyl ethylenediamine dihydrochloride in 5 % H_3PO_4) was added and immediately mixed. After 10 min, the product of the reaction was read at 540 nm. The concentration of nitrite was calculated from a standard curve using known concentrations of sodium nitrite. Cellular toxicity of crude drugs and herbal medicines was evaluated using WST-1 assay (DOJIN) according to the procedure of the manufacturer. Treatments with Kampo medicines and their ingredients were carried out at nontoxic doses as determined by the cell viability defined by WST-1 assay.

Northern blot hybridization : Total RNA was extracted from culture cells by a modified guanidinium thiocyanate method using ISOGEN (Nippon Gene, Tokyo, Japan) according to the procedure of the manufacturer. Northern blot hybridization was carried out essentially as described by Sambrook *et al.*[14] Briefly, samples 10 μg each of total RNA were denatured and subjected to electrophoresis on a 1 % agarose-formaldehyde gel. After electrophoresis, the gel was stained with ethidium bromide and photographed under UV illumination to verify even loading and determine ribosomal RNA positions. RNAs were transferred to Hybond-N nylon membranes (Amersham Life Science, Tokyo, Japan) and fixed by UV irradiation. Each membrane was hybridized with ^{32}P-labeled iNOS cDNA probe. After hybridization, the membranes were washed under high-stringency conditions.

The washed blots were autoradiographed using Kodak XAR-5 film with an intensifing screen at -70 °C. The probe was a 1033-bp cDNA fragment of the mouse macrophage iNOS gene prepared by reverse transcriptase polymerase chain reaction from LPS-stimulated RAW264 cells as previously reported.[15] The iNOS cDNA fragment was labeled with [α-^{32}P] dCTP by random priming.

Results

Inhibition by Kampo medicines of cytokine-induced NO production from McA-RH7777 cells

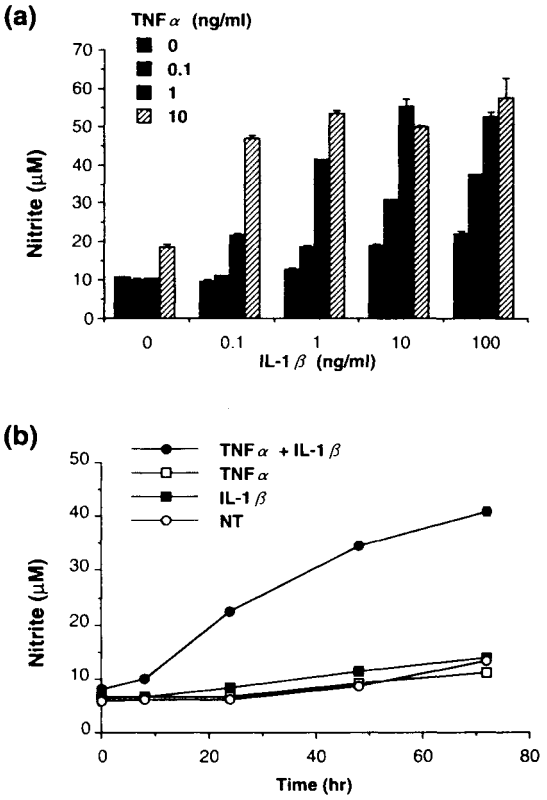


Fig. 1 Cytokine-induced nitric oxide production in rat hepatoma McA-RH7777 cells.

(a) Cells were incubated for 48 hr in the presence of varying concentrations of TNF-α and/or IL-1β, and accumulated nitrite in the medium was measured. Synergistic effects by these cytokines on the NO production are demonstrated.

(b) Cells were incubated for varying times in the presence of 1 ng/ml of TNF-α and/or 1 ng/ml of IL-1β. Increased NO production in a time-dependent manner was observed when the cells were stimulated with both cytokines. Cells treated with either of TNF-α (1 ng/ml) or IL-1β (1 ng/ml) did not increase NO production beyond that of non-treated (NT) cells.

Significant increase in NO production was observed in McA-RH7777 cells when the cells were treated with TNF-α and IL-1β, but not with either alone. When McA-RH7777 cells were incubated in the presence of 100 ng/ml TNF-α or 10 ng/ml IL-1β, increase in NO production was only twice that of the control culture. The simultaneous administration of these cytokines resulted in increase in NO production more than 4 times that of the control culture even at 1 ng/ml, demonstrating the synergistic effect of these cytokines on NO induction from McA-RH7777 cells (Fig. 1). Using this *in vitro* assay model of NO production from cytokine-stimulated hepatoma cells, in which hepatoma cells were stimulated to produce NO with 1 ng/ml each of TNF-α and IL-1β, accumulated nitrite was measured 48 hr after treatment and aque-

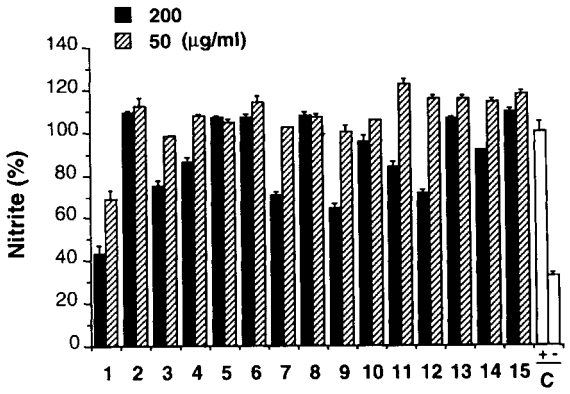


Fig. 2 Inhibitory effects of Kampo medicines on cytokine-induced nitric oxide production in McA-RH7777 cells.

Cells were incubated for 48 hr at 37°C in RPMI medium containing 10 % FBS in the presence of 1 ng/ml of TNF-α and IL-1β. Nitrite was quantified as described in the text. All data represent means ± S.E.M. for triplicate determination. Open columns indicate nitrite of the conditioned medium in the culture with (+) or without (−) cytokines. Nitrite in the culture treated with cytokines in the absence of added samples was set at 100 % and the relative ratio of nitrite is indicated as % nitrite of the cytokine-stimulated culture. Nitrite in the culture media after treatment with 200 μg/ml (shaded columns) or 50 μg/ml (hatched columns) of samples is demonstrated.

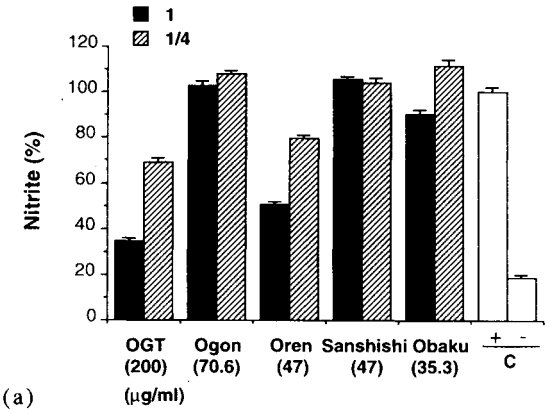
Samples of Kampo medicines : 1, Oren-gedoku-to ; 2, Mao-to ; 3, Moku-boi-to ; 4, Ryo-kei-jutsu-kan-to 5, Keishi-ka-shakuyaku-to ; 6, Tokaku-joki-to ; 7, Choi-joki-to ; 8, Keishi-ninjin-to ; 9, Daio-kanzo-to ; 10, Ji-daboku-ippo ; 11, Daiken-chu-to ; 12, San'o-shashin-to ; 13, Ryo-kyo-jutsu-kan-to ; 14, Oren-to ; 15, Inchinko-to (1, 黄連解毒湯 ; 2, 麻黄湯 ; 3, 木防已湯 ; 4, 苓桂朮甘湯 ; 5, 桂枝加芍薬湯 ; 6, 桃核承気湯 ; 7, 調胃承気湯 ; 8, 桂枝人参湯 ; 9, 大黄甘草湯 ; 10, 治打撲一方 ; 11, 大建中湯 ; 12, 三黄瀉心湯 ; 13, 苓姜朮甘湯 ; 14, 黄連湯 ; 15, 茵蔯蒿湯)

ous extracts of 129 Kampo medicines were investigated. The first screening was conducted by treating the cytokine-stimulated hepatoma cells with 400 μg/ml of each Kampo medicine. Fifteen Kampo medicines decreased cytokine-induced NO production from McA-RH7777 cells by more than 30 %. These Kampo medicines were further investigated for inhibitory activity at lower doses. As shown in Fig. 2, Oren-gedoku-to (OGT) inhibited NO production most effectively. OGT decreased NO production to 45 % that of the cytokine-stimulated culture at 200 μg/ml. No Kampo medicine except OGT inhibited NO production at 50 μg/ml. No significant cellular toxicity, as determined by the cell viability defined by WST-1 assay, was observed in the 15 Kampo medicines at 400 μg/ml (data not shown).

Because OGT consists of a mixture of 4 medicinal plants Oren (*Coptidis Rhizoma*), Ogon (*Scutellariae Radix*), Sanshishi (*Gardeniae Fructus*), Obaku (*Phellodendri Cortex*), the effects of each crude drug were evaluated in the same experimental model as follows. Aqueous extracts of each crude drug were added to the culture at doses proportional to the weight ratio of each crude drug in OGT, and inhibitory activity on NO production was evaluated. Treatment of cytokine-stimulated hepatoma cells with 47 μg / ml of Oren decreased NO production to essentially that of 200 μg/ml OGT (Fig. 3a), suggesting the inhibition of NO production from cytokine-stimulated hepatoma cells to ultimately be due to Oren. As shown in Fig. 3b, berberin, a major ingredient of Oren, showed dose-dependent inhibition of the NO production, while baicalin and baicalein, major ingredients of Ogon, did not significantly inhibit cytokine-induced NO production. Although the amount of berberin in Oren was less than 10 %, 100 μg/ml Oren showed more inhibition than 40 μg/ml berberin, indicating an ingredient(s) other that berberin to possibly have inhibitory effect.

Inhibitory effects of herbal medicines in LPS-stimulated RAW264 cells

Murine macrophage cell line RAW264 cells were stimulated with 1 μg/ml LPS for 48 hr to produce NO, and nitrite in the conditioned medium was measured. The inhibitory effects of 129 Kampo medicines were initially screened at 200 μg / ml. Oren-gedoku-to



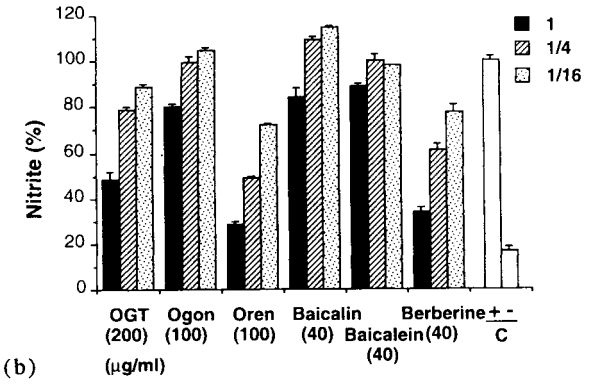


Fig. 3 Inhibition by Oren-gedoku-to (OGT), its constituent crude drugs and some ingredients on cytokine-induced NO production in McA-RH7777 cells.

(a) OGT contains Ogon (*Scutellariae Radix*), Oren (*Coptidis Rhizoma*), Sanshishi (*Gardeniae Fructus*) and Obaku (*Phellodendri Cortex*) at a weight ratio of 3:2:2:1.5. Doses of each medicinal plant in 200 μg/ml OGT are indicated in parentheses. Nitrite in the culture treated with doses in the parentheses is demonstrated by the shaded columns. Results obtained at 1/4 doses are shown by hatched columns. Open columns indicate nitrite of conditioned medium in the culture with (+) or without (−) cytokines. Nitrite in the culture treated with cytokines in the absence of added samples was set at 100 % and the relative ratio of nitrite is indicated as % nitrite of the cytokine-stimulated culture. All data represent means ±S.E.M. for triplicate determinations.

(b) Shaded columns exhibit nitrite in the culture treated with substances at the doses indicated in the parentheses. Nitrite produced by treatment with 1/4 and 1/16 doses is demonstrated by hatched and stippled columns, respectively. All data represent means±S.E.M. for triplicate determinations.

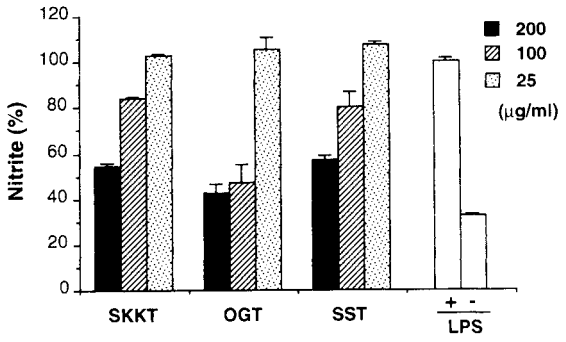


Fig. 4 Inhibitory effects of Kampo medicines on LPS-induced nitric oxide production from RAW264 cells.

Dose-dependent inhibition by Saiko-keishi-kankyo-to (SKKT), Oren-gedoku-to (OGT) and San'o-shashin-to (SST) on NO production is demonstrated. All data represent means ± S.E.M. for triplicate determination. Open columus indicate nitrite of conditioned medium in the culture with (+) or without (−) LPS. Nitrite in the culture treated with LPS in the absence of added samples was set at 100 % and the relative ratio of nitrite is indicated as % that of the LPS-stimulated culture. Nitrite in the culture media after treatment with 200 μg/ml (shaded columns), 100 μg / ml (hatched columns) or 25 μg / ml (stippled columns) is shown.

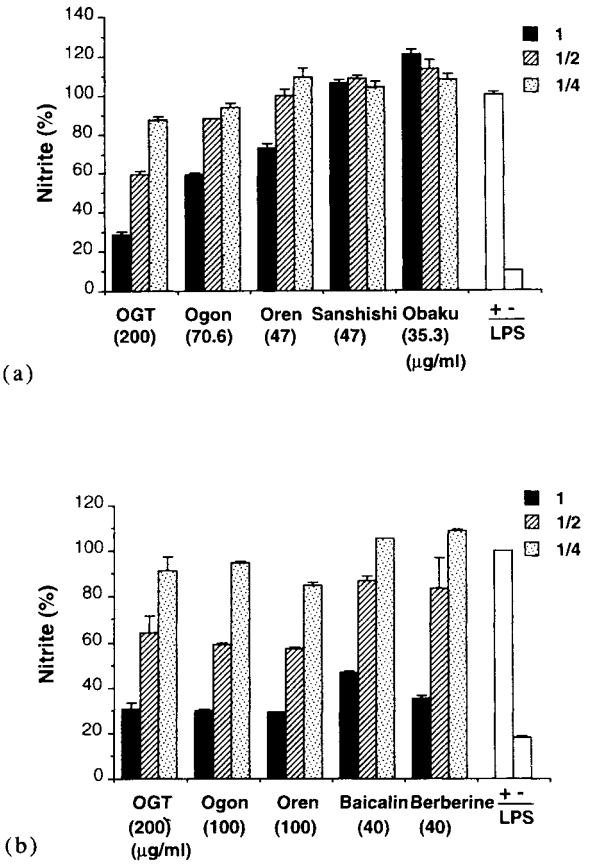


Fig. 5 Inhibition by OGT, its constituent crude drugs and some ingredients on LPS-stimulated NO production from RAW264 cells.

(a) Doses of each crude drug in 200 μg/ml OGT are indicated in parentheses. Nitrite in the culture treated with crude drugs at doses in the parenthese is demonstrated by the shaded columns. Results obtained at 1/2 and 1/4 doses are demonstrated by hatched and stippled columns, respectively. Open columns indicate nitrite of conditioned medium in the culture with (+) or without (−) LPS. Nitrite in the culture treated with LPS alone was set at 100 % and relative ratio of nitrite is indicated as % of the LPS-stimulated culture. All data represent means±S.E.M. for triplicate determinations.

(b) Shaded columns show nitrite in the culture treated with the reagents at doses indicated in the parentheses. Nitrite obtained at 1/2 and 1/4 doses is demonstrated by hatched and stippled columns, respectively. All data represent means±S.E.M. for triplicate determinations.

(OGT), Saiko-keishi-kankyo-to (柴胡桂枝乾姜湯, SKKT) and San'o-shashin-to (三黄瀉心湯, SST) inhibited NO production to less than 60 % of that of LPS-stimulated culture cells at 200 μg/ml (Fig. 4). No significant cellular toxicity, as determined by the cell viability defined by WST-1 assay, was observed in these Kampo medicines at 200 μg/ml. OGT showed the strongest inhibitory activity, and therefore the effects of Oren, Ogon, Sanshishi, Obaku, which constitute OGT, were investigated (Fig. 5a). Aqueous extracts of each crude drug were added to the culture at doses proportional to the weight ratio in OGT and inhibitory activity on NO production was evaluated. Oren and Ogon inhibited NO production but to an extent less than that of OGT. Baicalin and berberin inhibited NO production in LPS-stimulated RAW264 cells at more than 40 μg/ml (Fig. 5b).

Effects of OGT and its constituent crude drugs on transcription of the iNOS gene

For clarification of the effects of Kampo medicines and crude drugs on transcription of the iNOS gene, the expression of iNOS mRNA was assessed by Northern blot hybridization using ^{32}P-labeled mouse iNOS cDNA as the probe. As shown in Fig. 6, iNOS mRNA could hardly be detected in total RNA prepared from unstimulated McA-RH7777 or RAW264 cells (lane 1). Intracelluar iNOS mRNA markedly increased at 48 hours after treatment with TNF-α/IL-1β for McA-RH7777 or LPS for RAW264 cells (lanes 2). OGT significantly inhibited the transcriptional

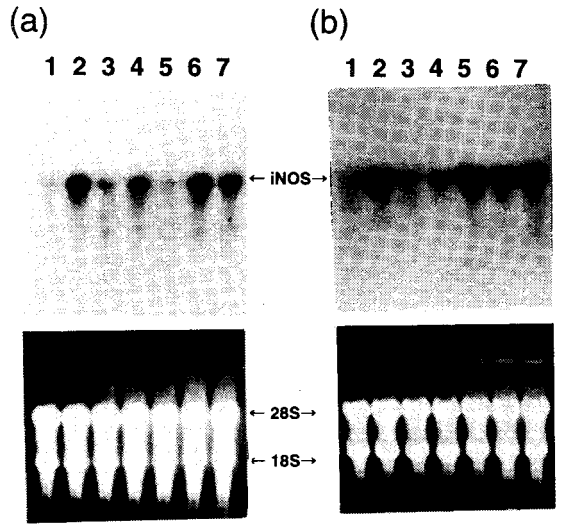


Fig. 6 Northern blot analysis showing effects of Orengedoku-to and its constituent crude drugs on iNOS mRNA expression in McA-RH7777 hepatoma cells (a) and RAW264 cells (b). Ten μg total RNAs isolated from cells after 48 hr treatment were separated by 1 % agarose-formaldehyde gel electrophoresis following Northern blot hybridization using the ^{32}P-labeled mouse iNOS cDNA probe. An autoradiograph of Northern blot analysis showing the expression of iNOS mRNA appears on the upper panel. A photograph of the ethidium bromide-stained gel prior to transfer of RNAs to the nylon membrane appears at the bottom of the figure to demonstrate even loading and positions of ribosomal RNAs. Lane 1 represents non-treated culture cells. Cells in lanes 2-7 were treated with TNF-α/IL-1β(a) or LPS (b) to stimulate NO production. OGT (lane 3), Ogon (*Scutellariae Radix*), Oren (*Coptidis Rhizoma*), Sanshishi (*Gardeniae Fructus*) and Obaku (*Phellodendri Cortex*) were added at the same doses indicated in Fig. 3 (a) and 5 (a).

induction of the iNOS gene in both experimental systems (lane 3). Oren inhibited iNOS gene transcription in TNF-α/IL-1β-treated McA-RH7777, but not LPS-stimulated RAW cells (lane 5). Ogon inhibited iNOS gene transcription in LPS-stimulated RAW264, but not TNF-α/IL-1β-stimulated McA-RH7777 cells (lane 4). Sanshishi and Obaku failed to inhibit iNOS gene expression (lanes 6,7).

Discussion

NO is a multifunctional mediator with various important physiological functions such as neurotransmission, non-specific immune defence and vasodilation.[16,17] NO is derived from L-arginine by isoforms of nitric oxide synthase (NOS) : constitutive (cNOS) and inducible (iNOS).[18,19] Although the iNOS pathway was first characterized in macrophages activated by LPS and IFN-γ, NO production is not limited to the reticuloendothelial system. Hepatocytes also express iNOS following exposure to inflammatory cytokines such as TNF-α, IL-1, and IFN-γ.[2,5] Thus, NO can be produced in large amount from inflammatory cytokine-stimulated hepatocytes as well as activated inflammatory cells, including Kufpper cells, in chronic hepatitis.[2,4,13,20]

The role of NO in inflammatory liver diseases is a matter of controversy. There are several reports that NO acts as a cytoprotective factor against liver injury such as endotoxin-induced liver damage. For instance, Harbrecht *et al.* reported that administration of a competitive inhibitor of NOS aggravates LPS-induced hepatic damage in *Corynebacterium Parvum*-sensitized mice.[21] However, it is widely accepted that the induction of NO in large amount may possibly be a causative factor of liver cell damage as well as carcinogenesis in inflammatory liver diseases.[11-13] NO causes deterioration of cellular functions by inhibiting enzymes involved in cell respiration and DNA synthesis.[6,7] An increased production of NO is probably responsible for the detrimental decrease in blood pressure seen in septic shock,[20] and the enhanced generation of NO has been implicated in the hyperdynamic state of cirrhosis, where elevated concentrations of circulating endotoxins may be responsible for its induction.[13] Investigations using mutant mice lacking iNOS gene have recently confirmed that NO produced by iNOS is a causative factor for LPS-induced lethality.[22,23]

Chronic infection and inflammation are well-recognized risk factors for a variety of human cancers.[24] Reactive oxygen species, such as hydroxyl radical and hydrogen peroxide, produced by inflammatory cells have been proposed to contribute to the multistage carcinogenesis process by inducing DNA or tissue damage, mutations, DNA strand breaks and chromosomal aberrations in inflamed tissues.[25] Recent studies show that reactive nitrogen intermediates, such as nitric oxide and its derivatives, i.e. peroxynitrite and nitrogen dioxide, also play an important role in the inflammatory process and pos-

sibly in carcinogenesis.[8,12,26,27] Nitric oxide and superoxide anion, both formed in inflamed tissue, react rapidly to form the peroxynitrite anion ($ONOO^-$).[27] Peroxynitrite is a strong oxidant which may cause DNA base modifications and tissue damage, which may lead to increased cell proliferation and mutation.[26] Liu *et al.* reported that hepatic formation of N-nitroso compounds and/or NO could be an etiologic factor in hepatocellular carcinoma.[11,12] Recent reports also suggested that inhibition of NO production in inflammation may contribute to prevention of carcinogenesis.[8-12] Evidence is thus accumulating that the effective inhibition of inducible NO production in inflammatory liver tissue is beneficial for the treatment of hepatitis and prevention of liver cell carcinogenesis.

The authors thus established *in vitro* NO production models using rat hepatoma and murine macrophage cell lines in which NO production is induced by inflammatory stimuli such as TNF-α/IL-1 β or LPS, and the inhibitory effects of Kampo medicines on NO production were investigated. OGT was found to be the most effective for inhibiting NO production in LPS-stimulated macrophages and TNF-α/IL-1β-stimulated hepatoma cells at nontoxic doses. Of 4 crude drugs consisting of OGT, Oren and Ogon expressed some inhibitory activity on NO production. The mechanisms of inhibition by Oren and Ogon may differ. Oren was mainly effective in cytokine-stimulated hepatoma cells, and Ogon in LPS-stimulated RAW264 cells. This indicates that contributory components to the inhibitory effects of OGT on NO induction differ depending on cell type and/or type of stimuli. Additive or synergistic effects of a multitude of inhibitors are likely to provide better effect than a single inhibitor. Our results thus suggest that a mixture of different medicinal plants, i.e. Kampo medicines, is much more effective than a single crude drug or component for inhibiting NO production and probably preventing cellular injury and hepatocarcinogenesis in inflammatory liver diseases.

Oren and Ogon are often used in combination for treating inflammatory conditions and digestive organ disorders in some Kampo prescriptions. The present results may thus provide mechanistic basis for clinical usage of combination of Oren and Ogon and suggest the usefulness of mixtures of crude drugs for effectively inhibiting NO production in inflammatory conditions. A crude drug is comprised of many ingredients. Its effects are quite complicated and not necessarily attributable to just one component. The major ingredients of Oren and Ogon, berberin, baicalin and baicalein, showed some inhibitory activity, but their efficacy cannot explain the total activity of these crude drugs, suggesting some other contributory ingredients and/or synergistic effects by these components. Although OGT suppressed the transcription of the iNOS gene in this study, the mechanism of transcriptional inhibition remains to be elucidated. Further study should be conducted to identify the active component(s) responsible for the inhibitory effects of OGT on NO production induced by inflammatory stimuli in macrophages and hepatoma cells.

和文抄録

一酸化窒素（Nitric oxide, NO）は生体内で重要な伝達物質であるが，炎症の場での過剰なNO産生は，組織障害や発癌の原因となっている。今回，肝癌細胞およびマクロファージ細胞を用いて，それぞれIL-1β/TNFα処理およびLPS処理によりNO産生を誘導する*in vitro*の実験モデルを作製し，129処方の漢方方剤エキス粉末および構成生薬の作用を検討した。黄連解毒湯（OGT）が両方の実験モデルにおいて最も強いNO産生抑制活性を示し，NO合成酵素の遺伝子発現レベルを抑制することを確認した。OGTの構成生薬のうち，黄連はサイトカイン刺激肝癌細胞からのNO産生を，黄芩はLPS刺激マクロファージからのNO産生に対して強い抑制作用を示した。これらの結果は，炎症における過剰なNOの産生の抑制において，単一の生薬より漢方薬のほうが，より有効であることを示唆している。

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