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Modulation of nitric oxide production by crude drugs and Kampo medicines

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Abstract

Nitric oxide (NO) is a multi-functional mediator which plays an important role in the regulation of various biological functions *in vivo*. Dysregulation of NO production is therefore causatively related to the pathogenesis of various diseases which include inflammation, cancer, immunological disorders and vascular diseases.This paper summarizes our previous studies which investigated the effects of aqueous extracts of crude drugs and Kampo medicines on NO production in murine macrophage cell line RAW264 cells, rat hepatoma McA-RH7777 cells and rat vascular smooth muscle cells (VSMC) *in vitro*. The inhibitory effect on NO production from activated macrophages was demonstrated in some crude drugs with anti-inflammatory properties. *Scutellariae Radix* (Ogon) was shown to most effectively inhibit NO production from LPS-stimulated macrophages. *Coptidis Rhizoma* (Oren) significantly inhibited NO production from TNF-α/IL-1β-stimulated hepatoma cells. These decreases of NO production were shown to be accompanied by a decrease of the cellular amount of iNOS protein and mRNA. On the other hand, the inducing effect on NO production from non-stimulated macrophages was demonstrated in some crude drugs with immunostimulatory properties, which include *Astragali Radix* (Ougi) and *Ginseng Radix* (Ninjin). *Scutellariae Radix, Astragali Radix* and *Ginseng Radix* were also shown to induce NO production and consequently increase the cellular amount of cGNP in VSMC. These effects were shown to be directly mediated by modulation of the transcription of iNOS gene. Modulatory effects on NO production from macrophages have been suggested to be associated with their anti-inflammatory or immunostimulatory effects. Enhancing effects on NO production from VSMC were suggested to be partly responsible for their vasodilatory and anti-atherosclerotic effects. It was thus suggested that a mixture of different crude drugs, i.e. Kampo medicines, might exhibit diverse effects on NO production *in vivo* and these effects may contribute to the pharmacological effects of Kampo medicines on homeostasis and defense mechanisms in the human body.

Key words nitric oxide, nitric oxide synthase, macrophages, vascular smooth muscle cells, Scutellariae Radix, Ginseng Radix, Astragali Radix.

Abbreviations NO, nitric oxide; NOS, nitric oxide synthase; iNOS, inducible nitric oxide synthase; cNOS, constitutive nitric oxide synthase; VSMC, vascular smooth muscle cell; cGMP, guanosine 3′,5′-cyclic monophosphate; sGC, soluble guanylate cyclase; IFN α/β, interferon α and β; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; SST, Sho-saiko-to; OGT, Oren-gedoku-to.

Introduction

Nitric oxide (NO) is a multi-functional mediator which is implicated in a wide range of biological functions such as neurotransmission, non-specific immune defense and vasodilation as summarized in Fig. 1.[1] NO is derived from L-arginine by isoforms of nitric oxide synthase (NOS) : constitutive (cNOS) and inducible (iNOS).[2] Of constitutive types of NOS, eNOS is expressed in vascular endothelial cells and plays an important role in the regulation of vascular

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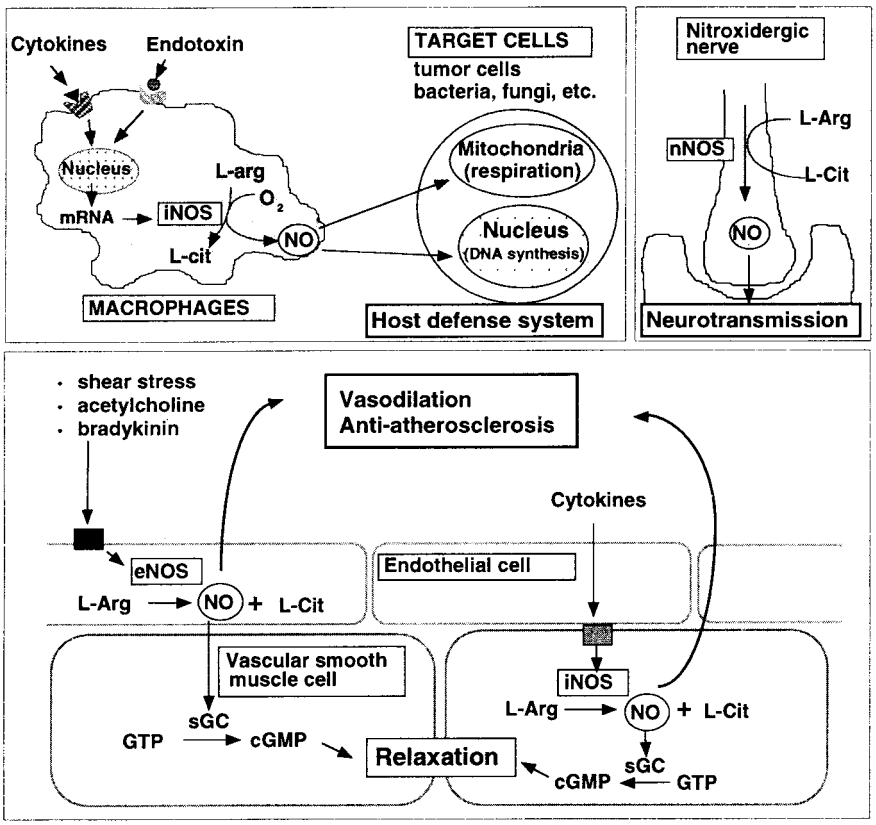

Fig. 1 Biological functions of nitric oxide (NO) in macrophages, nitroxidergic nerves, and the vascular wall. Three isoforms of nitric oxide synthase (NOS), endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS) are shown.

tone and blood flow. nNOS is the other type of cNOS and is constitutively expressed in some neural cells. Modulation of the activity of eNOS or nNOS by crude drugs is an important target of investigation in the study of pharmacological effects of Kampo medicines in cardiovascular and nervous systems. On the other hand, iNOS is induced in macrophages, vascular smooth muscle cells (VSMC), liver cells, and so on by various stimuli such as inflammatory cytokines and lipopolysaccharide (LPS). NO production by iNOS in macrophages is essential for the defense mechanisms against microorganisms and tumor cells.[3-5] NO exhibits cytoprotective properties to some cells such as hepatocytes and endothelial cells.[6,7] However, its excessive production in inflammation is thought to be a causative factor for septic shock, cellular injury and carcinogenesis.[8-11] Thus, iNOS has the character of a two-faced sword (Fig. 2), and the appropriate regulation of NO production from iNOS is very important in the maintenance of homeostasis and defense mechanisms in the human body.

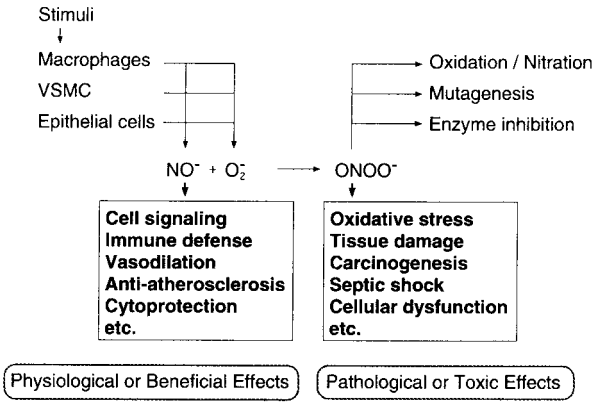

Fig. 2 Two-faced (beneficial and toxic) characters of nitric oxide (NO) produced by inducible nitric oxide synthase in macrophages, vascular smooth muscle cells (VSMC) and epithelial cells.

The aim of this paper is to summarize our previ-

ous reports concerning the modulatory effects of crude drugs on NO production from macrophages,[12-14] hepatoma cells [15] and VSMC,[16,17] especially focusing on the *in vitro* effects of *Scutellariae Radix* (Ogon), *Coptidis Rhizoma* (Oren), *Astragali Radix* (Ougi) and *Ginseng Radix* (Ninjin).

Materials and Methods

Crude drugs 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 were prepared by Tsumura & Co. (Tokyo, Japan). More than 200 kinds of crude drugs were screened for each experiment. Some crude drugs were subjected to further investigation based on the results of the screening experiments. Many kinds of Kampo medicines were also investigated. Details of each experimental method such as cell culture, measurement of NO production, and analysis of iNOS gene expression have been described in our previous reports.[12-17]

Results and Discussion

Inhibition of NO production by an aqueous extract of Scutellariae Radix in LPS-stimulated macrophages[12]

Besides its function as a physiological mediator, evidence is accumulating that NO participates in inflammatory- and autoimmune-mediated tissue destruction.[1,8,11,18] In order to investigate the anti-inflammatory effects of crude drugs from the standpoint of modulation of NO production from inflammatory cells, investigation was conducted on the effects of various crude drugs on NO production from LPS-stimulated macrophages. Murine macrophage cell line RAW264 cells were stimulated with lipopolysaccharide (LPS) to induce NO production, and simultaneously incubated with various concentrations of aqueous extracts of crude drugs. *Scutellariae Radix* extract

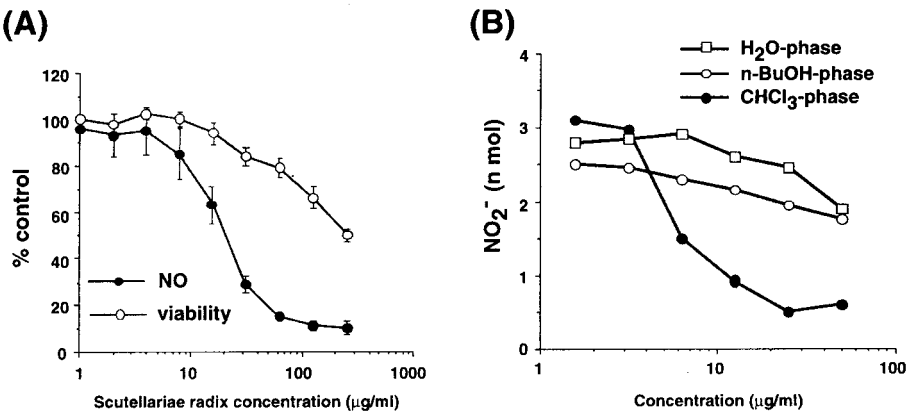

Fig. 3 Inhibitory effect of *Scutellariae Radix* on the nitric oxide (NO) production in LPS-stimulated RAW264 cells.
(A) Cultures were incubated with 100 ng/ml of LPS in the presence of various concentrations of *Scutellariae Radix* extract. The extracellular accumulation of nitrite (closed circles) and the cell viability (open circles) were determined after 36 hr incubation period. The data represent % of the control value, in which cells were incubated with 100 ng/ml of LPS in the absence of *Scutellariae Radix* extract (mean±S.D., n=8)
(B) Effects of the fractionated extracts on the NO production from LPS-stimulated RAW264 cells are shown. An aqueous extract of *Scutellariae Radix* was fractionated using a partition extraction method into water (H_2O)-phase, n-butanol (n-BuOH)-phase and chloroform ($CHCl_3$)-phase. Cultures were incubated with 100 ng/ml of LPS in the presence of various concentrations of fractionated extracts. The extracellular accumulation of nitrite was determined after 36 hr incubation period. Major components of the chloroform-phase fraction are flavonoids such as baicalein and wogonin.

was found to be the most effective in the suppression of NO production from LPS-stimulated RAW264 cells. The extract suppressed the LPS-induced NO production from RAW264 cells at non-toxic doses. Treatment of stimulated RAW264 cells with *Scutellariae Radix* suppressed the NO production dose dependently at concentrations higher than 4 μg/ml, with 50 % inhibitory concentration of 20 μg/ml (Fig. 3A). Analysis of the fractionated extracts indicated that the flavonoids in *Scutellariae Radix* have inhibitory effects on NO production from stimulated RAW264 cells (Fig. 3B). Although the inhibitory activities varied among the flavonoids, baicalein was demonstrated to have the strongest inhibitory activity on NO production from LPS-stimulated macrophages. Because baicalein is a major ingredient of *Scutellariae Radix,* baicalein was suggested to be the major contributory component to the suppressive effect of *Scutellariae Radix* extract on the inducible NO production from LPS-stimulated macrophages, although other flavonoids also partly contribute to the inhibitory effect. iNOS mRNA and iNOS protein levels were significantly increased in RAW264 cells after treatment with LPS, but the treatment with *Scutellariae Radix* extract decreased both iNOS protein and mRNA levels in parallel with reduction of NO release, suggesting that the extract effects the iNOS gene transcription.

Inhibitory effects of Coptidis Rhizoma on NO production in cytokine-stimulated hepatoma cells[15]

In inflammatory liver diseases such as viral hepatitis, Kupffer cells and infiltrating lymphocytes are activated to produce many cytokines, oxyradicals and NO radicals.[19,20] 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).[20-23] In order to investigate the mechanisms of the pharmacological effects of crude drugs on chronic liver diseases, an *in vitro* experimental model of cytokine-induced NO production from hepatoma cells was established. Rat hepatoma cell line McA-RH7777 cells were stimulated with TNF-α and IL-1β to induce nitric oxide production, and crude drugs were investigated. An aqueous extract of *Coptidis Rhizoma* significantly inhibited NO production from TNF-α/IL-1β-stimulated hepatoma cells. The decrease of NO production was shown to be accompanied by a decrease of the cellular amount of iNOS protein and mRNA.

Screening of Kampo medicines for inhibition of NO production[15]

Using these *in vitro* NO production models, effects of Kampo medicines were also investigated. Screening of 129 Kampo medicines revealed that Orengedoku-to (黄連解毒湯, OGT; Huang-Lian-Jie-Du-Tang in Chinese) showed the strongest inhibitory activity for the NO production both from macrophages and hepatoma cells. Of 4 constituent medicinal herbs (*Coptidis Rhizoma, Scutellariae Radix, Gardeniae Fructus, Phellodendri Cortex*), *Coptidis Rhizoma* and *Scutellariae Radix* inhibited NO production but to an extent less than that of OGT (Fig. 4), suggesting the usefulness of Kampo medicines rather than each constituent crude drug. As already described, *Coptidis Rhizoma* was mainly effective in cytokine-stimulated hepatoma cells, and *Scutellariae Radix* 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 a better effect than a single inhibitor.

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 cell damage.[6] However, it is widely accepted that the induction of NO in large amounts may possibly be a causative factor of liver cell damage as well as carcinogenesis in inflammatory liver diseases.[24,25] NO causes deterioration of cellular functions by inhibiting enzymes involved in cell respiration and DNA synthesis.[26,27] An increased production of NO is probably responsible for the detrimental decrease in blood pressure seen in septic shock,[11] 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.[25] Chronic elevation of NO synthesis, which may occur in chronic viral hepatitis and liver cirrhosis, could increase the risk of hepatocellular carcinoma.[24] The effective inhibition of NO production induced by inflammatory stimuli from he-

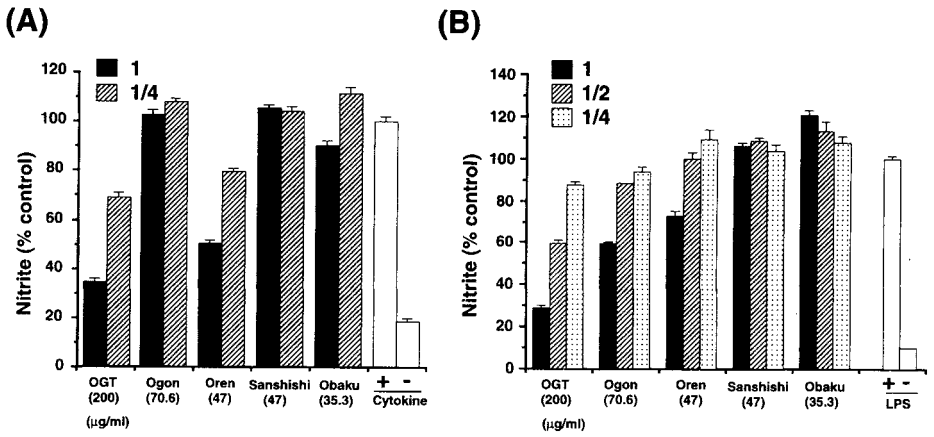

Fig. 4 Effects of Oren-gedoku-to (OGT) and its constituent crude drugs on NO production from cytokine-stimulated McA-RH7777 hepatoma cells (A) and LPS-stimulated RAW264 macrophages (B).
(A) OGT contains *Scutellariae Radix* (Ogon), *Coptidis Rhizoma* (Oren), *Gardeniae Fructus* (Sanshishi) and *Phellodendri Cortex* (Obaku) at a weight ratio of 3:2:2:1.5. Doses of each crude drugs 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 mean±S.E.M. for triplicate determinations.
(B) 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 parentheses 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 mean±S.E.M. for triplicate determinations.

patocytes, hepatoma cells and macrophages may thus prove beneficial for the prevention of liver cell carcinogenesis as well as for damage to tissue components. Our results thus suggest that a mixture of different crude drugs, i.e Kampo medicines, is much more effective than a single crude drug or component in inhibiting NO production in inflammatory diseases.

Coptidis Rhizoma and *Scutellariae Radix* are often used in combination for treating inflammatory conditions and digestive organ disorders in some Kampo prescriptions. Our findings may thus provide a mechanistic basis for the clinical usage of a combination of *Coptidis Rhizoma* and *Scutellariae Radix* and, in addition, 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 *Coptidis Rhizoma* and *Scutellariae Radix,* berberin, baicalin and baicalein, showed some inhibitory activity, but their efficacy cannot explain the total activity of these crude drugs, suggesting possibly some other contributory ingredients and/or synergistic effects by these components.

Stimulation of NO production by aqueous extracts of crude drugs in RAW264 Cells[13]

Some crude drugs are well recognized to have immunostimulatory effects which are associated with the enhancement of nonspecific resistance of the host to pathogenic organisms and neoplastic cells.[28,29] Macrophage activation appears quite likely to be involved in the immunostimulatory effect of crude drugs, since macrophages serve as important effector cells in the host defense system. Recent work shows NO to be essential in macrophage-induced cytotoxicity against microorganisms and tumor cells.[3-5] Therefore, crude drugs were screened for their stimulatory effects on NO production from murine macrophage RAW264 cells. The first screening was conducted by treating the RAW264 cells with 100 μg/

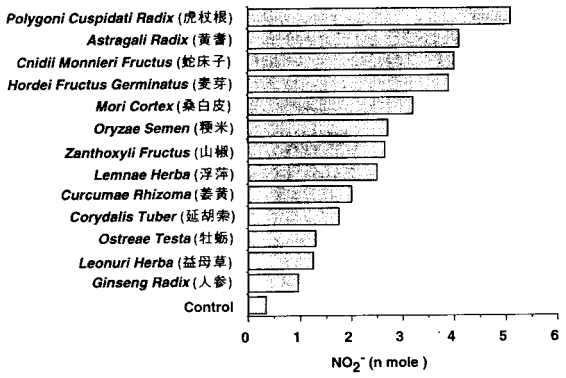

Fig. 5 Stimulation of NO production by aqueous extracts of crude drugs in RAW264 cells. RAW264 murine macrophage cells were cultured in the presence of 100 μg/ml of each crude drugs and the accumulated nitrite amounts were measured 48 hr after stimulation. Crude drugs showing stimulatory effects on NO production are demonstrated.

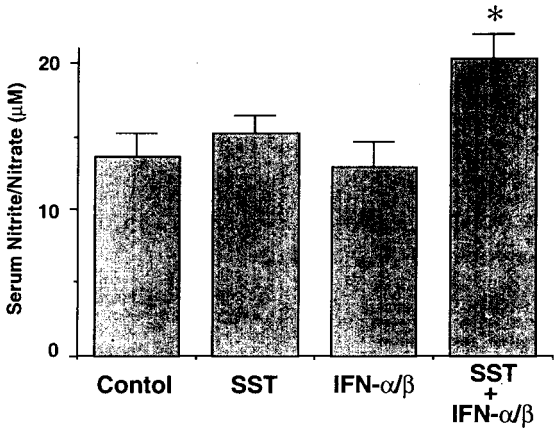

Fig. 6 Effects of Sho-saiko-to (SST) and/or IFNα/β on serum nitrite and nitrate in mice. Serum nitrite and nitrate (NO_2^-/NO_3^-) was measured in mice treated with SST and/or IFNα/β for 3 weeks as described in the text. *$p<0.05$ compared with control.

ml of each crude drug and the accumulated nitrite levels were measured 48 hours after stimulation. Several crude drugs, which include *Astragali Radix* and *Ginseng Radix*, were found to significantly stimulate NO production from RAW264 cells (Fig. 5). Transcriptional induction of iNOS gene and subsequent increase of cytoplasmic iNOS protein were demonstrated by immunohistochemical and immunoblot analyses. To distinguish the effect of crude drugs from that of LPS which might be contaminated in the crude drug preparation, NO inducing effects were also analyzed with crude drug samples pretreated with perchloric acid. Treatment with perchloric acid significantly decreased the NO-inducing effects of crude drugs, but not that of LPS. This indicates that carbohydrate components of crude drugs may be responsible for the NO induction from macrophages.

In vivo effects of Kampo medicines on NO production were also investigated. The effects of the Kampo medicine Sho-saiko-to (小柴胡湯, SST) and interferon α/β (IFN α/β) on *in vivo* NO generation were investigated.[14] SST (0.92 g/kg of body weight) was orally administered to Balb/c mice for 3 weeks with or without intraperitoneal injection of IFN α/β (10^5 unit/kg of body weight, 2 times per week). Serum nitrite/nitrate was measured as an index of *in vivo* NO generation. A significant increase in serum nitrite/nitrate was observed in mice treated with SST and IFN α/β, but not with either alone (Fig. 6). SST and IFN α/β are thus shown to synergistically increase NO generation *in vivo*. This result demonstrates that the oral administration of SST stimulates NO production *in vivo* in combination with IFN α/β effects. IFN α/β are endogenously produced in various infectious diseases and express potent anti-viral activity. The result may provide clues to explain the mechanism by which SST acts as an immunomodulatory drug, especially in infectious diseases.

Induction of NO production in vascular smooth muscle cells by treatment with aqueous extracts of Astragali Radix, Ginseng Radix and Scutellariae Radix[16,17]

In the vasculature, NO was originally identified as the endothelium-derived relaxing factor (EDRF) and has been shown essential to the control of vascular tone and peripheral blood flow.[30,31] Endothelium-derived NO relaxes vascular smooth muscle cells (VSMC) and inhibits platelet adhesion and aggregation. These features of NO are attributed to its ability to activate soluble guanylate cyclase (sGC) and thus increase intracellular guanosine 3′,5′-cyclic monophosphate (cGMP).[30,31] VSMC not only responds to endothelium-derived NO but also possesses enzymes to produce NO from L-arginine (Fig. 1). Charpie *et al.* found VSMC-derived NO to function as an autocrine factor in the regulation of vascular tone.[32] Thus, NO plays a fundamental role in the control of blood

pressure and regional blood flow through the activation of sGC and the resultant increase in cGMP in VSMC. NO also has many anti-atherosclerotic properties.[30] These include inhibition of the adhesion and aggregation of platelets, superoxide anion production, leukocyte adhesion, and VSMC proliferation. Hogg *et al.* found NO to inhibit LDL oxidation by acting as a chain-breaking antioxidant.[33] The oral administration of L-arginine, the nitric oxide precursor, showed anti-atherosclerotic effects in hypercholesterolemic rabbit and inhibited development of intimal hyperplasia following balloon catheter-induced injury.[34,35]

The effects of aqueous extracts of various crude drugs on the NO production in cultured rat VSMC were therefore investigated. Aqueous extracts of *Astragali Radix, Ginseng Radix* and *Scutellariae Radix* were shown to induce NO synthesis in VSMC (Fig. 7A). Aqueous extracts of *Astragali Radix* and *Ginseng Radix* induced NO production in VSMC in a dose-dependent manner at concentrations over 400 μg/ml, and a 10- to 20-fold increase over the basal level in the NO production was observed when 1 mg/ml of the crude drugs was applied. Aqueous extract of *Scutellariae Radix* also showed a dose-dependent induction of NO synthesis at concentrations between 20 to 100 μg/ml. The mechanism for this was then investigated. Northern blot analysis showed that treatment with aqueous extracts caused an increase in the transcription of the iNOS gene in VSMC. Immunohistochemical and immunoblot analyses demonstrated a significant increase of iNOS protein in VSMC after treatment with aqueous extracts. These extracts increased dose-dependently intracellular cGMP in VSMC (Fig. 7B). This increase was inhibited by N^G-monomethyl-L-arginine, an inhibitor of NOS, or methylene blue, an inhibitor of soluble guanylate cyclase (sGC), indicating intracellular cGMP increase to be due to stimulation in the NO-sGC pathway (Fig. 8). Modulation of cellular functions of VSMC by way of NO-sGC-cGMP-mediated signal transduction pathways may be one of the mechanisms by which these crude drugs exhibit vasodilation and anti-atherosclerotic effects.

Bidirectional effects of Scutellariae Radix on NO production in macrophages and VSMC

Scutellariae Radix has been known to reduce prostaglandin and leukotrien production, to scavenge superoxide, and to inhibit the release of chemical mediators from inflammatory cells. These activities are suggested to be responsible for its anti-inflammatory and anti-allergic effects. *Scutellariae Radix* contains large amounts of flavonoids, and it has recently

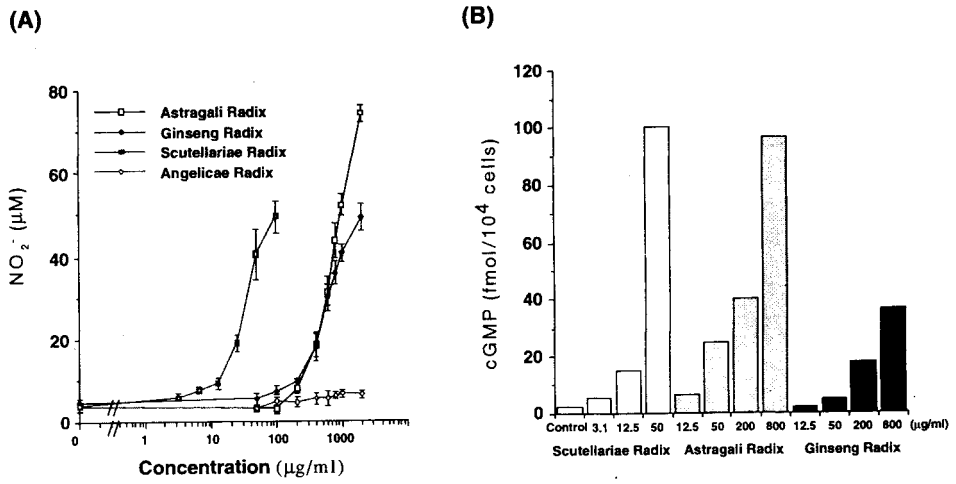

Fig. 7 Stimulation of nitric oxide (A) and cGMP (B) production by aqueous extracts of *Astragali Radix, Ginseng Radix,* and *Scutellariae Radix* in rat vascular smooth muscle cells (VSMC).
(A) Cultures were incubated for 144 hours in the presence of various concentrations of the crude drugs. Effect of aqueous extract of *Angelicae Radix* is shown as a negative control. Nitrite levels in the conditioned media were quantified using Griess colorimetric assay. (mean±S.D., n=6)
(B) cGMP levels in VSMC cultured for 24 hours in the presence of the crude drugs. Intracytoplasmic cGMP is expressed in femtomoles per 10^4 cells.

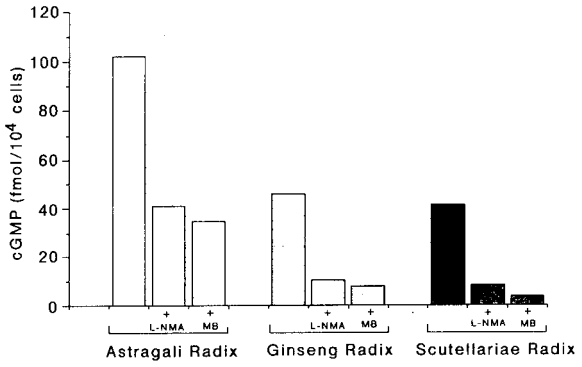

Fig. 8 Effects of N^G-monomethyl-L-arginine (L-NMA) and methylene blue (MB) on cGMP production in VSMC induced by aqueous extracts of *Astragali Radix* (500 μg/ml), *Ginseng Radix* (500 μg/ml) or *Scutellariae Radix* (100 μg/ml). Increase in intracellular cGMP induced by crude drugs was markedly reduced by the simultaneous incubation with 1 mM L-NMA or 5 μM MB. Intracytoplasmic cGMP is expressed in femtomoles per 10^4 cells. Data shown are representative of three independent experiments, which gave similar results. Values represent the average of two samples with duplicate measurements.

been reported that flavonoids are effective scavengers of both NO and peroxynitrite.[36,37] Our study demonstrated that *Scutellariae Radix* flavonoids inhibit NO production in LPS-stimulated macrophages. It is therefore reasonable to speculate that these effects on NO production may be related to the effectiveness of *Scutellariae Radix* against inflammatory diseases. *Scutellariae Radix* exerts vasodilatory and anti-atherosclerotic effects. The induction by *Scutellariae Radix* of iNOS expression from VSMC may possibly be associated with its vasodilation and anti-atherosclerotic effects. Our recent study demonstrated that some crude drugs including *Scutellariae Radix* increase the promoter activity of the eNOS gene in endothelial cells (unpublished data). It was thus suggested that the modulatory effects of *Scutellariae Radix* on NO production may be associated with some of its pharmacological activities. The effects of *Scutellariae Radix* on NO production in macrophages and the vascular wall and the possible relation to its pharmacological effects are shown in Fig. 9.

It appears that an aqueous extract of *Scutellariae Radix* contains both inhibitory and stimulatory components for NO synthesis, depending on the cell types. Baicalein, a flavone of *Scutellariae Radix,* was demonstrated to be the major contributory component for

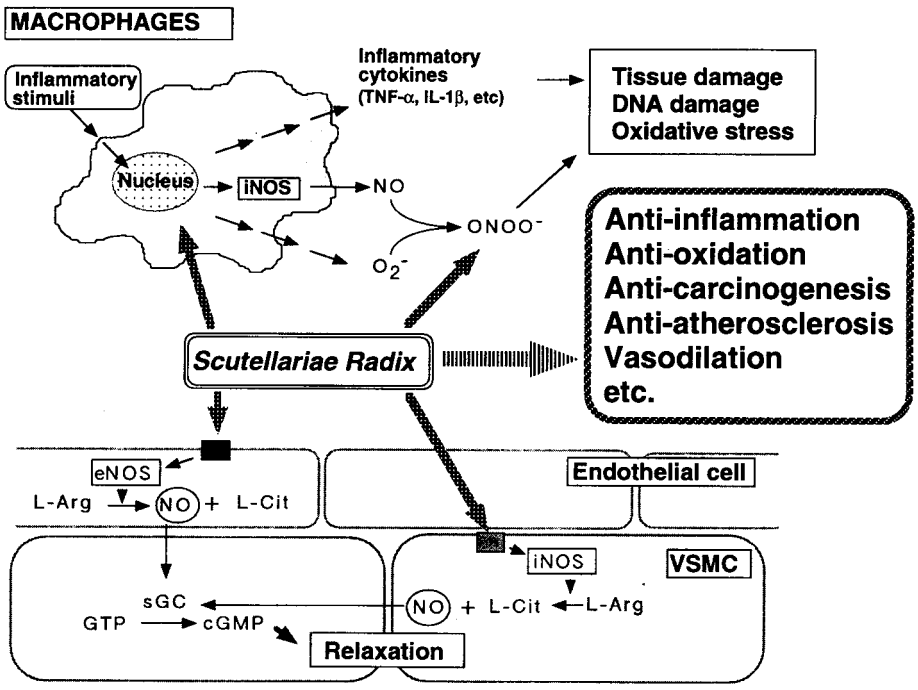

Fig. 9 Summary of the effects of *Scutellariae Radix* on nitric oxide (NO) production in macrophages and the vascular wall. The possible relation to its pharmacological effects is shown.

the inhibition of NO synthesis in LPS-stimulated macrophages. Our preliminary experiments suggested that additive or synergistic effects by several components, which include some kinds of flavonoids and polysaccharides, are involved in the NO induction from VSMC (unpublished data).

Modulation of NO production in association with the pharmacological effects of Kampo medicines

The inhibitory effect on NO production from activated macrophages was demonstrated in some crude drugs having anti-inflammatory effects such as *Scutellariae Radix*. On the other hand, the inducing effects on NO production from non-stimulated macrophages was demonstrated in some crude drugs having immunostimulatory effects such as *Astragali Radix* and *Ginseng Radix*. Therefore these activities were suggested to be closely related to their pharmacological effects against inflammatory diseases and immunological disorders. Kampo medicines consist of a mixture of many kinds of crude drugs. The effects of Kampo medicines on various pathological conditions are very complicated. For example, Sho-saiko-to (SST) contains both *Scutellariae Radix* and *Ginseng Radix,* and its immunomodulatory effects appear to be different depending on the condition of the host. SST was demonstrated to enhance *in vivo* NO production.[14] Some components in SST appear to stimulate the reticuloendothelial system with consequent NO-inducing activity. Kondo and Takano reported glycyrrhizin, a major ingredient of *Glycyrrhizae Radix* (Kanzo), to activate macrophages *in vivo* and stimulate NO production in response to LPS.[38] Our studies found an aqueous extract of *Ginseng Radix* to be capable of inducing NO production from macrophages. These components of SST may possibly be involed in *in vivo* NO production and immunostimulatory effects. In addition to its immunopotentiating effects, SST exerts an anti-inflammatory and anti-allergic action. An aqueous extract of *Scutellariae Radix* was shown to inhibit NO production from LPS-stimulated macrophages. SST is often used to normalize and treat unbalanced conditions of the host in various pathological conditions.[39,40] SST has bidirectional, i,e., immunostimulatory and anti-inflammatory, effects on the immune system. These conflicting effects of SST as an immunomodulator may be partly explained by its component complexity in which both inducing and inhibitory components on NO production are present.

The effects of Kampo medicines on the activity and expression of constitutive NOS (cNOS) are also important. cNOS in endothelial cells, eNOS, generates low levels of NO in response to hormones or sheer stress. Some crude drugs show vasodilatory action by way of stimulation of the endothelium-derived NO production.[41] Recent studies demonstrated that eNOS expression is also inducible in endothelial cells.[42] The effects of crude drugs and their constituent ingredients on signal transduction and gene expression are now an important target in the investigation of the pharmacological effects of crude drugs and Kampo medicines. In our recent study, the transcriptional promoter activities of human iNOS and eNOS were analyzed using reporter plasmids transfected in macrophages and endothelial cells of human origin. These experiments revealed that some crude drugs are indeed effective in stimulating transcriptional activity of the iNOS or eNOS gene in the human cells (unpublished observations). The importance of NO in the regulation of a wide range of biological functions is well recognized, and therefore the modulatory effects of Kampo medicines on NO production in the human body should be further investigated in association with their clinical usefulness on various pathological conditions.

和文抄録

一酸化窒素（Nitric Oxide, NO）は生体内における重要な伝達物質であり，循環器系，免疫系，神経系，消化器系など多くの生体機能の調節に関与している。その作用においてNOは両刃の剣としての2面性を持っており，その調節においてはバランスが重要である。生体機能調節に対する和漢薬の作用メカニズム研究の一環として，誘導性NO合成酵素（iNOS）の活性やNO産生に及ぼす作用を多くの生薬や漢方方剤について検討した。

1）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。そこで，抗炎症及び発癌抑制作用との関連から，培養細胞を用いてNO産生を誘導する実験系にて検討した。サイトカイン刺激肝癌細胞からのNO産生に対しては黄連が，また，LPS刺激マクロファージからのNO産生に対しては黄芩が最も強い抑

制作用を示した。一方，NO産生刺激を指標としてマクロファージ活性化作用を検討したところ，黄耆，人参など免疫賦活作用が指摘されている生薬にNO産生刺激活性を認めた。これらの作用がNO合成酵素の遺伝子発現の調節であることも確認した。NO産生に対して，抗炎症作用を有する黄芩や黄連に抑制作用を認め，免疫賦活作用を有する生薬に刺激作用を認めたことより，これらの薬効にNO産生に対する作用が関与している可能性が示唆された。また，方剤では両方向の作用を示す生薬を組み合わせることにより，生体機能のバランス調節を行っている可能性も示唆された。

2) NOは血圧や血流のコントロールにおいて重要な役割を果たす。そこで，血管平滑筋細胞に対するNO産生刺激作用について検討し，人参，黄耆，黄芩が，血管平滑筋細胞のNO合成酵素発現を転写レベルで誘導し，さらに，NOによるguanylate cyclaseの活性化により細胞内のcGMPを増加させることを明らかにした。これらの生薬による血管拡張作用や抗動脈硬化作用などの循環系に及ぼす薬理作用の機序の一つとして，血管平滑筋細胞のNO-guanylate cyclase-cGMP系に対して直接作用している可能性が示唆された。

3) 黄芩エキスはマクロファージと血管平滑筋細胞からのNO産生に対して異なる作用を示したが，その機序として，フラボノイドや多糖成分など複数の成分による複合的な作用による事を明らかにした。炎症性疾患や循環器系などに多彩な作用を有する黄芩の薬効におけるNO産生調節の関与と，生体機能調節における多成分系薬剤の有用性が示唆された。

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