Search for anti-inflammatory plant constituents by monitoring suppression of nitric oxide production in activated macrophages(活性化マクロファージの一酸化窒素産生の抑制を指標とする抗炎症性植物成分の探索研究)

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論 文 内 容 要 旨

Nitric oxide (NO) is one of the critical mediators in inflammation produced by inducible isoform of NO synthase (iNOS) in macrophages when stimulated by lipopolysaccharide (LPS) and some cytokines. NO generated by the iNOS plays a role in non-specific immune defense against tumor, parasite, bacteria and protozoa. On the other hand, NO is known to be responsible for the hypotension observed in endotoxin shock. Since glucocorticoides, representative anti-inflammatory agents, strongly reduce NO production in activated macrophages, one of the beneficial methods for evaluating anti-inflammatory drug is thought to measure NO production in LPS-stimulated macrophages. Along this idea, the author examined suppressive effects of 44 Mongolian plants and two immunosuppressive crude drugs on the LPS-stimulated NO production in mouse peritoneal macrophages induced by bacillus Calmette-Guérin (BCG).

Among the methanol extracts of 44 Mongolian plants checked in this study, *Schizonepeta multifida, Halenia corniculata and Artemisia sieversiana* significantly (>50%) inhibited the NO production at a concentration of 100 μg/ml, and *Stellera chamaejasme, Artemisia vulgaris, Geranium pratense and Leontopodium ochroleucum* moderately (30-49%) inhibited it at the same concentration. These plants might be promising sources for NO reducing agents. Furthermore, the methanol extracts of *Artemisia sieversiana* and *Schizonepeta multifida* showed a concentration-dependent inhibition on LPS-stimulated NO production in BCG-induced mouse peritoneal macrophages within the concentration range of 10-200 μg/ml without any toxic action (Fig. I), indicating both plants to be specially attractive sources for NO reducing agents.

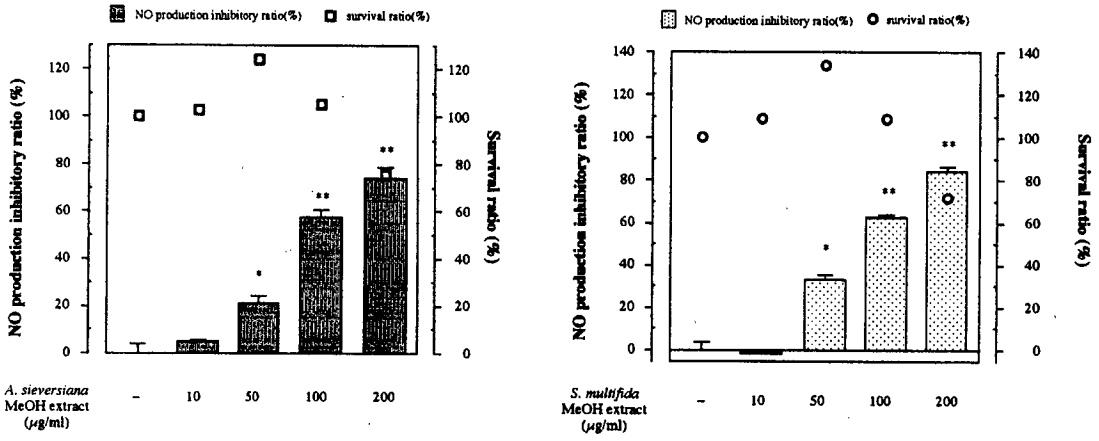
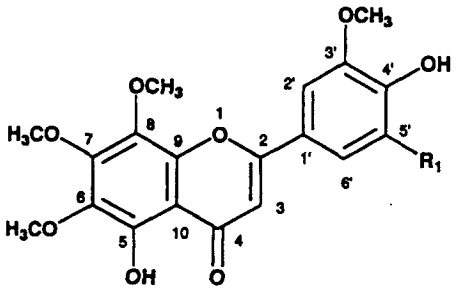


Fig. I. Effects of MeOH extracts of *A. sieversiana* and *S. multifida* on LPS-stimulated NO production and cell viabilities in BCG-induced mouse peritoneal macrophages. Data for suppression of LPS-stimulated NO production are expressed as the mean(%) ± s.e of each sample compared with LPS alone-treated control culture. Data for cell viability are indicated as the ratio of absorbance of sample-treated Mϕ to that of non-treated Mϕ as percentage. Significantly different from non-treated control group, *P<0.05 and **P<0.01

The methanol extract of *Cleome droserifolia,* an anti-inflammatory Egyptian medicinal plant, reduced the NO production, and two flavonoids (**1**,**2**) were isolated as the active components. The new flavonoid (**1**) was determined to be 5,4′-dihydroxy-6,7,8,3′, 5′-pentamethoxyflavone and the other (**2**) was identified as 5,4′-dihydroxy-6,7,8,3′-



R_1= OCH_3 (1)
R_1= H (2)

tetramethoxyflavone (8-methoxy-cirsilineol). Compound **1** suppressed the NO production in a concentration-dependent manner in the concentration range of 0-20 μg/ml, but showed a cytotoxic action toward the macrophages at concentrations over 25 μg/ml (Fig. Ⅱ). Compound **2** was also toxic to the macrophages at the same concentrations. IC_{50} values for the suppressions of NO production by **1** and **2** were 50.5 and 85.5 μM, respectively. Genistein also concentration-dependetly suppressed the NO production and did not show any significant toxicity to the macrophages in the concentration range of 0-50 μg/ml. The IC_{50} value for genistein was 9.6 μM (Fig. Ⅱ). Compounds **1** and **2** have the potential to be NO reducing agents.

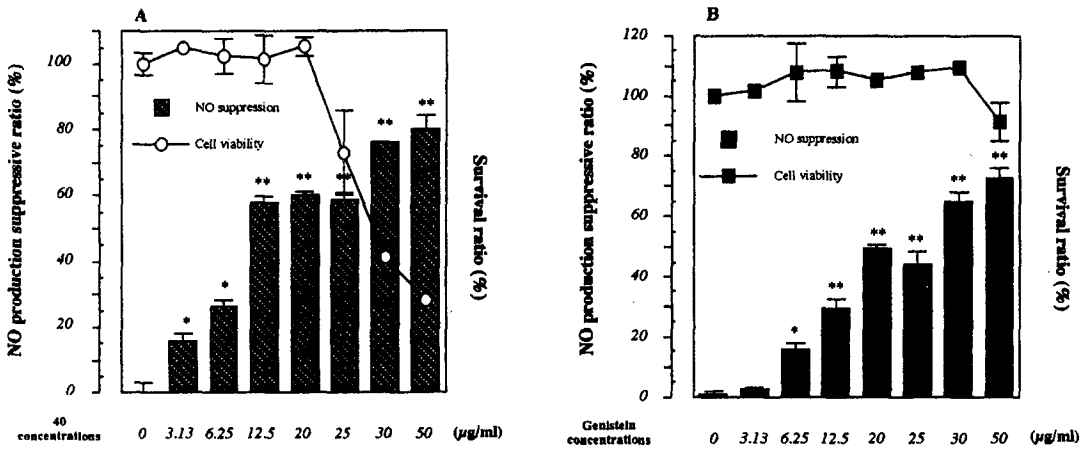
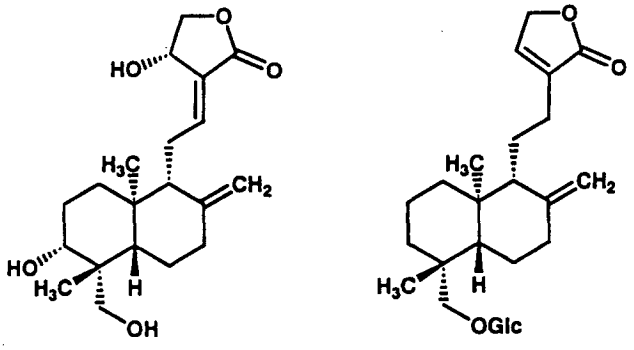


Fig. II. Concentration dependent suppression by 40 (panel **A**) and genistein (panel **B**) on NO production and their cell viabilities 24 hour after culture of BCG-induced mouse peritoneal macrophages activated by LPS. Data for cell viability are indicated as the ratio of absorbance of sample-treated macrophages to that of non-treated macrophages as percentage. Statistically significant from non-treated control group, *P<0.01 and **P<0.001.

Oral treatment of methanol extract of *Andrographis paniculata,* one of the anti-inflammatory Chinese herbs, reduced the NO production, and a diterpene lactone neoandrographolide (**3**) was identified as an active component. Neoandrographolide (**3**) suppressed the NO production by 35 and 40% at doses of 5 and 25 mg/kg/day, respectively (Fig. Ⅲ). Neoandrographolide (**3**) also suppressed the NO production with an IC_{50} value of 35.5 μM when directly added to the cultured macrophages (Fig. Ⅳ). On the other hand, andrographolide (**4**), the main constituent of this plant, suppressed the NO production *in vitro* with an IC_{50} value of 7.9 μM, but did not suppress it *in vivo.* These results indicate that neoandrographolide might play an important role in the anti-inflammatory action of *A. paniculata.*



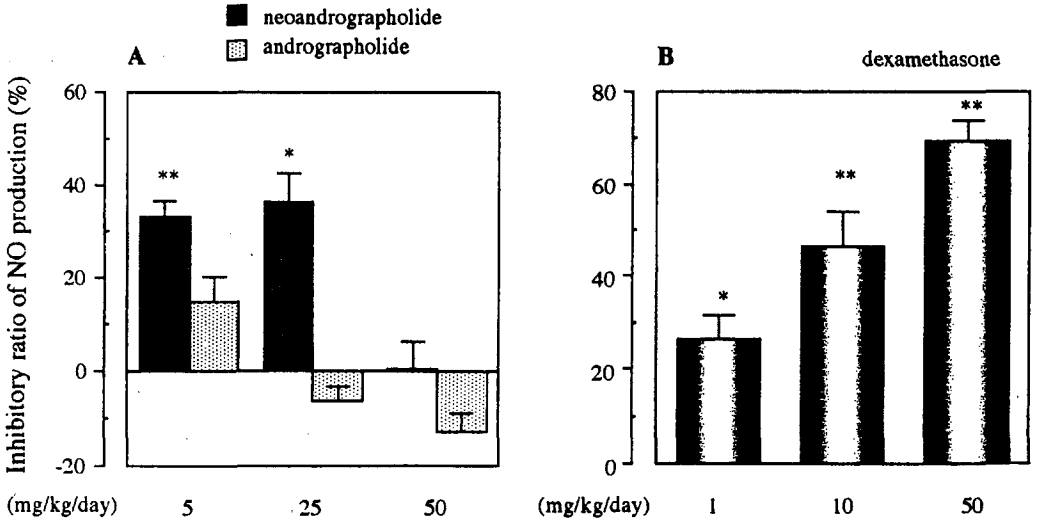


Fig. III. Effects of orally administered andrographolide (panel **A**), neoandrographolide (panel **A**), and dexamethasone (panel **B**) on LPS-activated NO production in BCG-induced mouse peritoneal macrophages. Data for inhibition of NO production 24 h after LPS triggering are expressed as the mean(%) ± s.e. of each sample compared with control culture (NO production, LPS alone: 58.0 ± 6.6 μM). Statistically significant from control mice, *P<0.05 and **P<0.01.
Number of mice=9 to 10

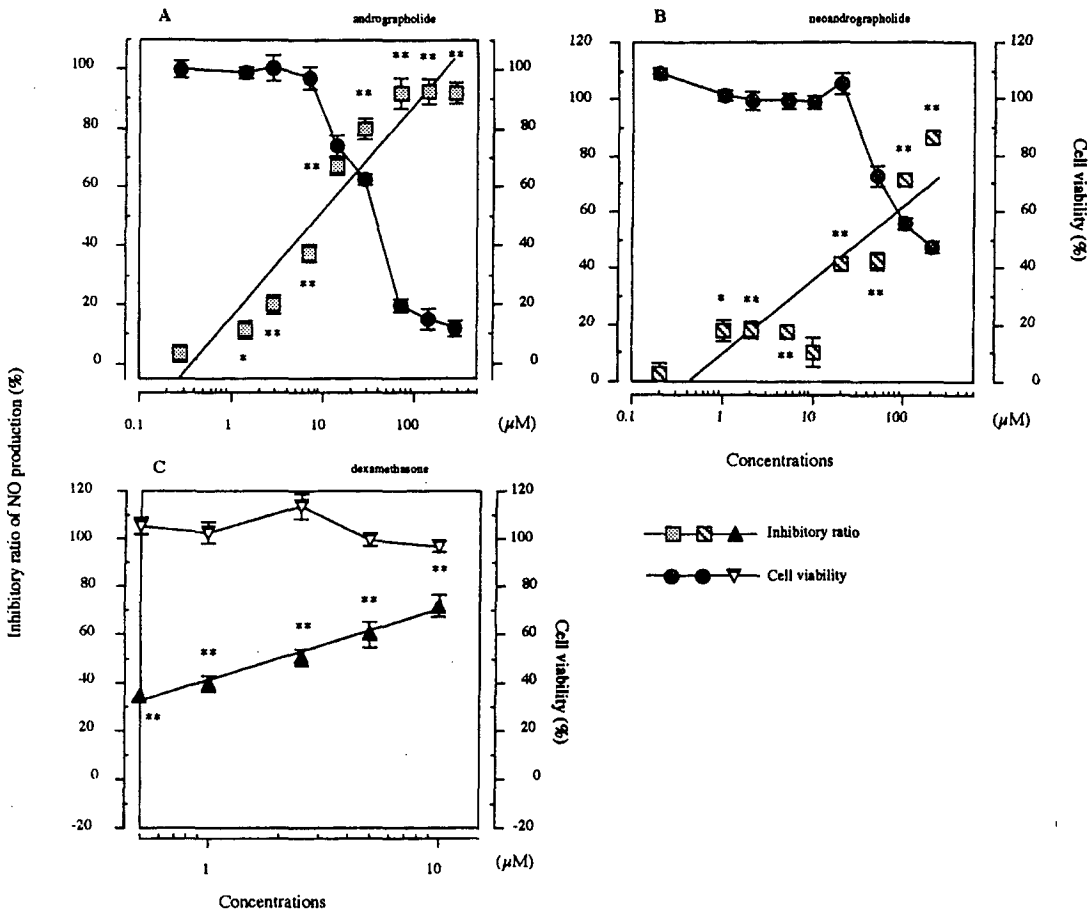


Fig. IV. Concentration-dependent suppression by andrographolide(panel **A**), neoandrographolide (panel **B**), and dexamethasone(panel C) on NO production and their cell viabilities 24 hour after culture of BCG-induced macrophages stimulated with LPS. Data for the cell viability are indicated as the ratio of absorbance of test sample-treated macrophages to that of non-treated macrophages as a percentage. Statistically significant from control group, * $P< 0.005$ and ** $P< 0.001$.

審査結果の要旨

本論文は，免疫系の異常な亢進が関係している肝炎などの炎症性疾患に対する予防・治療薬を探索する際の初期スクリーニング法として，活性化マクロファージが産生する一酸化窒素（NO）の抑制効果を指標とする評価系を用いて種々の植物エキスをスクリーニングするとともに，NO産生を抑制する数種の植物成分を単離して，それらのNO産生抑制効果を詳しく調べたものである。

はじめに，44種のモンゴル産植物のエキスについて，BCG誘導マウス腹腔マクロファージのLPS刺激によるNO産生に対する抑制効果が検討された。その結果，*Scizonepeta multifida*, *Halenia corniculata*, *Artemisia sieversiana*に強いNO産生抑制効果が，*Stellera chamaejasme*, *Artemisia vulgaris*, *Geranium pratens*, *Leontopodium ochroleucum*に中程度のNO産生抑制効果があることが明らかにされ，これらの植物がNO産生抑制物質を探索する材料として有望であることが示された。

つぎに，エジプトで抗炎症を目的に民間薬として使われている*Cleome droserifolia*のNO産生抑制効果が検討された。その結果，NO産生を抑制する2種のフラボノイドが単離され，一方は新規化合物の5,4′-dihydroxy-6,7,8,3′,5′-pentamethoxyflavoneと決定され，他方は5,4′-dihydroxy-6,7,8,3′-tetramethoxyflavoneと同定された。これらのフラボノイドは，0-20 μg/mlの濃度範囲で濃度依存的にNO産生を抑制し，IC_{50}値はそれぞれ50.5，85.5 μMであることが明らかにされた。この実験結果から，これらのフラボノイドが*C. droserifolia*の抗炎症効果に関与している可能性が示唆された。

さらに，インドネシアで炎症性疾患，高血圧，糖尿病などの治療に広く使われている*Andrographis paniculata*のNO産生抑制効果が検討され，*A. paniculata*のメタノールエキスが*in vitro*系だけでなく経口投与した場合の*in vivo*系でもNO産生を抑制することが明らかにされた。*In vitro*系でNO産生を抑制する物質として，本植物の主成分であるandrographolideとneoandrographolideが単離，同定され，IC_{50}値はそれぞれ7.9，35.5 μMであることが明らかにされた。一方，サンプルを経口投与する*in vivo*系ではneoandrographolideのみにNO産生抑制効果が認められ，andrographolideにはNO産生抑制効果が認められなかった。これらの実験結果から，*A. paniculata*の抗炎症効果にはneoandrographolideの方の寄与が大きい可能性が示唆された。

このように，本論文はNO産生抑制効果を指標とする方法が炎症性疾患に対する予防・治療薬を探索する際の初期スクリーニング法として有効であることを示している。よって，本論文は博士（薬学）の学位論文として合格と認める。