細胞資源工学

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本部門では，和漢薬資源の恒久的維持，育成を図るため，薬用生物に関する細胞工学的研究を行なうと同時に，動植物細胞の持つ遺伝情報を解析して，その薬用資源開発への応用，あるいは微生物および動物細胞を用いて生理活性物質の探索，和漢薬の薬効発現機構の解明を行なうことを目指している。本年度の主な研究テーマと成果は下記の通りである。

Ⅰ．腸内嫌気性菌によるバイオトランスフォーメーション

腸内細菌によるサイコサポニンの変換反応を検討し，*Eubacterium* sp. A-44, *Bifidobacterium* sp. Saiko-1, Saiko-2 などの菌株がサイコサポニンをプロサイコゲニンに変換することを見いだした。*Eubacterium* sp. A-44 からサイコサポニン加水分解酵素 (β-D-グルコシダーゼの一種)，プロサイコゲニン加水分解酵素 (β-D-フコシダーゼの一種) を精製し，その基質特異性，至適 pH などを調べた。

Ⅱ．腸内細菌による薬物の代謝活性化に関与する遺伝子

Sennoside の加水分解に関与する *Bifidobacterium* sp. 由来の酵素遺伝子の塩基配列を決定した。

Ⅲ．抗ウイルス薬の開発

霊芝およびその胞子から種々のラノスタン型のトリテルペンを単離し，それらの抗 HIV 作用，HIV-プロテアーゼ阻害作用を見いだした。また，韓国産，スーダン産の植物の抗 HIV 作用，HIV-プロテアーゼ阻害作用を検索した。

Ⅳ．腎疾患における病態の解明と治療薬の開発

活性酸素，フリーラジカルが直接的，間接的に腎疾患に関わっていることから，丹参から単離した magnesium lithospermate B，緑茶，大黄牡丹皮湯，温脾湯を中心に腎不全モデル，虚血-再灌流による腎障害の *in vitro* 評価系，cisplatin, cephaloridine による細胞障害系で検討し，その有効性を明らかにした。また漢方方剤，生薬，フラボノイドの抗酸化活性についても検討した。

◇ 著　書

1）服部征雄 (分担)：『漢方薬理学』高木敬次郎監修，木村正康編集，南山堂，東京，1997.
2）横澤隆子，大浦彦吉，西岡五夫：腎摘ラットで増加する活性酸素を magnesium lithospermate B が軽減した。「腎とフリーラジカルー第3集」加藤克巳，長瀬光昌監修，山田晴生，中村一路編，93-96，東京医学社，東京，1997.
3）横澤隆子，藤塚直樹，大浦彦吉，柏木　寛：腎不全ラットにおけるフリーラジカルの産生と消去能について。「腎とフリーラジカルー第3集ー」加藤克巳，長瀬光昌監修，山田晴生，中村一路編，114-117，東京医学社，東京，1997.

◇ 原　著

1）**Lim Y. A., Ma C., Kusumoto I. T., Miyashiro H., Hattori M., Gupta M. P. and Correa M.: HIV-1 Reverse Transcriptase Inhibitory Principles of *Chamaesyce hyssopifolia*. Phytother. Res. 11, 22-27, 1997.**

In the course of our studies on the development of anti-acquired immunodeficiency syndrome (AIDS) agents, we isolated corilagin, quercetin 3-*O*-β-D-

glucopyranoside and 1,3,4,6-tetra-*O*-galloyl-β-D-glucopyranose from *Chamaesyce hyssopifolia,* as the main inhibitory sbstances against human immunodeficiency virus (HIV) reverse transcriptase, an enzyme essential for the proliferation of HIV. The IC_{50} of these substances were 20, 50 and 86 μM, respectively, their modes of inhibition being non-competitive with respect to the substrate.

2) Helal A. M., Nakamura N., Meselhy R. M., El-Fishawy A. M., Hattori, M. and Mahran G. H.: Guaianolides from *Centaurea scoparia*. Phytochemistry, 45, 551-554, 1997.

3β, 8α-*O*-Di(4-hydroxytigloyl)-1αH, 5αH, 6βH, 7αH-guai-4(15), 10(14), 11(13)-triene-6,12-olide; a new guaianolide, has been isolated from the aerial parts of *Centaurea scoparia* together with nine known ones.

3) Heikal O. A., Miyashiro H., Akao T. and Hattori M.: Quantitative Determination of Paeoniflorin and Its Major Metabolites, Paeonimetabolin I, in the Rat Plasma by Enzyme Immunoassay. J. Trad. Med., 14, 15-19, 1997.

After oral administration of paeoniflorin (PF) at a dose of 20 mg/kg in rats, the plasma concentrations of PF and its major metabolite paeonimetabolin I (PM-I) were determined by using the enzyme immunoassay method. The plasma concentration of PF rapidly reached a C_{max} (95 ng/ml) at 30 min, then decreased to 0.2 ng/ml at 240 min after administration of PF. On the other hand, the plasma concentration of PM-I reached a C_{max} (400 ng/ml) at 140$\pm$24.7 min, then decreased to 0.5 ng/ml at 480 min. The *AUCs* were 7700$\pm$900 and 52400$\pm$17500 ng$\cdot$min$\cdot$ml^{-1}, for PF and PM-I, respectively, indicating that the latter was a major compound present in the plasma. These findings suggest that orally administered PF is scarcely absorbed from the gastrointestinal tract (GIT), while the unabsorbed one is transformed to PM-I by intestinal bacteria, which is subsequently absorbed from GIT. This is the first report showing the presence of PM-I in the plasma after oral administration of PF.

4) Lim Y. A., Kida H., Miyaji M., Kusumoto I. T., Miyashiro H., Hattori M., Shimotohno K., Gupta M. P. and Correa M.: Inhibitory Effects of Some Panamanian Plants on Human Immunodeficiency Viral Reverse Transcriptase and Protease. J. Trad. Med., 14, 54-58, 1997.

In the course of our studies on the development of anti-HIV agents, we have screened thirty-nine Panamanian plants for their inhibitory activity against HIV-1 reverse transcriptase (RT) and protease (PR), essential enzymes for proliferation of HIV. Water extracts of *Chamaesyce hyssopifolia* (whole plant), *Cordia spinescens* (leaves), and *Hyptis lantanifolia* (aerial parts), and the methanol extracts of *Tetrapteris macrocarpa* (aerial parts) and *Xylopia frutescens* (leaves) appreciably inhibited the activity of HIV-1 RT, with IC_{50} of 8, 6, 7, 8, and 11 μg/ml, respectively. Furthermore, the methanol extracts of *Erythroxylum citrifolium* (trunk), *Serjania mexicana* (whole plant), *Waltheria indica* (branches), and a water extract of *Lindackeria laurina* (leaves) showed appreciable inhibition against HIV-protease, with IC_{50} of 58, 48, 46, and 54 μg/ml, respectively.

5) Kida H., Nakamura N, Meselhy R. M., Akao T. and Hattori M.: Isolation and Identification of Human Intestinal Bacteria Capable of Hydrolyzing Saikosaponins. J. Trad. Med., 14, 34-40, 1997.

For studying biotransformation of saikosaponins by human intestinal bacteria, thin-layer chromatography-densitometric analysis was performed on the metabolites obtained by anaerobic incubation of saikosaponins with a human fecal suspension. It revealed that saikosaponins a, b_1, b_2 and d were converted to the corresponding prosaikogenins and saikogenins in order. In the case of saikosaponin c, saikogenin E was obtained as a sole product. Of 31 defined human intestinal bacterial strains, only *Eubacterium* sp. A-44. could metabolize saikosaponins a, and b_1 to the corresponding prosaikogenins and saikogenins. Saikosaponins d and b_2 were hydrolyzed to the respective prosaikogenins, but no saikosaponin c by this strain.

After screening bacterial colonies from fresh human feces for metabolizing activity of saikosaponins, two of 60 isolates showed appreciable

ability of hydrolyzing saikosaponins a, b_1, b_2 and d, except for c, to the corresponding prosaikogenins. However, both strains did not further hydrolyze the prosaikogenins to sakiogenins. They were identified as *Bifidobactereim* spp., named *Bifidobacterium* sp. Saiko-1 and Saiko-2, close species to *Bifidobacterium breve* ss *breve* and *Bifidobacterium adolescentis,* respectively.

6) Takeda S., Isono T., Wakui Y., Mizuhara Y., Amagaya S., Maruno M. and Hattori M.: *In Vivo* Assessment of Extrahepatic Metabolism of Paeoniflorin in Rats : Relevance to Intestinal Floral Metabolism. J. Pharm. Pharmacol., 49, 35-39 (1997).

The extraction ratios of paeoniflrin in gut wall (E_G), liver (E_H) and lung (E_L) were assessed by comparing AUCs after various routes of its administration to estimate the first-pass effects and the metabolism by intestinal flora.

Pulmonary extraction ratio of paeoniflorin was assessed by comparing AUCs calculated from venous and arterial plasma concentrations after its intravenous administration (0.5 mg kg^{-1}). The mean pulmonary extraction ratio was estimated to be 0·06. The hepatic extraction ratio (E_H was assessed by comparing AUCs after intraportal and intravenous administrations (0.5 and 5 mg kg^{-1}). The plasma concentration profiles of paeoniflorin after intraportal administration were very close to those after intravenous administration, suggesting a negligible hepatic extraction ratio of paeoniflorin. The AUC value after intraperitoneal administration (0.5 mg kg^{-1}) was greater than that after intraportal or intravenous administration. This finding suggests that paeoniflorin is not metabolized in the gut wall. The transference of paeoniflorin from the serosal side to the mucosal side was evaluated by the *in vitro* everted sac method. The low intestinal permeability (19.4 % at 60 min) was demonstrated by the comparison with phenobarbital (63.1 % at 60 min).

We conclude that paeoniflorin is not metabolized by gut wall, liver and lung, its poor absorption from the intestine results in extremely low bioavailability and the unabsorbed fraction of paeoniflorin is degraded by the intestinal flora.

7) Meselhy M. R., Nakamura N. and Hattori M.: Biotransformation of (-)-Epicatechin 3-*O*-Gallate by Human Intestinal Bacteria. Chem. Pharm. Bull., 45, 888-893, 1997.

The biotransformation of (-)-epicatechin 3-*O*-gallate (**1**) and related compounds was undertaken using a human fecal suspension. Of fifteen metabolites isolated, four compounds were new, namely, two epimers of 1-(3′-hydroxyphenyl)-3-(2″,4″,6″-trihydroxyphenyl)propan-2-ols (**6, 19**) ; 2″,3″-dihydroxyphenoxyl 3-(3′,4′-dihydroxyphenyl)propionate (**14**) and 1-(3′,4′-dihydroxyphenyl)-3-(2″,4″,6″-trihydroxyphenyl)propan-2-ol (**18**).

(-)-Epicatechin (**2**), (-)-epigallocatechin (**16**) and their 3-*O*-gallates (**1, 17**) were extensively metabolized by a human fecal suspension after incubation for 24 h, whereas the gallates (**1, 17**) resisted any degradation by a rat fecal suspension, even after a prolonged incubation time (48 h), suggesting a difference in metabloic ability between two intestinal bacterial mixtures from different species.

8) Arima T., Matsumoto J., Hori H., Kitamura Y., Namba T., Hattori M., Kadota S. and Nomura Y.: Ameliorating Effects of Calcium/Magnesium Lithospermate B on Cognitive Deficiencies in Senescence Accelerated Mouse. Kor. J. Gerontol, 7, 17-24, 1997.

Effect of long-term treatment with calcium/magnesium lithospermate B (LSB), a major ingredient of Dan-Shen (Salviae Miltiorrhizase Radix) methanol extract (DME), on memory and learning in the scenescence-accelerated mouse was investigated by maens of Morris's water maze task. LSB treatment significantly decreased both escape latency and path length in the P8 strain of senescence-accelerated mouse (SAMP8), which strain spontaneously develops learning deficits. These results suggest that LSB treatment improved spatial learning. In neurochemical investigation, no effect on choline acetyltransferse activity or [^{3}H]QNB (quinuclidinyl benzilate) binding was observed in the cortex and hippocampus. Similarly, no effect was observed in [^{3}H] MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo [a,b] -cyclohepten-5,10-imine maleate) binding, [^{3}H] NNA (N^G-nitro-L-arginine) binding, or [^{3}H] PDBu (phorbol 12,13-dibutyrate) binding

in the membrane fractions. However [^{3}H] PDBu binding in the cytosolic fraction was increased in the hippocampus. Thus our data suggest that long-term LSB treatment improves spatial learning in SAMP8. As to the mechanism, LSB treatment alters the hippocampal functions mediated by protein kinase C (PKC).

9) Heikal O. A., Akao T., Takeda S. and Hattori M.: Pharmacokinetic Studies of Paeonimetabolin I, a Major Metabolite of Paeoniflorin from Paeony Roots. Biol. Pharm. Bull., 20, 517-521, 1997.

Plasma concentrations of paeoniflorin (PF) and its major metabolite, paeonimetabolin I (PM-I), were estimated after oral administration of PF to rats at doses of 0.5 and 5 mg/kg. The maximal plasma concentrations (C_{max}) of PF were 9.9 and 20.3, and those of PM-I were 16.5 and 101.7 ng/ml at each dose, respectively. The times to C_{max} (t_{max}) of PF were 11.6 and 13.3, and those of PM-I were 60 and 80 min, respectively. The AUC_{0-180} of PM-I were 1873 and 12358, and those of PF were 300 and 1174 ng/ml, respectively.

On the other hand, after intravenous administration of PM-I to rats at doses of 0.2 and 2 mg/kg (equal in molar ratio to 0.5 and 5 mg/kg PF), the plasma concentration of PM-I decreased rapidly and the plasma concentration-time curve profile of it fitted well with the two-compartment model at each dose, with terminal half lives ($t_{1/2}$) of 90.9 and 90.6 min. The V_{dss} values were 0.91 and 3.79l/kg, the CL_{tot} values were 8.7 and 39.9 ml/min kg, and the AUC_{0-180} values were 5614.1 and 13176.0 ng min/ml, at each dose, respectively. The significant increase in V_{dss} and CL_{tot} with increasing doses suggested dose-dependent pharmacokinetics.

When PM-I was given orally at the same doses, the following parameters were shown: C_{max} of 102.2 and 285 ng/ml at t_{max} 6.2 and 7.5 min and $AUCs$ of 4145.6 and 14182.1 ng min/ml, at each dose. The bioavailability (F) values were 0.8 and 1.07, respectively.

These findings indicated that the high percentage of PM-I transformed by intestinal bacteria was rapidly absorbed from the gastrointestinal tract, and a significantly high concentration of PM-I, rather than PF, was present in the plasma after oral administration of PF.

10) Heikal O. A., Kanaoka M., Akao T., Hattori M.: Effects of Spacer Homologous and Heterologous Combinations on Enzyme Immunoassay for Paeonimetabolin I, a Major Metabolite of Paeoniflorin. J. Trad. Med., 14, 105-113 (1997).

In the course of developing enzyme immunoassay for paeonimetabolin I, a metabolite of paeoniflorin, we investigated the effects of spacer homologous and heterologous haptens on the sensitivity and specificity in the assay. Three paeonimetabolin-I derivatives possessing spacers of different length at C-8 were employed as haptens for the preparation of antisera and labeled antigens. These were 8-(carboxymethylthio) paeonimetabolin I (CMP), 8-(2′-carboxyethylthio) paeonimetabolin I (CEP) and 8-(5′-carboxypentylthio) paeonimetabolin I (CPP). The N-succinimide esters of the respective carboxythio derivatives were coupled with β-galactosidase (β-Gal) and bovine serum albumin (BSA) to give enzyme-labeled antigens and BSA conjugates (immunogens). Antisera 1CEP and 2CPP were prepared by immunization of rabbits with the CEP- and CPP-BSA conjugates. The sensitivity was higher in heterologous combinations using the labeled antigens linked with a shorter spacer arm than that used for antibody production. The cross reactivities of 2CPP with the paeonimetabolin-I related compounds were higher than those of 1CEP.

11) Nakamura N., Kojima S., Lim Y. A., Meselhy R. M., Hattori M., Gupta M. P. and Correa M.: Dammarane-type Triterpenes from *Cordia spinescens*. Phytochemistry, 46, 1139-1141 (1997).

Two new triterpenes, 3α,6β,25-trihydroxy-20(S),24(S)-epoxydammarane (**1**) and 3α-acetoxy-6β,25-dihydroxy-20(S),24(S)-epoxydammarane (**2**), were isolated from the methanol extract of the leaves of *Cordia spinescens*, together with cabraleadiol (**3**).

12) Matsuse I. T., Nakabayashi T., Lim Y. A., Hussein G. M. E., Miyashiro H., Kakiuchi N., Hattori M., Stargeo S. and Shimotohono K.:

A human Immunodeficiency Virus Protease Inhibitory Substance from *Swietenia mahagoni*. *Phytother*. Res., 11, 433-436, 1997.

For the purpose of finding anti-HIV agents from natural sources, various plant extracts were screened for their inhibitory activity against HIV-protease, and on enzyme essential for viral proliferation. By bioassay-directed fractionation of the methanol extract of *Swietenia mahagoni* (bark) which had shown inhibitory activity against this enzyme, we isolated and identified chlorogenic acid methyl ester as a inhibitory substance, its 50 % inhibitory concentration being less than 40 μg/ml.

13) **Lim Y. A., Kojima S., Nakamura N., Miyashiro H., Fushimi H., Komatsu K., Hattori M., Shimotohno K., Gupta M. P., Correa M. : Inhibitory Effects of *Cordia spinescens* Extracts and Their Constituents on Reverse Transcriptase and Protease from Human Immunodeficiency Virus. Phytother. Res., 11, 490-495, 1997.**

By bioactive-guided fractionation of a water extract of *Cordia spinescens,* magnesium lithospermate (**1**), calcium rosmarinate (**2**) and magnesium rosmarinate (**3**) were isolated as potent inhibitory substances against HIV-1 reverse transcriptase (RT) with IC_{50} values of 0.8, 5.8 and 3.1μM, respectively. However, they were weak HIV-1 protease (PR) inhibitors with $IC_{50}>100\ \mu$M. The RT inhibition by these compounds was noncompetitive with respect to dTTP substrate.

14) **Kida K., Akao T., Meselhy M. R. and Hattori M : Enzymes Responsible for the Metabolism of Saikosaponins from *Eubacterium* sp. A-44, a Human Intestinal Anaerobe. Biol. Pharm. Bull., 20, 1274-1278, 1997.**

From a human intestinal bacterium, *Eubacterium* sp. A-44, which is capable of hydrolyzing saikosaponins to saikogenins, two glycosidases, β-D-glucosidase and a novel type of β-D-fucosidase, were isolated and characterized as saikosaponin-hydrolyzing β-D-glucosidase and prosaikogenin-hydrolyzing β-D-fucosidase.

Relative to the hydrolyzing activities toward saikosaponins a, b_1 and b_2, the β-D-glucosidase showed lower ability to hydrolyze saikosaponin d, but no ability to hydrolyze saikosaponin c or prosaikogenins.

By Sephacryl S-300 column chromatography, the molecular weight of prosaikogenin-hydrolyzing β-D-fucosidase was estimated to be about 130 kDa. The β-D-fucosidase could hydrolyze prosaikogenins A and F, but not prosaikogenins D and G or saikosaponins. Relative to *p*-nitrophenyl β-D-fucoside-hydrolyzing activity, this enzyme had 32.0 % and 22.2 % of its hydrolyzing ability toward *p*-nitrophenyl β-D-glucoside and *p*-nitrophenyl β-D-galactoside, respectively. *p*-Nitrophenyl β-D-fucoside-hydrolyzing activity was inhibited by D-fucose, and was weakly inhibited by D-glucose, D-glucono δ-lactone, D-galactose and D-galactono δ-lactone.

By combining these two glycosidases, saikosaponins a and b_1 were converted to their saikogenins *via* the corresponding prosaikogenins.

15) **Saito K., Oku T., Ata N., Miyashiro H., Hattori M., and Saiki I. : A Modification and Conventional Method for Assessing Tumor Cell Invasion and Migration and Its Application to Screening Inhibitors. Biol. Pharm. Bull., 20, 345-348 (1997).**

In order to screen potent inhibitors of tumor invasion and metastasis, we here devised a simple and reproducible *in vitro* assay for tumor invasion and migration. A conventional cell-counting assay using a Transwell chamber with a microporous membrane filter is troublesome and time-consuming, involving visually counting the cells under a microscope, and the invaded or migrated cells are sometimes distributed unevenly in predetermined fields on the lower surface of the filter. Therefore, it is difficult to evaluate the invasive and migratory abilities of tumor cells easily and quantitatively by the cell counting method. In the present study, crystal violet dye was used for staining the invaded cells and colorimetrically assessing the invasive ability per filter as an absorbance. In this crystal violet assay, tumor cell invasion into a reconstituted basement membrane Matrigel was proportional to both the cell number added into the chamber and the incubation period, and inversely proportional to the amount of Matrigel barrier on the upper surface of filter. The results obtained by this dye-uptake

method were highly consistent with those of a conventional cell-counting assay. Using this crystal violet assay, the anti-invasive effect of doxorubicin (DOX) was detected more easily and found to be highly proportional to that by the conventional cell-counting method. We therefore applied this convenient assay method to screen anti-invasive and anti-metastatic compounds. As a result, caffeic acid was found to be more active in the inhibition of both tumor cell invsion and migration without showing direct cytotoxicity *in vitro* than other related compounds.

16) Yokozawa T., Dong E., Oura H., Kashiwagi H., Nonaka G. and Nishioka I.: Magnesium Lithospermate B Suppresses the Increase of Active Oxygen in Rats after Subtotal Nephrectomy. Nephron, 75, 88-93, 1997.

Subtotally nephrectomized rats were found to have decreased activities of superoxide dismutase (SOD) and catalase, and spin trapping with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) showed that the amount of hydroxyl radical in the residual kidney tissue was greater than that in normal rat kidney. This indicated both direct and indirect involvement of free radicals in renal failute. In contrast, rats given magnesium lithospermate B (10 mg/kg body weight) orally for 30 days after subtotal nephrectomy showed restoration of SOD and catalase activities to almost normal levels. Hydroxyl radical, which is highly reactive and for which there is no scavenger system in the body, was decreased markedly in kidney homogenates obtained from rats given magnesium lithospermate B and in an experimental system for hydroxyl radical production to which magnesium lithospermate B was directly added. The increased levels of uremic toxins in the blood were also low in rats given magnesium lithospermate B. This indicates that magnesium lithospermate B helps to inhibit the progression of renal failure by scavenging radicals.

17) Yokozawa T., Fujitsuka N., Oura H., Ienaga K. and Nakamura K.: *In vivo* Effect of Hydroxyl Radical Scavenger on Methylguanidine Production from Creatinine. Nephron, 75, 103-105, 1997.

Both the serum and urinary creatol/Cr and creatol+MG/Cr molar ratios were markedly higher in rats given Cr than in control rats, indicating an increased rate of conversion of Cr to creatol and MG (increased production of creatol and creatol+MG) under Cr loading. In contrast, when DMTU was administered in advance, the serum creatol level decreased in a dose-dependent manner, the level being 74 % lower in rats given a dose of 100 mg/kg body weight than in controls. The urinary excretion of creatol was also decreased in rats given DMTU, but unlike the serum levels there was no dose dependency. Since there were similar changes in MG, it was speculated that DMTU blocked the production of creatol from Cr or production of creatol and MG from Cr. However, there were no significant changes in the molar ratio of MG/creatol except for urinary MG/creatol in rats given 50 mg/kg, indicating that DMTU inhibited the production of creatol from Cr. Thus, it is apparent that ·OH is involved in the production of creatol from Cr. The creatol/Cr molar ratio may serve as an indirect index of the amount of ·OH generated in the body.

18) Yokozawa T., Dong E., Chung H.Y., Oura H. and Nakagawa H.: Inhibitory Effect of Green Tea on Injury to a Cultured Renal Epithelial Cell Line, LLC-PK_1. Biosci. Biotech. Biochem., 61, 204-206, 1997.

When cells from a cultured renal epithelial cell line, LLC-PK_1, were cultured under hypoxic conditions (oxygen concentration of 2 % or less) before reoxygenation was applied (95 % air, 5 % CO_2), the leakage of lactate dehydrogenase (LDH) into the medium increased. This phenomenon was inhibited in the presence of dimethyl sulfoxide, a hydroxyl radical scavenger, suggesting the involvement of free radicals. Such oxidative stress was significantly inhibited by a green tea extract, and more potently by a tannin mixture. On the other hand, under ordinary culture conditions (95 % air, 5 % CO_2), there was cell injury, although the LDH leakage was less than that under hypoxia/reoxygenation, and such injury was inhibited by the green tea extract and the tannin mixture.

19) Yokozawa T., Dong E. and Oura H.: Proof that Green Tea Tannin Suppresses the

Increase in the Blood Methylguanidine Level Associated with Renal Failure. Exp. Toxic. Pathol., 49, 117-122, 1997.

The effects of a green tea tannin mixture and its individual tannin components on methylguanidine were examined in rats with renal failure. The green tea tannin mixture caused a dose-dependent decrease in methylguanidine, a substance which accumulates in the blood with the progression of renal failure. Among individual tannin components, the effect was most conspicuous with (-)-epigallocatechin 3-*O*-gallate and (-)-epicatechin 3-*O*-gallate, while other components not linked to gallic acid showed only weak effects. Thus, the effect on methylguanidine was found to vary among different types of tannin.

20) Yokozawa T., He L.Q., Muto Y., Nagasaki R., Hattori M. and Oura H.: Effects of Rhubarb Extract in Rats with Diabetic Nephropathy. Phytother. Res., 11, 73-75, 1997.

Rhubarb extract was given orally to rats with diabetic nephropathy induced by subtotal nephrectomy and injection of streptozotocin. As a result, high blood and urinary glucose levels, which were conspicuous in similarly treated rats that were not given rhubarb extract, were ameliorated. Furthermore, improvement of hyperlipidaemia, and accelerated excretion of urinary urea nitrogen and creatinine were observed. These findings indicate that rhubarb extract has potential as a new therapeutic agent for inhibiting the progression of diabetic nephroparhy.

21) Yokozawa T., Dong E., Liu Z.W. and Oura H.: Antiperoxidation Activity of Traditional Chinese Prescriptions and Their Main Crude Drugs *in Vitro*. Nat. Med., 51, 92-97, 1997.

The antiperoxidation activities of 80 traditional Chinese prescriptions and 30 of their constituent crude drugs were studied in detail using a lipid peroxidation generation system mediated by addition of H_2O_2/Fe^{2+} to rat liver homogenate. It was found that the prescriptions with the greatest protective activity against peroxidation were, in order to effectiveness, Daio-botampi-to, Dai-saiko-to, Mashi-nin-gan, Otsuji-to, Saiko-keishi-kankyo-to, Shakuyaku-kanzo-to and Shishi-hakuhi-to, of which the first 5 showed relatively lower IC_{50} values. In an addition, among the crude drugs tested, Glycyrrhizae Radix, Rhei Rhizoma, Scutellariae Radix, Cinnamomi Cortex and Ephedrae Herba showed the strongest inhibition of lipid peroxidation formation. Almost all the prescriptions which had antioxidant effects were composed of one or more of these crude drugs.

22) Dong E., Yokozawa T., Liu Z.W., Oda S., Muto Y., Hattori M. and Watanabe H.: Protective Effects of Daio-botampi-to and Its Three Major Components on Rat Kidney and Renal Proximal Tubule Cells Subjected to Ischemia (Hypoxia)-Reperfusion (Reoxygenation). J. Trad. Med., 14, 41-48, 1997.

The effects of Daio-botampi-to (大黄牡丹皮湯) and its three major components-Rhei Rhizoma, Moutan Cortex and Persicae Semen-in ischemic-reperfused rats were examined. When Daio-botampi-to and Rhei Rhizoma were given orally at a dose of 200 mg/kg body weight/day for 20 consecutive days prior to ischemia and reperfusion, the conversion of xanthine dehydrogenase (XDH) to xanthine oxidase (XOD) was inhibited. In addition, both were able to sustain or increase the activities of the antioxidation enzymes superoxide dismutase, catalase and glutathione peroxidase, while malondialdehyde levels in the serum and renal tissue were lower. Decreased levels of urea nitrogen and creatinine in serum demonstrated a protective action against the renal dysfunction caused by ischemia and recirculation. On the other hand, it was demonstrated that Daio-botampi-to and Rhei Rhizoma could affect proximal tubule cells cultured under hypoxia-reoxygenation, probably by preventing oxygen free radicals from attacking cellular membranes. Although Moutan Cortex and Persicae Semen did not produce the same effects, being blood circulation-facilitating agents, it is believed that their action may occur through another mechanism, i.e. improving impaired reflow of blood on recirculation, resulting in prolonged ischemia.

23) Yokozawa T., Liu Z.W., Shibata T. and Hasegawa M.: Role of Oriental Medicines in the Treatment of Acute Renal Failure : Carthami Flos, Rhei Rhizoma and Astragali

Radix. J. Trad. Med., 14, 49-53, 1997.

Rats with renal failure induced by injection of glycerol show increased levels of urea nitrogen and creatinine (Cr) in serum and decreased creatinine clearance (Ccr). Fractional excretion of sodium (FE_{Na}) increases with the increase in urinary volume, while urinary osmotic pressure decreases. In glycerol-treated rats, Carthami Flos, Rhei Rhizoma and Astragali Radix all caused a decrease in urea nitrogen, Cr and FE_{Na}. In addition, Carthami Flos increased the urinary osmotic pressure, Rhei Rhizoma decreased the urinary volume, and Astragali Radix increased the Ccr, suggesting that these drugs ameliorate the abnormalities of acute renal failure through different modes of action.

24) Yokozawa T., Dong E., Chung H.Y., Oura H. and Nishioka I.: A Protective Action of Magnesium Lithospermate B on Renal Function after Ischemia-Reperfusion of Rats and Cultured Proximal Tubular Cells. Nat. Med., 51, 287-292, 1997.

The effect of magnesium lithospermate B in ischemic-reperfused rats was examined. In the control rats, blood and renal parameters and the activities of antioxidative enzymes in the renal tissue deviated from the normal range, indicating dysfunction to the kidneys. In contrast, when magnesium lithospermate B was given orally at dose of 10 mg/kg body weight/day for 30 consecutive days prior to this ischemia and reperfusion, the creatinine levels in the serum and renal tissue were lower, while guanidinoacetic acid levels were higher in the treated rats compared to controls; in addition, superoxide dismutase and catalase activity in the renal tissue remained almost as high as that in normal untreated rats. This indicates that the improvement in renal function caused by magnesium lithospermate B is closely related to its antiperoxidation activity, which was confirmed by electron spin resonance findings. In an *in vitro* model with LLC-PK_1 cells exposed to hypoxia and reoxygenation incubation, magnesium lithospermate B effectively inhibited the leakage of lactate dehydrogenase from the cells, and the formation of malondialdehyde, in a concentration-dependent pattern. Results from both *in vivo* and *in vitro* studies have indicated that magnesium lithospermate B can not only protect but also prevent renal dysfunction as a result of ischemia-reperfusion by inhibiting lipid peroxidation. In addition, its membrane-repairing effect suggests that the proximal tubule is one of its possible sites of action.

25) Yokozawa T., Dong E., Kashiwagi H., Kim D. W., Hattori M., Kadota S. and Namba T.: *In Vitro* and *in Vivo* Studies on Anti-Lipid Peroxidation Effect of Extract from Luobuma Leaves. Nat. Med., 51, 325-330, 1997.

Three Luobuma extracts prepared from *Apocynum venetum* L. were investigated for their antioxidant activities using non-enzymatic and enzymatic oxygen free radical generation systems *in vitro,* and an *in vivo* system using animals treated with carbon tetrachloride. The results demonstrated that Luobuma leaf extract I efficiently inhibited the lipid peroxidation reaction generated either non-enzymatically or enzymatically *in vitro.* Spin-trapping also revealed the same ability. In the *in vivo* experiment, a significant decrease in thiobarbituric acid-reactive substance in serum and liver was recognized after treatment with Luobuma leaf extract III. The activities of superoxide dismutase, catalase and glutathione peroxidase were also higher in rats given Luobuma leaf extract III.

26) Liu Z.W., Yokozawa T., Dong E. and Yamamura H.: Effects of Daio-botampi-to and Its Component Drugs on Cephaloridine-Induced Renal Injury. J. Trad. Med., 14, 129-135, 1997.

Daio-botampi-to (大黄牡丹皮湯) has been proved to decrease the severity of renal injury induced by cephaloridine, in which proximal uriniferous tubules represent the main site of injury. Variations in the nitrite/nitrate ratio and the activity of radical scavenger enzymes also suggested the protective effect of Daio-botampi-to against oxygen stress. In addition, a cell-protective effect on a cultured renal epithelial cell line, LLC-PK_1, was observed. These effects are considered to result from combined actions of the component drugs of this preparation, i.e., Rhei Rhizoma, Moutan Cortex and Persicae Semen.

27) Yokozawa T., Dong E., Liu Z.W. and Shimizu

M.: Antioxidative Activity of Flavones and Flavonols *in Vitro*. Phytother. Res., 11, 446–449, 1997.

Forty-one flavonoids of the flavone and flavonol types were investigated for their antioxidative activity using a lipid peroxidation generation system mediated by the addition of H_2O_2/Fe^{2+} to rat liver homogenate. The results showed that both types specifically and markedly reduced the production of peroxidants induced by H_2O_2, Fe^{2+} or a Fenton-type reaction. The scavenging ability of these compounds was found to be associated closely with their chemical structure, especially the number of hydroxyl groups linked to the basic skeleton. However, there were some exceptions, implying that not only hydroxyl groups but also their configuration determines the activity.

28) Yokozawa T., Dong E., Oura H., Nishioka I., Kawai Y. and Gemba M.: Protective Effects of Wen-Pi-Tang against Cultured Renal Epithelial Cellular Injury. Phytomedicine., 4, 245–250, 1997.

A study was conducted to clarify whether extracts of Wen-Pi-Tang and its component crude drugs ameliorate renal cellular injury by assaying lactate dehydrogenase and malondialdehyde leakage from LLC-PK_1 cells in culture. The cells were cultured with various concentrations of the samples under two sets of conditions: routine and hypoxia-reoxygenation. The results demonstrated that Wen-Pi-Tang, Rhei Rhizoma, Glycyrrhizae Radix exerted marked protective effects on the cells; Ginseng Radix showed moderate activity, whereas Zingiberis Rhizoma and Aconiti Tuber had virtually no such effect. Two pure compounds, epicatechin 3-*O*-gallate and licochalcone A isolated from Rhei Rhizoma and Glycyrrhizae Radix, respectively, exerted the same marked effects as the parent crude drugs. In the light of these findings, we concluded that Wen-Pi-Tang and its major components protect renal epithelial cells against injury mediated by hypoxia-reoxygenation and/or prevent such injury. The primary mechanism of these effects appeared to be antilipid peroxidant activity present in the preparation.

29) Yokozawa T. and Dong E.: Influence of Green Tea and Its Three Major Components upon Low-Density Lipoprotein Oxidation. Exp. Toxic. Pathol., 49, 329–335, 1997.

The abilities of green tea extract and its three major components to inhibit lipid peroxidation in low-density lipoprotein (LDL) catalyzed by copper were tested *in vitro* using malondialdehyde as a parameter of antioxidant activity. The results demonstrated that green tea extract markedly delays peroxidation with a dose-dependent pattern. Of the three components, polyphenols had the strongest action. Similar action was also shown in the theanine-treated group but was weaker than in the former, whereas caffeine had a very limited effect. Based on these data, it is concluded that green tea extract can effectively inhibit peroxidation and that this activity is due largely to the polyphenols it contains. According to the ultraviolet spectra, copper chelation is suggested to be one of the possible mechanisms of LDL antiperoxidation.

30) Yokozawa T., Dong E., Liu Z.W., Shibata T., Hasegawa M., Watanabe H. and Oura H.: Magnesium Lithospermate B Ameliorates Cephaloridine-Induced Renal Injury. Exp. Toxic. Pathol., 49, 337–341, 1997.

To determine whether magnesium lithospermate B ameliorates renal injury induced by cephaloridine, the effect of cephaloridine was investigated in rats given magnesium lithospermate B for 20 days preceding cephaloridine administration and in control rats given no magnesium lithospermate B. In the control rats, blood and urinary parameters and the activity of radical-eliminating enzymes in the renal tissue deviated from the normal range, indicating damage to the kidneys. In contrast, rats given magnesium lithospermate B showed decreased urine volume, increased urinary osmotic pressure, and decreased urinary levels of glucose, protein, sodium and potassium, denoting less damage to the kidney. In this group, the urinary nitrite/nitrate ratio, and the activities of superoxide dismutase and catalase in the renal tissue were increased, while the malondialdehyde levels were decreased, suggesting the involvement of radicals in the normalizing of kidney function. The increased levels of urea nitrogen in the blood of rats with induced renal failure

were also lowered by administering magnesium lithospermate B.

31) Yokozawa T., Dong E., Oura H., Nonaka G. and Nishioka I.: Magnesium Lithospermate B Ameliorates Cisplatin-Induced Injury in Cultured Renal Epithelial Cells. Exp. Toxic. Pathol., 49, 343-346, 1997.

A study was conducted to clarify whether magnesium lithospermate B ameliorates cisplatin-induced renal injury in terms of lactate dehydrogenase and malondialdehyde leakage from LLC-PK_1 cells in culture. Magnesium lithospermate B was shown to suppress the cytotoxicity of cisplatin, the suppressive effect increasing with the dose of magnesium lithospermate B.

◇ 総　説

1) 服部征雄：腸内嫌気性菌による代謝反応について，Methods in Kampo Pharmacology, Vol. 1, 15-22, 1997, ライフサイエンスメディカ，東京。

2) 服部征雄：LC/MS およびEIAによる微量代謝物の分析，Methods in Kampo Pharmacology, Vol. 2, 101-110, 1997, ライフサイエンスメディカ，東京。

3) Chung H.Y., Shim K.H., Song S.H., Huh J.I., Kim Y.J., Kim K.W., Im K.S., Lee K.H. and Yokozawa T.: Action Mechanisms of Ginseng against Oxidative Stress and Aging. Progress in Medicine, 17, 903-914, 1997.

◇ 学会報告

1) 来田浩明，服部征雄，赤尾光昭：柴胡サポニンの代謝に関与するヒト腸内細菌由来の酵素について．日本薬学会第117年会，1997，3，町田．

【目的】 柴胡の成分であるサイコサポニンはヒト腸内細菌叢により容易に代謝される。しかし，ヒト腸内分離菌株中1株（*Eubacrerium* sp. A-44）およびヒト糞便より2菌種（*Bifidobacterium* sp. Saiko-1, Saiko-2）のみにその代謝活性が検出されたにすぎない。後者の2種はサイコサポニンをプロサイコゲニンに代謝したが，*E.* sp. A-44はプロサイコゲニンを経てサイコゲニンまで代謝した[1]。今回，*E.* sp. A-44のサイコサポニンの代謝に関与する酵素について検討したので報告する。

【実験・結果】 上記の3菌種ともβ-D-グルコシダーゼ活性を有していたが，*E.* sp. A-44にのみに強いβ-D-フコシダーゼ活性が認められた。*E.* sp. A-44の粗抽出液のButyl-Toyopearl 650Mカラムクロマトグラフィーによってこの両活性は分離され，それぞれ2つずつのピークとして得られた。2つのβ-D-グルコシダーゼフラクションの内1つがサイコサポニンaをプロサイコゲニンFに水解し，2つのβ-D-フコシダーゼフラクションの内1つがプロサイコゲニンFをサイコゲニンFに水解した。ヒト腸内においてサイコサポニンはこれらの酵素の協同作用によりゲニンにまで代謝されると考えられる。

【文献】

1) 来田ら，第13回和漢医薬学会大会要旨集　p. 83 (1996)

2) A. Nawawi, 中村憲夫 G. Hussein, A. S. Ahmed, 服部征雄, 黒川昌彦, 白木公康：Screening of Traditional Medicinal Plants for Anti-Herpes Simplex Virus Type 1 (HSV-1) Activity. 日本薬学会第117年会．1997，3，町田．

【Purpose】 For investigating naturally occuring antiviral agents, various traditional medicinal plants were examined for antiviral activity against herpes simplex virus type 1 (HSV-1) by plaque formation assay.

【Experimental】 Plaque formation assay was performed using monolayer cells in 6.0 cm diameter dish infected with Seibert strain at 100 plaque forming units (PFU) per dish, and using methyl cellulose (0.8 %) medium overlay with 100 μg/ml of the samples. The anti-HSV-1 activity of each test sample was determined as the percent plaque formation of control.

【Result】 Of 57 traditional medicinal plants collected in Indonesia, Egypt and Sudan, the water and/or methanol extracts of 11 plants i.e. *Acacia nilotica* (pods), *Eurycoma longifolia* (stem), *Eucalyptus alva* (fruits), *Filicium decipiens* (stem bark), *Haplophyllum tuberculatum* (roots and aerial parts), *Garcinia mangostana* (leaves), *Melaleuca leucadendra* (fruits), *Nephelium lappaceum* (pericarp), *Punica granatum* (pericarp), *Schleichera oleosa* (fruits), and *Toona sureni* (leaves), showed potent inhibitory activity. The methanol extract of *Toona sureni* and the tannins fraction isolated thereof showed potent HSV-1 inhibitory activity (IC_{50} 38 μg/ml and 15 μg/ml, respectively).

3) 金　東郁，横澤隆子，服部征雄，門田重利，難波

恒雄：LDLの酸化における羅布麻葉（*Apocynum venetum* L.）の関与．日本薬学会第117年会，1997，3，町田．

【目的】 動脈硬化症の重要な因子として，酸化低密度リポタンパク(酸化LDL)が注目されている。筆者らは昨年の本大会において，羅布麻葉水エキスに高コレステロール血症低下作用を有することを報告したが，[1] 本研究ではLDLの酸化における関与を検討した。

【方法】 Wistar系雄性ラット（体重約170 g）を用い，高コレステロール食（1.0 % cholesterol, 0.5 % cholic acid）で20日間飼育した。その間，中国山東省で採取した羅布麻葉の未焙煎ならびに2回焙煎した水エキス（投与量，70 mg/rat/day）を経口投与した。各種コレステロールは比色法で定量した。LDLは超遠心分離法にて分離し，0.15M NaCl溶液（pH 7.4, 4℃）で透析した。LDLの酸化はKuzuyaらの方法に従い，透析したLDLに10 μM $CuSO_4$を添加して，37℃で4時間インキュベーション後，生成したチオバルビツール酸反応物質（TBARS）をNaitoらの方法で測定した。

【結果】 羅布麻水エキスはLDLの酸化を著しく抑制し，この作用は焙煎したエキスで強力であった。また，血清遊離コレステロールとLDL-コレステロール，TBARSレベルの有意な低下と焙煎エキスにおいて肝組織中の総コレステロール量が有意に低下していた。

【結果】 羅布麻葉水エキスがLDLの酸化を抑制し，動脈硬化進展の予防に有用である可能性が示唆された。

【文献】

1）金　東郁ら。羅布麻（*Apocynum venetum* L.）に関する研究。－高コレステロール食投与ラットを用いての検討－。日本薬学会，第116年会，講演要旨集2，192P．1996.

4）横澤隆子，武藤靖子，董　而博，劉　中武，服部征雄：酸素ストレスにおける甘草の役割．日本薬学会第117年会，1997，3，町田．

【目的】 甘草の腎における役割を虚血-再灌流障害ラットと低酸素・再酸素化を施した培養腎上皮細胞を用い検討した。

【方法】 1. 動物実験：Wistar系雄性ラット（体重150 g前後）に甘草エキスを62.5と125 mg/kg体重/日をそれぞれ経口投与した。40日後に左腎動・静脈，尿管を45分間遮断，その後2時間再灌流し，腹部下大動脈から採血した。次いで氷冷した生理食塩水で灌流後，腎臓を採取し，血清成分，腎組織中のMDA，ラジカル消去酵素活性を測定した。2. 細胞培養実験：LLC-PK_1細胞をD-MEM/F-12に5 % FCSを添加した培地で培養し，コンフルエンスに達してから細胞を1穴あたり10^4個ずつseedした。2時間後にサンプルを添加し，41時間培養した。次いで低酸素下（酸素濃度2 %未満）で6時間培養後，通常の条件下（95 % air, 5 % CO_2）で1時間培養することで再酸素化した。細胞障害の指標として培地中へ漏出したLDH活性を測定した。

【結果】 虚血-再灌流後のblood urea nitrogen，血中MDAレベルは甘草エキス投与群で有意に低下していた。一方，虚血-再灌流を施した左腎のラジカル消去酵素活性は62.5 mg投与群では有意な低下を示したが，125 mg投与群では対照群とほとんど変わらず，右腎のSOD, catalase活性は逆に有意に上昇していた。両方の腎のMDAレベルはいずれも低下していたが，特に125 mg投与群で著しく低下，培養細胞実験においても甘草エキスに細胞障害を抑制する知見が得られた。

【結論】 甘草に酸素ストレスを軽減する作用が認められた。

5）Park, H. Miyashiro, M. Hattori, T. Hatano, T. Yoshida：薬用植物抽出物および化合物のHIV-1プロテアーゼ抑制活性研究，1997年度大韓薬学会，1997，4，大邱，韓国．

6）横澤隆子，大浦彦吉：大黄牡丹皮湯の腎における関与．第40回日本腎臓学会学術総会，1997，5，新潟．

【目的】 大黄牡丹皮湯並びにその主構成生薬の大黄，牡丹皮，桃仁の作用を検討した。

【方法及び結果】 腎の虚血-再灌流前に大黄牡丹皮湯あるいは大黄を20日間経口投与（200 mg/kg体重/日）した群において，XDHのXODへの変換を抑制，血清並びに腎組織中のMDAレベルの低下，SOD, catalase, GSH-Px活性の上昇を認めた。またblood urea nitrogen, s-Crレベルの低下も認め，虚血-再灌流障害に対する保護作用を有していた。一方，培養腎上皮細胞LLC-PK_1における低酸素-再酸素化による細胞障害を大黄牡丹皮湯と大黄が軽減した。しかし牡丹皮，桃仁にはこのような作用は認められなかった。

【結論】 大黄牡丹皮湯に虚血-再灌流障害を軽減する作用が認められ，その作用発現に大黄が重要な役割を担っていることが示唆された。

7）横澤隆子，大浦彦吉，河合悦子，玄番宗一：培養腎上皮細胞LLC-PK_1の細胞障害を緑茶が軽減した．第40回日本腎臓学会学術総会，1997，5，新潟．

【目的】 腎不全ラットにおける酸化的ストレス状態の緩和作用が認められている緑茶の効果について，ブタ腎由来培養上皮細胞株を用い検討した。

【方法】 LLD-PK_1細胞を用いた。細胞障害の指標とし

て培地中へ漏出するLDH活性を測定した。

【結果】 LLC-PK_1細胞を低酸素下で培養後，再酸素化を試みた結果，培地へのLDH漏出は顕著に増加した。一方，緑茶エキス（終濃度1.25 μg）をあらかじめ添加した場合，再酸素化による酵素の漏出は有意に抑制され，添加量を増加した場合，これら作用はさらに増強した。エキスで認められた作用はタンニンでも認められ，特に添加量を増加した場合に，その作用はエキスより強力であった。しかしこのような作用はcaffeineでは認められず，theanineによる作用は非常に弱かった。

【結論】 腎上皮細胞障害を緑茶エキス並びにタンニンが軽減した。

8) Ienaga K., Mikami H., Nishibata R., Nakamura K. and Yokozawa T.: NZ-419, A Novel Intrinsic Anti-Oxidant, and Its Inhibitory Effect on the Progression of Chronic Renal Failure in Rats. XIVth International Congress of Nephrology, 1997, 5, Sydney.

NZ-419, 5-hydroxy-1-methylhydantoin, is a newly recognized intrinsic anti-oxidant. We investigated its inhibitory effect on progression of the adenine-induced chronic renal failure (CRF) in rats. The effect was evaluated three weeks after adenine-loading when the GFR value decreased to ca. 5-10 % of normal one. First, NZ-419 was administered from the beginning: NZ-419 prevented the initiation and/or progression of CRF clearly, because the decrease in creatinine (Cr) clearance was significantly inhibited and levels of biochemical parameters in serum were significantly retained: serum Cr level was restored significantly in a dose dependent manner, and serum calcium and phosphate levels were maintained in normal levels. Moreover, an inhibitory effect of NZ-419 on the progression of CRF was also observed by two weeks administration using the adenine-induced CRF rats whose GFR has been already decreased into ca. 25-50 % of normal values. Similar effects of NZ-419 showed significant decreases in serum concentrations of Cr, MG, GSA and creatol® (CTL). The molar ratio CTL/Cr, an index of hydroxyl radical (oxidative stress) *in vivo,* in both serum and urine decreased significantly by NZ-419, indicating that this agent decreased oxidative stress. Our results indicate that NZ-419, an anti-oxidant, prevents and inhibits the progression of CRF in rats.

9) Ienaga K., Nakamura K., Yokozawa T., Aoyagi K. and Nakano K.: Oxidative Metabolic Pathways of Creatinine to Form NZ-419 and Creatol in Chronic Renal Failure. XIVth International Congress of Nephrology, 1997, 5, Sydney.

During our screening for low-molecular weight bio-active substances accumulated in mammals under stress conditions, we isolated 5-hydroxy-1-methylhydantoin (NZ-419) first from the inflamed skin tissues and then from urine of patients with chronic renal failure (CRF) together with creatol® (CTL: 5-hydroxycreatinine). We report herein discovery of two new oxidative pathways for Cr in mammals: Cr ⇒ NZ-419 ⇒ methylurea and Cr ⇒ CTL⇒ methylguanidine (MG). First, we show that ca. 0.2 % of Cr in normal subjects was catabolized in each pathway. Second, we show that CTL level and molar ratio CTL/Cr in both serum and urine increased up to ca. 2 % in CRF patients, and both indices in serum were highly correlated with renal dysfunction. Third, we show that serum NZ-419 levels of patients with CRF were significantly higher than those of normal subjects, but any correlation with severity of CRF could not be found. Since we have already shown that CTL levels and the ratio CTL/Cr in physiological fluids can be indices for oxidative stress and/or hydroxyl radical generation *in vivo,* our results show that oxidative stress increases in proportion to the severity of CRF. If we take the notion that oxidative stress can be one of promoting factors for the progression of CRF, some safe anti-oxidants could be candidates for medicines against CRF. We are now evaluating NZ-419 as such a candidate.

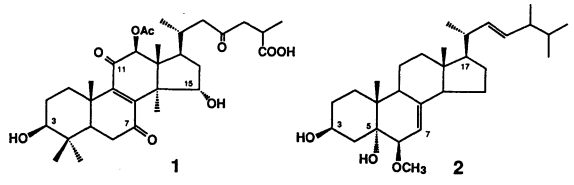
10) S. El-Mekkawy, M. R. Meselhy, N. Nakamura, M. Hattori : Triterpenes from the fungus *Ganoderma lucidum*. 日本薬学会北陸支部第96回例会，1997，6，金沢

【Purpose】 The fruiting bodies of the fungus *Ganoderma lucidum* (Polyporaceae) have been used as one of the valuable traditional Chinese medicines for the treatment of various kinds of diseases. Recently, several biologically active polyoxygenated triterpenes have been isolated from this mush-

room and proved effective as cytotoxic, antiviral and hepatoprotective agents. In the course of our continuing search for natural products as anti-HIV agents, the MeOH extract of the fruiting bodies was found to show moderate inhibitory activity *in vitro* against HIV-1 PR. The isolation and structure elucidation of two new triterpene and sterol are reported herein.

【Method】 The fruiting bodies of *G. lucidum* was extracted with MeOH. The MeOH extract was filtered through a column of Diaion HP-20, washed with water and then with MeOH. Repeated CC. of the MeOH eluate followed by MPLC and HPLC afforded thirteen compounds.

【Results】 Structural elucidation of two new compounds (**1** and **2**) and eleven known ones was accomplished by careful inspection of their spectroscopic data. Testing the HIV-1 PR inhibitory activity of the identified compounds is in progress in our laboratory.



11）谷川聖明，後藤博三，中村憲夫，田中宣充，服部征雄，寺澤捷年：桂皮含有タンニンにおける内皮依存性血管弛緩作用，第14回和漢医薬学会大会，1997，8，大阪．

【目的】 我々は本学会において，桂皮及びその主成分であるケイヒアルデヒド，ケイヒ酸に内皮依存性血管弛緩作用があることを報告した。また，桂皮より精油成分を除去した水溶成分にも同様の弛緩反応が存在することを明らかにし，それが桂皮含有タンニンであることを示唆した。今回我々は，桂皮よりタンニン成分を分取し，各画分における血管弛緩反応をラットの摘出血管を用いて検討した。

【方法】 Wistar 系雄性ラット（300～400 g）の胸部大動脈を摘出し，輪状標本（内皮保存標本と内皮除去標本）を作製した。これを 95% O_2-5% CO_2 で通気した 37℃ Krebs-Ringer 液の入った 5 ml の tissue bath 内に固定し用いた。桂皮より二量体から四量体までのタンニンを分取し，各画分につき弛緩反応を検討した。また単量体である Epicatechin についても同様の検討を行った。

【結果】 内皮保存血管においては，二量体である Procyanidin B-2 では 10^{-4}M 以上の高濃度において弛緩反応を認めた。また三量体である Procyanidin C-1，四量体である Cinnamtannin A_2 は，10^{-6}M の低濃度より濃度依存性に弛緩反応を認めた。しかし，内皮除去血管においては，それぞれの弛緩反応は消失した。さらに，Nitric Oxide（NO）の合成阻害薬である N^G-nitro-l-arginine methyl ester（L-NAME）を内皮保存血管に前処置したところ，同様にそれぞれの弛緩反応は消失した。Epicatechin には内皮保存血管，内皮除去血管，L-NAME で前処置した血管，全てにおいて血管弛緩反応は認められなかった。

【考察・結論】 桂皮含有タンニンには ACE 阻害剤類似の血管弛緩作用が報告されている。[1] 桂皮含有タンニンに，内皮依存性の血管弛緩作用を認め，その活性成分は，二量体以上の重合したタンニンであることが明らかとなった。さらに三量体，四量体と重合度が高くなるに従い内皮依存性弛緩作用が増強された。

1）Inokuchi J, Okabe H, *et al* : *Chem. Pharn. Bull,* 33(1), 264-269(1985).

12）M. R. Meselhy, O. A. Heikal, T. Akao, M. Hattori, H. Ono, C. Sadakane : Determination of Paeoniflorin and Paeonimetabolin l in Rat Plasma after Oral Administration of Toki-Shakuyaku-San(TS) and Shakuyaku-Kanzo-To (SK). 第14回和漢医薬学会大会，1997，8，大阪

【Purpose】 The present study was designed to investigate plasma concentrations of paeoniflorin (PF) and paeonimetabolin I (PM-I) after *p.o.* administration of two different Kampo prescriptions containing paeony roots, relative to that obtained after *p.o.* and/or *i.v.* administration of PF and/or PM-I, to emphasize the role of PM-I in the therapeutic effect of PF and Kampo prescriptions containing paeony roots.

【Method】 Doses of 100 and 500 mg/kg of the Kampo prescriptions TS and SK were orally administered to male Crj : CD (SD) rats. Similarly, PF (at doses of 0.5 and 5 mg/kg) and PM-I (in equal molar doses) were administered. Blood samples were obtained at time intervals after administration. Concentrations of PF amd PM-I in rat plasma were determined by the respective EIA methods.

【Results and Conclusion】 Following *p.o.* admini-

stration of PF, the maximal plasma concentrations (C_{max}) of PF were 9.9 and 20.3 (with *AUC* of 300 and 1174 ng·min/ml) and those of PM-I were 16.5 and 101.7 ng·min/ml at 0.5 and 5 mg/kg, respectively. The times to C_{max} (t_{max}) of PF were 11.6 and 13.3, and those of PM-I were 60 and 80 min, respectively. When PM-I was given orally at the same doses, C_{max} of 102.2 and 285 ng/ml were reached at t_{max} 6.2 and 7.5 min. The *AUC* values of PM-I after *p.o.* administration of PF (1873 and 12358 ng·min/ml) were comparable with those after *p.o.* and *i.v.* administration of PM-I (4145.6 and 14182.1, and 5614 and 13176 ng·min/ml, respectively) indicated that most of PF was transformed to PM-I by intestinal bacteria and PM-I was rapidly absorbed from the intestine. Following *p.o.* administration of TS, the C_{max} of PF (146.3 and 165.1 ng/ml) were reached at 60 and *ca.* 45 min (with *AUC* of 14305 and 19385 ng·min/ml), and those of PM-I were 184 and 400 ng/ml at 120 and 180 min (with *AUC* of 98497 and 182188 ng·min/ml) at 100 and 500 mg/kg, respectively. When SK was given orally, PF and PM-I were detected at considerable concentrations (C_{max} of 100.1 and 144 ng/ml, and 141.6 and 726.5 ng/ml, respectively. Relative to TS, the C_{max} of PF and PM-I were reached both at 5 min, and at 360 and 480 min, respectively, and retained in plasma for a longer period of time. From these observations, it seems likely that administration of TS of SK potentially affects absorption from GIT, plasma concentrations and elimination of both PF and PM-I.

13) 横澤隆子，董　而博，劉　中武，柏木　寛，中川孝子，服部征雄，鄭　海泳：茶のラジカル産生抑制作用についての検討．第14回和漢医薬学会大会，1997，8，大阪．

【緒言】フリーラジカルは種々の疾患の発症，進展に関与しているが，腎疾患も例外ではなく，なかでも糸球体腎炎，糖尿病性腎症，急性腎不全，慢性腎不全の病態に直接的，間接的に関わっていることが知られている。先に筆者らは緑茶に腎不全の進行を抑制する結果を明らかにし，緑茶にフリーラジカル消去作用が示唆された。[1-3] 本研究では，茶の製造工程の違いがラジカル消去能にどのような影響を及ぼすかについて，*in vitro* の実験系で検討した。

【材料と方法】緑茶（不発酵茶），烏龍茶（半発酵茶），紅茶（発酵茶）をそれぞれ熱水抽出して濾過後，凍結乾燥したものを以下の実験に供した。実験1：腎ホモジネートに活性酸素惹起剤（H_2O_2, $FeSO_4$），サンプルを添加し，Buege と Aust の方法に従い，生成した TBA-reactive substance を比色定量した。実験2：LLC-PK_1 細胞を DMEM/F-12 に5％ FCS を添加した培地で培養し，コンフルエンスに達してから1穴あたり 10^4 個ずつ細胞を seed 2 時間後に cisplatin，サンプルの順に添加し，48時間培養した。細胞障害の指標として培養液中へ漏出した LDH 活性と MDA 含量を測定した。実験3：ホモアルギニン水溶液にサンプル，5,5-dimethyl-1-pyrrorine-1-oxide (DMPO) を加えて激しく撹拌し，偏平セルを用い，生成したラジカルの DMPO-adduct の ESR スペクトルを測定した。測定条件は microwave power : 8.0 mW, field : 3350±50 Gauss, modulation : 100kHz 1 Gauss, sweep time: 0.5 min である。

【結果】フェントン反応による脂質過酸化を緑茶が最も強力に抑制し，次いで紅茶であった。一方，腎上皮細胞にcisplatinを添加した場合，細胞障害をひき起こし，DMTUで軽減したが，類似した作用は緑茶にも認められ，紅茶，烏龍茶も緑茶よりは弱いながらも認められた。また緑茶が DMPO-OH を著しく消去する作用を有していることを，ESR スペクトルより確認した。

【結論】緑茶，烏龍茶，紅茶のいずれにもラジカル消去作用が認められたが，緑茶においてその作用は強力であった。

1) Yokozawa T, Oura H, Hattori M, Iwano M, Dohi K, Sakanaka S, Kim M : *Nephron* **65**, 596 (1993).
2) Yokozawa T, Chung HY, He LQ, Oura H: *Biosci. Biotech. Biochem.* **60**, 1000 (1996).
3) Yokozawa T, Dong E, Chung HY, Oura H, Nakagawa H : *Biosci. Biotech. Biochem.* **61**, 204 (1997).

14) 劉　中武，横澤隆子，服部征雄，柴田　透，長谷川真常：Cephaloridine による急性腎不全における大黄牡丹湯の作用．第14回和漢医薬学会大会，1997，8，大阪．

【目的】先に筆者らは，虚血・再灌流障害によって生じる腎機能不全に対し，大黄牡丹皮湯が保護作用を有することを明らかにした。虚血・再灌流による腎のおもな障害部位は近位尿細管であることから，本研究ではこの部位を選択的に障害する cephaloridine を用い検討するとともに，近位尿細管由来細胞株を用い大黄牡丹皮湯の作用を検討した。

【方法】1. 動物実験：Wistar 系雄性ラット（6 週齢，体重 150 g 前後）に各エキス（大黄牡丹皮湯，大黄，牡丹皮，桃仁）を 200 mg/kg B.W./day 経口投与した。20 日後に cephaloridine（1 g/kg B.W.）を静注し，投与後 1～2 日目の尿を採取，次いで採血，腎を摘出し，実験に供した。2. 細胞培養実験：LLC-PK_1 細胞を D-MEM/F-12 に 5％ FCSを添加した培地で培養し，コンフルエンスに達してから細胞を 1 穴あたり 10^4 個ずつ seed した。2 時間後に cephaloridine（0.1 mM），各エキス（2.5～125 mg/ml）を添加し，通常の条件下（95％air, 5％ CO_2）で 48 時間培養した。細胞障害の指標として培地中へ漏出した LDH 活性を測定した。

【結果と考察】前もって大黄牡丹皮湯を投与した群の Na, グルコース排泄量は有意に低下し，尿量，浸透圧，蛋白排泄量も正常化傾向を示し，大黄牡丹皮湯に腎障害を軽減する作用を有していた。このことは血清成分にも反映されていた。一方，生体内の NO の指標の尿中 NO_2^-/NO_3^- 比は有意に増加，SOD, catalase 活性も有意に上昇し，酸素ストレスに対して大黄牡丹皮湯が防御的に作動し，cephaloridine による腎障害の改善に寄与しているものと考えられた。構成生薬では大黄投与群の NO_2^-/NO_3^- 比，ラジカル消去酵素活性，Na, グルコース，蛋白排泄量が大黄牡丹皮湯と類似した作用を認め，尿量，尿浸透圧も正常値に近い値まで回復していた。その他，牡丹皮は尿浸透圧の上昇作用を示し，桃仁は尿浸透圧を逆に低下してはいたが，グルコース，蛋白排泄量の著しい正常化傾向にあり，大黄，牡丹皮，桃仁はそれぞれ異なった機序のもとに，大黄牡丹皮湯の薬効発現に影響を及ぼしていることが予測された。cephaloridine の *in vitro* 評価系を LLC-PK_1 細胞を用いて構築した結果，cephaloridine 添加群では LDH の遊離が亢進し，細胞膜障害を呈していた。これに対し大黄牡丹皮湯を同時に添加した群では，添加量の増加とともに LDH の遊離を抑制し，このような作用は大黄，牡丹皮，桃仁のいずれにおいても認められた。

15）金　東郁，横澤隆子，服部征雄，門田重利，難波恒雄：羅布麻葉（*Apocynum venetum*）エキスの血圧に及ぼす効果．第14回和漢医薬学会大会，1997，8，大阪．

【目的】先に，我々は羅布麻葉エキスが高コレステロール血症低下作用，抗動脈硬化作用を有することを報告した。本研究では血圧に及ぼす効果について，3 種類の高血圧モデルを用い検討した。

【方法】実験 1：自然発症高血圧ラット（SHR/Izm 雄性，7 週齢）に羅布麻 A（未焙煎），B（2 回焙煎）をそれぞれ 70 mg/rat/day, Captopril を 30 mg, 40 日間飲水として投与した。実験 2：3/4 腎摘ラット（Wistar 系雄性，7 週齢）に羅布麻 A, B をそれぞれ 70 mg, 100 日間投与した。実験 3：Wistar 系雄性ラット（7 週齢）に 5％ 食塩，1％ コレステロール含有飼料を投与し，羅布麻 B（70 mg），釣藤散（70 mg），Captopril（30 mg）はそれぞれ飲水として投与した。血圧はラット・マウス用非観式血圧測定装置 MK-1030 を用い，無麻酔下で tail-cuff method で測定した。Na, K はイオン電極法で測定した。

【結果】実験 1：対照ラットの収縮期，平均，拡張期血圧が徐々に上昇するのに対し，羅布麻 A, B を投与した群では，いずれも 20 日目から血圧の上昇を抑制する知見が得られた。これら作用は Captopril 群よりは弱かったが，作用の持続性が認められた。尿中への Na, K排泄量に有意な変化は認めなかったが，増加傾向にあった。しかし血清 Na, K レベルは変化しなかった。実験 2：腎摘ラットの血圧は実験期間を通じてわずかに上昇していたが，羅布麻 A, B 投与群では 20 日目から有意な血圧下降作用を示し，その下降程度は投与期間の延長とともに増強する傾向にあった。血圧の下降とともに尿量並びに尿中への Na, K 排泄量が増加したが，血清レベルは変化しなかった。なお，羅布麻 A, B を 100 日間投与した BUN 値は有意に低下していた。実験 3：対照ラットの血圧は 40 日目以降，わずかに上昇していたが，羅布麻 B, 釣藤散，Captopril を投与した血圧はいずれも下降したが，羅布麻Bは釣藤散と匹敵した効果を，Captopril はこれらより強い効果を示した。しかし，60 日間投与した血清総コレステロール値は有意な変化を示さなかった。

【結論】羅布麻葉の水エキスに血圧上昇を抑制する作用が認められた。

16）横澤隆子，劉　中武，董　而博：腎虚血-再灌流モデルにおける甘草並びに Glycyrrhizin の役割．第14回和漢医薬学会大会，1997，8，大阪．

【目的】先に甘草に酸素ストレスを軽減する作用を明らかにしたが，本研究では腎における役割を虚血-再灌流腎障害モデルを用い検討した。

【方法】1. 動物実験：Wistar 系雄性ラット（6 週齢，体重 150 g 前後）に甘草エキス（62.5，125 mg/kg B.W./day, 経口）あるいは glycyrrhizin（2.5, 10 mg/kg B.W./day, 腹腔）を連日投与した。20 日後に左腎動・静脈，尿管を 45 分間遮断，その後 2 時間再灌流し，腹部下大動脈から採血した。次いで氷冷した生理食塩水で灌流後，腎臓を採取し，各種血清成分，腎組織中のラジカル消去酵素活性を測定した。2. 細胞培養実験：

LLC-PK_1 細胞を D-MEM/F-12 に 5％ FCS を添加した培地で培養し，コンフルエンスに達してから細胞を 1 穴あたり 10^4 個ずつ seed した。2 時間後にサンプルを添加し，通常の条件下（95％ air, 5％ CO_2）で 41 時間培養した。次いで低酸素下（酸素濃度 2％ 未満）で 6 時間培養後，通常の条件下で 1 時間培養することで再酸素下とした。細胞障害の指標として培地中へ漏出した LDH 活性と MDA 含量を測定した。

【結果】 甘草エキス投与群では BUN, 血清，腎組織中の MDA が低下，抗酸化酵素の SOD, catalase, GSH-Px 活性も低用量でいずれも有意に低下していた。しかし高用量では対照値と変わらず，glycyrrhizin 投与群では逆に catalase, GSH-Px 活性の有意な上昇作用が認められ，BUN, Cr, MDA に対する作用もエキス群より強力であった。LLC-PK_1 細胞を用いた実験でも，エキスと glycyrrhizin に細胞障害を抑制する知見が認められ，glycyrrhizin では低濃度から認められた。

【結論】 腎における酸素ストレスを軽減する作用が甘草エキスで認められ，glycyrrhizin が重要な役割を担っていることが示唆された。

17）横澤隆子，劉　中武，董　而博，大浦彦吉：腎障害における大黄牡丹皮湯の関与について．第 9 回腎とフリーラジカル研究会，1997，9，福井．

【目的】 先に筆者らは，腎の虚血-再灌流障害に対し，大黄牡丹皮湯が保護作用を有することを報告した。本研究では近位尿細管を選択的に障害する cephaloridine モデルを用い検討した。

【方法】 Wistar 系雄性ラット（6 週齢，体重 150 g 前後）に各エキス剤（大黄牡丹皮湯，大黄，牡丹皮，桃仁）を 200 mg/kg B.W./day 経口投与した。20 日後に cephaloridine (1 g/kg B.W.) を静注し，尿，血液，腎を採取した。

【結果と考察】 大黄牡丹皮湯投与群の Na, グルコース，蛋白排泄量は有意に低下，尿量，浸透圧も正常化傾向を示し，大黄牡丹皮湯に腎障害を軽減する作用を有していた。このことは血清成分にも反映されていた。一方，尿中 NO_2^-/NO_3^- 比は有意に増加，SOD, catalase 活性も有意に上昇し，酸素ストレスに対して大黄牡丹皮湯が防御的に作動し，cephaloridine による腎障害の改善に寄与しているものと考えられた。構成生薬では大黄投与群の NO_2^-/NO_3^- 比，ラジカル消去酵素活性，グルコース・蛋白排泄量が大黄牡丹皮湯と類似した作用を認め，尿量，尿浸透圧も正常値に近い値まで回復していた。その他，牡丹皮は尿浸透圧の上昇作用を示し，桃仁は尿浸透圧を逆に低下してはいたが，グルコース，蛋白排泄量は正常化傾向にあり，大黄，牡丹皮，桃仁はそれぞれ異なった機序のもとに，大黄牡丹皮湯の薬効発現に影響を及ぼしていることが予測された。

【文献】 Dong E, Yokozawa T, Liu ZW, et al., *J. Trad. Med.*, **14**, 41-48 (1997).

18）Chung H.Y., Baek B.S., Sung D.Y., Kim K. W., Lee K.Y. and Yokozawa T.: Green Tea Components as a Peroxynitrite Inhibitor. International Symposium on Antioxidant Food Supplements in Human Health, 1997, 10, Yamagata.

Peroxynitrite ($ONOO^-$) can oxidize methionine residues in proteins and peptides as well as thiols and thioethers. Peroxynitrite or its proposed-decomposition products have recently been defined as a potent oxidant and potential mediator of vascular injury and been suggested to initiate lipid peroxidation and cellular -SH oxidation. Green tea, next to water, is the most popular and commonly consumed beverage in the world. Most of the green tea polyphenols are flavonols, commonly known as catechins. Some major green tea catechins were isolated; (-)-epicatechin 3-*O*-gallate (ECG), (+)-gallocatechin 3-*O*-gallate (GCG). (-)-epigallocatechin 3-*O*-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin and (+)-catechin. Green tea has been reported to possess various biologic activities and pharmacologic effects, many of which remain to be verified.

In the present study, these catechins were screened as an antioxidant, an inhibitor of peroxynitrite generation, and a peroxynitrite scavenger. Especially, GCG, EGCG, and ECG showed a potent antioxidant activity, inhibition of peroxynitrite generation, and a remarkable peroxynitrite scavenging activity. These results suggest that GCG, EGCG, and ECG ameliorate oxidative damage from peroxynitrite as well as reactive oxygen species.

19）Chung H.Y., Kim J.W., Lee K.H. and Yokozawa T.: *In Vivo* Antioxidant Effects of *Salvia miltiorrhiza*. International Symposium on Antioxidant Food Supplements in Human Health, 1997, 10, Yamagata.

A phenolic compound, magnesium lithospermate B (MLS), was isolated and identified as a major biologically active component of an herbal material

called Salviae Miltiorrhizae Radix (SMR). The present study was carried out to elucidate the action mechanisms of MLS *in vivo* system. MLS significantly increased Cu, Zn-SOD, catalase and non-protein-SH levels compared to control in CCl_4-intoxicated ICR mice, while markedly decreased malondialdehyde in CCl_4-intoxicated ICR mice. These results suggest that antioxidative action of MLS is partly attributable to sustain endogenous antioxidants.

On the other hand, in senescence-accelerated mouse (SAM-R/1), MLS treatment inhibited free radical generation and lipid peroxidation and enhanced GSH/GSSG ratio, while it did not change antioxidant enzyme activities. Furthermore, there were no changes in gene expression, which were paralleled to enzyme activities.

These results suggest that antioxidative action of MLS is not due to antioxidant enzyme induction but due to direct radical-scavenging ability.

20) Ninomiya M., Sugiura K., Aoi N., Kim M. and Yokozawa T.: Suppressive Effect of Uremic Toxin (Methylguanidine) Formation by Green Tea Polyphenols. International Symposium on Antioxidant Food Supplements in Human Health, 1997, 10, Yamagata.

The patients with renal failure are generally subjected to a high oxidative stress. We previously reported the decrease of kidney functions by the accumulation of uremic toxin (uremia) due to oxidative stress. Methylguanidine (MG), a uremic toxin, is derived from creatinine (Cr) by the action of hydroxyl radicals. On the basis of above acknowledgement, we investigated the effect of green tea polyphenols on the serum MG and MG/Cr levels in human volunteers.

Two doses of 200 mg green tea polyphenols (SUNPHENON™) in a jelly form were given to 50 patients receiving chronic hemodialysis daily for six months. The serum MG was about 70 % of that at the beginning of the treatment. The MG/Cr ratio, 4.12×10^{-3} at the baseline, decreased significantly to 3.86×10^{-3} at 2 months and suppressive effect continued throughout the administration period. Therefore, green tea polyphenols could be explored as a potential material for suppression of uremic toxin.

21) Y. Yu, H. Miyashiro, J. Park, M. Hattori: Screening of Korean Plants for Inhibitory Effects on Essential Enzymes of HIV-1. International Symposium on Natural Medicines. 1997, 10, Kyoto.

The human immunodeficiency virus type 1 (HIV-1) is an aetiological agent for Acquired Immunodeficiency Syndrome (AIDS). HIV-1 is an enveloped virus with a single-stranded RNA genome that employs a reverse transcriptase (RT) enzyme in replication. The functional viral enzymes, including RT, protease (PR) and integrase, and structural proteins of HIV-1 are initially formed as a fused polyprotein, and acquire their activities after being cleaved by protease. The central role of RT and PR in HIV-1 replication and assembly makes them a logical traget for antiviral therapy. The maturation of envelope glycoproteins in the HIV-1 infected host cell is related with oligosaccharides processing by glucosidase and mannosidase. And an inhibition of glucosidase has a number of potential therapeutic uses including the treatment of cancer, diabetes and AIDS.

For the purpose of developing new anti-HIV agents from natural sources, extracts from various Korean plants were screened for inhibitory effects on RT, PR and α-glucosidase as essential enzymes for HIV-1.

The PR inhibitory activity was determined as the proteolytic activity of PR, by HPLC. Of the 102 extracts tested, the water extracts of *Aleurites fordii* Hemsl. (leaves), *Alnus firma* S. et Z. (stems), *Pyrachantha frotuneana* Maxim. (stems) and Rosa maximowicziana Regel (roots) inhibited the HIV-1 PR activity by more than 70 % at a concentration of 100 μg/ml.

In the RT inhibitory activity assay, determed by the ELOSA (enzyme-linked oligonucleotide sorbent assay) method, both the water and methanol extracts of *Actinidia chinensis* Planch. (leaves, stems), *Aleurites fordii* Hemsl. (fruit, leaves and stems) and *Rubus crataegifolius* Bunge (leaves and stems) showed more than 70 % inhibition at 100 μg/ml. Ten other plants were significantly lower in the RT activity at 100 μg/ml.

The α-glucosidase inhibitory activity was determined using p-nitrophenyl α-D-glucoside. The water extracts of *Aleurites fordii* Hemsl. (fruit), *Alnus firma* S. et Z. (leaves and stems), *Chaenomeles sinensis* Koehne (stems), *Pyrachantha frotuneana* Maxim. (stems) and *Rosa maximowiczziana* Regel (roots) inhibited the α-glucosidase activity by more than 80 % at a concentration of 100 μg/ml.

22) G. Hussein, N. Nakamura, H. Miyashiro, M. Hattori, T. Kawahata, T. Otake, K. Shimotohno : Inhibitory Effects of Sudanese Medicinal Plants on HIV-1 Protease and HIV Proliferation in MT-4 Cells. International Symposium on Natural Medicines. 1997, 10, Kyoto.

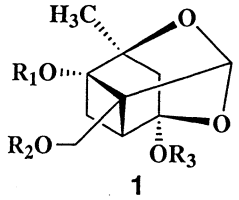
[Purpose] The vast and diverse flora of Sudan and its prominent traditional medicinal plants may be a promising source for anti-viral candidates. In the ongoing serch for anti-AIDS agents from natural products, a preliminary screening was carrid out on 48 extracts. Bioactivity-guided isolation of compounds from active extracts is being investigated. The results are presented herein. This is the first study to be reported on anti-HIV activities from Sudanese medicinal plants.

[Methods] Methanolic and aqueous extracts of 24 plant parts were tested for their inhibitory effects against HIV-1 protease by HPLC assay method. The principle of the method is to determine the inhibitory effects on the proteolytic activity of the enzyme on a substrate in the presence of the extract sample. The inhibitory effects on HIV-induced cytopathic effect (CPE) in MT-4 cells were investigated by microscopy.

[Results] 6 extracts showed high to moderate activities against HIV-1 protease [MeOH and H_2O extracts of *Acacia nilotica* (pods), MeOH extract of *A. nilotica* (stem-bark), MeOH and H_2O ectracts of *Maytenus senegalensis* (stem-bark) and MeOH extract of *Euphorbia granulata* (leaves)]. 5 extracts exhibited appreciable inhibitory effects on HIV-induced CPE in MT-4 cells [H_2O extracts of *A. nilotica* (pods), *Ambrosia maritima* (aerial parts), *E. granulata* (leaves) and MeOH extracts of *Balanites aegyptiaca* (bark) and *E. prostrata* (leaves) [IC ; $\geqq$31.25～$\geqq$62.5 μg/ml]. Among the active extracts, *A. nilotica* (pods) and *M. senegalensis* (stembark) extracts are being studied. Isolation of compounds from active fractions included triterpenes, flavan-3-ols and their glycosides and proanthocyanidins. Some of the isolated compounds had shown moderate inhibitory activity.

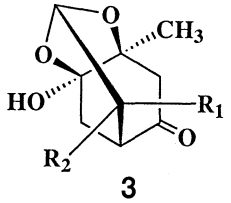
23) A. Abdel-Hafez, M. R. Meselhy, N. Nakamura, M. Hattori, T. A. Mohamed, N. M. Mahfpuz, M. A. El-Gendy : Modification of Paeoniflorin and Paeonimetabolin I by Chemical and Biochemical Means. International Symposium on Natural Medicines. 1997, 10, Kyoto.

Paeoniflorin (**1**) and its metabolite paeonimeta-



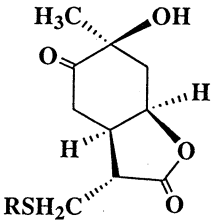
1

R_1 = H, Glc, Me_4-Glc or Ac_4-Glc
R_2 = H, Benzoyl or Valproyl
R_3 = H, CH_3, Ac

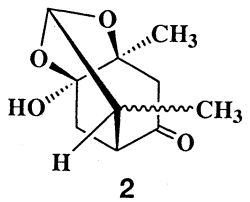


3

(7*R*) R_1= CH_2SR, R_2= H
(7*S*) R_1= H, R_2= CH_2SR



4



2

R = a = *n*-propyl
b = *iso*-propyl
c = *n*-butyl
d = *sec*-butyl
e = *iso*-butyl
f = *n*-hexyl
g = allyl
h = cyclopentyl
i = cyclohexyl
j = phenyl
k = *o*-methylphenyl
l = *m*-methylphenyl
m= *p*-methylphenyl
n = 2-naphthyl
o = acetyl
p = benzoyl
q = benzyl

bolin I (**2**) showed promising neuropharmacological activities such as improvment of aging-induced and nuleus basalis lesion induced learning deficits and of spatial working memory deficits, and anticonvulsant activity, respectively.

In the present work, chemical and biochemical modifications of **1** and **2** were undertaken in order to obtain the most active and less toxic compounds. A variaty of thiopaeonimetabloin I adducts (**3 a-q**) were prepared by incubation of **1** with *Lactobacillus brevis* in the presence of different thios for 6 hours in 0.5 M K-phosphate (pH 7.25). The products were purified by filtration through Diaion HP-20 column chromatography followed by repeated silica gel column chromatography (yields 20-30 %). During the incubation of **1** with different aromatic thiols, new paeonilactone A adducts (**4 j-n**) (yields 0.5-4.5 %) were formed together with **3 j-n**.

The biological activities of the prepared compounds are now under investigation.

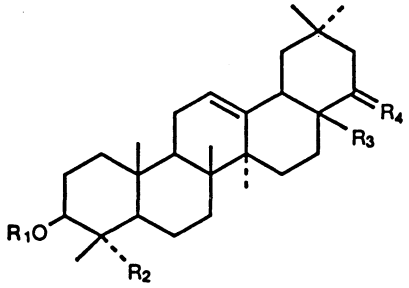
24) C. Ma, N. Nakamura, M. Hattori, K. Shimothono: Triterpene Saponins and Flavone *C*-Glycosides from the Seeds of *Abrus precatorius*. International Symposium on Natural Medicines. 1997, 10, Kyoto.

As part of our ongoing program for the discovery of anti-HIV agents from plants, we have studied the methanol extract of the seeds of *Abrus precatorius*, from which we isolated five new compounds (saponins **1, 3** and flavone *C-glycosides* **9-11**) together with abrine, *N,N,N*-trimethyl tryptophan and other known compounds. Their structures were determined by chemical and spectroscopic methods. The new saponins were oleanolic acid derivatives linked with a glucose-glc A unit at *C*-3. The new flovone *C*-glycosides with sugar(s) linked at *C*-6 exhibited extensive doubling of signals in their ^{1}H and ^{13}C-NMR spectra, possibly due to the presence of two conformers. The attachment of sugar(s) was unambiguously determined with HMBC measured in DMSO-d_6 by observing the correlations of C-5-OH proton with C-5 and C-6, as well as the correlations of C-5 and C-6 with the anomeric proton of the inner sugar.

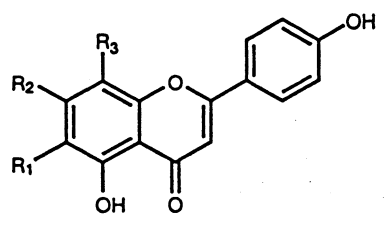
Of these compounds, a new saponin (**1**) and five known saponins (**2, 4, 5, 6** and 8) appreciably inhibited HIV-1 protease activity by more than 50 % at a concentration of 100 μM.

25) Kusano R., Tanaka T., Kouno I., Liu Z.W., Dong E. and Yokozawa T.: Synthesis and Radical Scavenging Effects of Amphipathic Derivatives of Green Tea Polyphenol. International Symposium on Natural Medicines. 1997, 10, Kyoto.

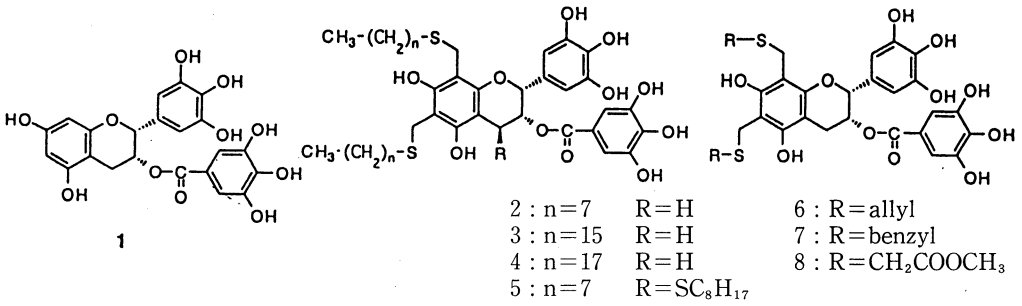
Radical scavenging effects of thirty plant polyphenols (MW=170−3738) were compared by measuring thiobarbituric acid reactive substances generated on lipid peroxidation in rat liver mi-



	R_1	R_2	R_3	R_4
1	glc²-glcA	CH_2OH	COOH	H,H
2	glc²-glcA	Me	COOH	H,H
3	glc²-glcA	Me	COOglc	H,H
4	glcA²-gal	Me	Me	H,β-OH
5	glcA²-gal²-xyl	Me	Me	H,β-OH
6	glcA²-gal²-rha	Me	Me	H,β-OH
7	glc AMe²-gal²-rha	Me	Me	H,β-OH
8	glcA²-gal²-rha	Me	Me	O



	R_1	R_2	R_3
9	glc	OMe	OMe
10	glc²-apiose	OMe	OMe
11	glc²-apiose	OMe	H
12	OMe	OMe	glc
13	OMe	OMe	glc²-apiose
14	OMe	OMe	H
15	OMe	OH	H



1

2 : n=7 R=H
3 : n=15 R=H
4 : n=17 R=H
5 : n=7 R=SC_8H_{17}

6 : R=allyl
7 : R=benzyl
8 : R=CH_2COOCH_3

crosomes with $FeSO_4-H_2O_2$. Polyphenols with larger molecular size tend to show stronger scavenging activity; however, despite of its small molecular size epigallocatechin 3-*O*-gallate (**1**), the major polyphenol of green tea, showed strong activity comparable to that of pentagalloylglucose (PGG). Although **1** and PGG are relatively hydrophobic ($P_{octanol/water}=70$ and 92, respectively) compared to other polyphenols, these polyphenols showed only low scavenging activity against radicals generated by lipophilic radical initiator (AMVN) in lipid bilayers of egg PC. On the other hand, the scavenging effects of these hydrophobic polyphenols against radicals generated by water soluble radical initiator (AAPH) were almost same as those of other hydrophilic polyphenols. Although it is known that polyphenols interact with lipid bilayers,[1] these results indicated that polyphenols did not penetrate into hydrophobic region of lipid bilayers. Taking these results into account, we have developed new amphipathic polyphenols having improved activity against radicals in both hydrophilic and hydrophobic environment. Derivatives **2**−**8** were synthesized from **1** or persimmon tannin by condensation with various thiol compounds and formaldehyde. Compounds **2** and **5** showed stronger activity than **1** (inhibition % at 70 mM : **1**, 12.7 % ; **2**, 48.6 % ; **5**, 60.4 %) against radicals generated by AMVN in lipid bilayer, while the activity of **6**, **7** and **8** was similar to that of **1**. Moreover, compound **2** showed improved activity (IC_{50} : 7.9 μM) against radicals generated by AAPH as well compared to **1** (21 μM) and α-tocopherol (41 μM). Since compounds **3** and **4** aggregate lipid bilayers even at low concentration, the activity could not be compared.

1) Huh N.-W., Porter N. A., McIntoch T. J., Simon S. A., *Biophys. J.*, **71**, 3261-3277 (1996).

26) 朴鍾 喆, 朴桂権, 朴基栄, 鄭信教, 李 甲然, 劉永法, H. Miyashiro, M. Hattori : 済州島産天然資源植物の HIV-1 プロテアーゼ抑制活性, The 46 th Annual Convention of the Pharmaceutical Society of Korea 1997.

27) M. Hattori, S. El-Mekkawy, M. R. Meselhy : Inhibitory Effects of Components from *Ganoderma lucidum* on the Growth of Human Immunodeficiency Virus (HIV) and the Protease Activity. The First International Symposium on *Ganoderma lucidum* in Japan, 1997. 11, Tokyo.

A new highly oxygenated triterpene has been isolated from the methanol extract of the fruiting bodies of *Ganoderma lucidum* together with twelve known compounds. The structures of the isolated compounds were determined by spectroscopic maens including 2D-NMR. Ganoderiol F and ganodermanontriol were found active as anti-HIV with an inhibitory concentration of 7.8 μg/ml for both, and ganoderic acid B, ganoderiol B, ganoderic acid C1, 3β, 5α-dihydroxy-6β-methoxyergosta-7, 22-diene, compound **1**, ganoderic acid H and ganoderiol A were moderately active inhibitors against HIV-1 PR with a 50 % inhibitory concentration of 0.17 mM.

28) 中村憲夫, 馬 超美, 服部征雄 : 相思子に含まれるサポニンの API-MS について. 第41回北陸質量分析談話会, 1997, 11, 富山.

29) 中川孝子, 横澤隆子, 劉 中武, 後藤博三, 中川眸, 竹内茂彌, 服部征雄, 寺澤捷年 : 食餌性 arginine が引き起こす生体内反応. 第19回グアニジノ化合物研究会, 1997, 12, 横浜.

【目的】 arginine (Arg) は, nitric oxide (NO) の生成と guanidinoacetic acid (GAA), creatinine (Cr),

methylguanidine（MG）などのグアニジノ化合物代謝の接点に位置する化合物である。本研究ではArgを負荷したラットのこれら成分の動態について検討した。

【方法】 Wistar系雄性ラット（5週齢，体重130 g前後）に，18％ カゼイン食(control群)，2％ Arg含有18％ カゼイン食(2％ Arg群)，4％ Arg含有 18％ カゼイン食（4％ Ar 群）をそれぞれ20日間投与し，血液，尿，肝，腎組織中のグアニジノ化合物，血液，尿中の NO_2^-, NO_3^- をHPLC，酸化窒素分析システムで測定した。また肝，腎組織中の superoxide dismutase（SOD），catalase，glutathione peroxidase (GSH-Px)活性は亜硝酸法，H_2O_2 の紫外部吸収法，GSH量を比色定量する方法で測定した。

【結果】 Argの投与量の増加とともに血清，尿中の NO_3^-, Arg, GAA. Crがいずれも有意に増加していた。BUN, 尿中のurea, protein排泄量も有意に増加，尿中のMG排泄量も増加傾向にあった。肝組織中のArg, GAA, Cr量も4％ Arg群で高く，尿と類似していたが，腎組織では逆に低下していた。一方，SOD, catalase, GSH-Px活性は腎では上昇，特にSOD活性における上昇が著しかった。これに対し，肝組織のSOD活性は有意に低下，GSH-Px活性も低下傾向にあったが，catalase活性は有意に上昇していた。

【結論】 Arg負荷ラットのNO，グアニジノ化合物，ラジカル消去酵素活性の動態を明らかにした。

◇ その他

1）服部征雄，渡邊裕司，済木育夫，難波恒雄，畑中保丸，倉石　泰，小橋恭一，小泉　徹：ヒト腸内細菌による変換反応の新しい医薬品開発への応用，平成7年度提案公募型最先端分野研究開発成果発表会，1997, 2，東京。

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3）服部征雄：成人病（生活習慣病）に有効な燕龍茶の働き，食品工業，40，55-59（1997）.

4）服部征雄，渡邊裕司，済木育夫，難波恒雄，畑中保丸，小橋恭一，倉石泰，小泉　徹，Meselhy Ragab Meselhy：ヒト腸内細菌による変換反応の新しい医薬品開発への応用，『平成7年度提案公募型・最先端（重点）分野研究開発』p.228-229, 新エネルギー・産業技術総合開発機構（NEDO），アンザイ，東京，1997.

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7）横澤隆子：腎不全，『緑茶まるごと健康法』p.66, 大森正司監修，マキノ出版，東京，1997.

8）横澤隆子：透析患者における緑茶タンニン投与の影響。第2回和漢薬研究所夏期セミナー，1997, 7，富山。