Effects of Oriental medicines on the production of advanced glycation endproducts

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Abstract

Advanced glycation endproducts (AGEs) are largely involved in the pathogenesis of diabetic nephropathy. It is apparent that inhibition of AGEs formation is important in preventing the occurrence and progression of nephropathy. Therefore, to seek possible AGEs inhibitors in Oriental medicines, we began our investigation with an *in vitro* evaluation system. Among the 12 Oriental medical prescriptions examined, Ompi-to inhibited AGEs formation to the greatest extent, followed by Tokaku-joki-to and Keishi-bukuryo-gan and Daio-botampi-to in that order. Among the 21 component galenicals examined, Rhei Rhizoma, Cinnamomi Cortex, Moutan Cortex and Paeoniae Radix all had a potent inhibitory action, indicating that Rhei Rhizoma, vascular system disturbance-eliminating drugs and tannin-containing crude drugs can all inhibit the formation of AGEs. These Oriental prescriptions and component galenicals proved to have more potent inhibitory activity than the positive control aminoguanidine.

Key words advanced glycation endproducts, diabetic nephropathy, Ompi-to, Rhei Rhizoma, aminoguanidine.

Abbreviations Ompi-to (Wen-Pi-Tang), 温脾湯; Tokaku-joki-to (Tao-He-Cheng-Qi-Tang), 桃核承気湯; Keishi-bukuryo-gan (Gui-Zhi-Fu-Ling-Wan), 桂枝茯苓丸; Daio-botampi-to (Da-Huang-Mu-Dan-Pi-Tang), 大黄牡丹皮湯; Shimbu-to (Zhen-Wu-Tang), 真武湯; Toki-shakuyaku-san (Dang-Gui-Shao-Yao-San), 当帰芍薬散; Hachimi-jio-gan (Ba-Wei-Di-Huang-Wan), 八味地黄丸; Gosha-jinki-gan (Niu-Che-Shen-Qi-Wan), 牛車腎気丸; Saiko-keishi-to (Chai-Hu-Gui-Zhi-Tang), 柴胡桂枝湯; Sho-saiko-to (Xiao-Chai-Hu-Tang), 小柴胡湯; Sairei-to (Chai-Ling-Tang), 柴苓湯; Hochu-ekki-to (Bu-Zhong-Yi-Qi-Tang), 補中益気湯.

Introduction

The population of dialysis patients in Japan has been increasing year by year. In 1999, about 186,000 patients are currently on dialysis treatment, and about 1/4 of these patients have diabetic nephropathy as the underlying disease. The recent increase in the population of patients on maintenance dialysis treatment is mainly attributable to the increased prevalence of diabetic nephropathy.[1] Under these circumstances, the development of a good remedy as well as elucidation of determing factors in the occurrence and progression of diabetic nephropathy is urgently needed.

The results of the Diabetes Control and Complications Trial (DCCT) showed that diabetic nephropathy is derived from persistent hyperglycemia.[2] It is now apparent that strict blood glucose control maintained for a prolonged period markedly inhibits the occurrence and progression of diabetic nephropathy. However, clinically it is not easy to practice strict blood glucose control in view of the side effects and the risk of hypoglycemia, as indicated by the DCCT.[2] Therefore, it is desirable to develop drugs which can correct

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disorders of carbohydrate metabolism resulting from a failure of strict blood glucose control and which will contribute to some extent in the prevention of complications. To this end, polyol metabolism disorder-improving agents and protein glycation inhibitors are promising. To improve polyol metabolism disorders, inhibitors of aldose reductase, which catalyzes the metabolic process from glucose to sorbitol, have been developed with the aim of correcting the acceleration of this process, and they are currently on the market for use in the treatment of diabetic neuropathy. Meanwhile, aminoguanidine is the only protein glycation inhibitor currently under development and there seems to be hardly any other promising drugs of this class at present. Although a large-scale phase-III clinical study of aminoguanidine in patients with diabetic nephropathy is now underway, careful investigations on the safety of this drug are desirable.[3] On the other hand, some Oriental medical prescriptions and galenicals have proved to be somewhat effective for diabetes and nephropathy, suggesting their probable role in multifaceted treatment of these diseases.[4-8]

To examine the possibility of their usefulness, we tested rhubarb prescriptions, bupleurum root prescriptions, some crude drugs used for eliminating disturbances of the vascular system, and rehmannia root prescriptions such as Hachimi-jio-gan for their effects on the reaction of protein glycation.

Materials and Methods

Prescriptions and crude drugs : The composition of Ompi-to used in the experiment was as follows : 15 g of Rhei Rhizoma (*Rheum officinale* BAILLON), 3 g of Ginseng Radix (*Panax ginseng* C.A. MEYER), 9 g of Aconiti Tuber (*Aconitum japonicum* THUNBERG), 3 g of Zingiberis Rhizoma (*Zingiber officinale* ROSCOE) and 5 g of Glycyrrhizae Radix (*Glycyrrhiza glabra* LINN. var. *glandulifera* REGEL et HERDER). As described previously,[9] an extract was obtained by boiling the above crude drugs gently in 1,000 ml water for 65 min. This yielded approximately 500 ml of decoction, which was then concentrated under reduced pressure to leave a brown residue with a yield of about 30 %, by weight, of the original preparation. Extracts of the other prescriptions were made according to the same standard (formula composition, dosage of each crude drug and production techniques, but without any excipient) as the commercial product from Tsumura Juntendo, Inc., Tokyo, Japan. The crude drug was treated in the same manner as that for a prescription. A voucher specimen is deposited in the Institute of Natural Medicine, Toyama Medical and Pharmaceutical University.

Determination of AGEs formation : According to the method of Vinson and Howard,[10] bovine serum albumin (10 mg/ml) in 50 mM phosphate-buffer (pH 7.4) with 0.02 % sodium azide to prevent bacterial growth was added to glucose (25 mM) and fructose (25 mM). This reaction mixture was mixed with different concentrations of the prescription, crude drug or aminoguanidine. After incubating at 37°C for 2 weeks, the fluorescent reaction products were assayed on a spectrofluorometric detector (Shimadzu RF-550, Kyoto, Japan) with the excitation at 350 nm and emission at 450 nm. All incubations were done in quadruplicate. The data is expressed in terms of IC_{50} value (concentration in μg/ml required to inhibit AGEs formation by 50 %) calculated from the log-dose inhibition curve.

Results

As shown in Table I, it was found that some of the prescriptions had very significant inhibiting effects on the AGEs formation. Four prescriptions (Ompi-to, Tokaku-joki-to, Keishi-bukuryo-gan, Daio-botampi-to) were found to have strong AGEs formation-inhibiting activity, and 5 prescriptions (Shimbu-to, Toki-shakuyaku-san, Hachimi-jio-gan, Gosha-jinki-gan, Saiko-keishi-to) had a moderate action (IC_{50}=30~55 μg/ml). Three of the 12 prescriptions showed only a weak inhibitory activity on the AGEs formation. Similar to the prescriptions, aminoguanidine also inhibited AGEs formation, but this effect was moderate (Table I).

Of the 21 crude drugs tested, Rhei Rhizoma showed the most active inhibition, with 50 % inhibitory activity at a concentration as low as 2.0 μg/ml (Table II). Following Rhei Rhizoma, the next most active drugs in order were Cinnamomi Cortex,

Table I IC_{50} values of prescriptions tested against AGEs formation.

Prescriptions	IC_{50} (μg/ml)
Ompi-to	7.85 ± 0.15
Tokaku-joki-to	10.96 ± 0.16
Keishi-bukuryo-gan	11.56 ± 0.14
Daio-botampi-to	18.18 ± 0.44
Shimbu-to	31.43 ± 0.14
Toki-shakuyaku-san	41.22 ± 1.38
Hachimi-jio-gan	45.55 ± 1.10
Gosha-jinki-gan	47.03 ± 1.12
Saiko-keishi-to	54.82 ± 1.44
Sho-saiko-to	80.81 ± 0.90
Sairei-to	81.44 ± 1.96
Hochu-ekki-to	127.35 ± 1.13
Aminoguanidine	59.20 ± 1.47

Table II IC_{50} values of crude drugs tested against AGEs formation.

Crude drugs	IC_{50} (μg/ml)
Rhei Rhizoma	2.0 ± 0.3
Cinnamomi Cortex	6.9 ± 0.2
Moutan Cortex	7.5 ± 0.1
Paeoniae Radix	13.9 ± 0.5
Zingiberis Siccatum Rhizoma	102.2 ± 2.6
Cnidii Rhizoma	310.6 ± 7.5
Zingiberis Rhizoma	459.2 ± 28.8
Aconiti Tuber	>500
Angelicae Radix	>500
Astragali Radix	>500
Atractylodis Rhizoma	>500
Benincasae Semen	>500
Bupleuri Radix	>500
Ginseng Radix	>500
Glycyrrhizae Radix	>500
Hoelen	>500
Persicae Semen	>500
Pinelliae Tuber	>500
Rehmanniae Radix	>500
Scutellariae Radix	>500
Zizyphi Fructus	>500

Moutan Cortex and then Paeoniae Radix. Zingiberis Siccatum Rhizoma, Cnidii Rhizoma and Zingiberis Rhizoma showed only weak inhibition, with IC_{50} values between 100~460 μg/ml. Fourteen of the 21 crude drugs appeared to have no action because their IC_{50} value exceeded 500 μg/ml.

Discussion

It is now apparent that the protein glycation reaction occurs in the living body, although not as fast as in food during cooking, and the relation between this reaction and some pathological conditions including diabetic nephropathy has been suggested.[11-15] Protein glycation can be broadly divided into the early-phase reaction in which Amadori rearrangement products are produced and the late-phase reaction in which these products are converted to AGEs.[16,17] AGEs have specific physicochemical properties including fluorescence, a brown color, and molecular bridge formation,[11,12,18-20] and has the biological characteristics of ligands recognized by cell membrane receptors expressed in vascular endothelial cells and macrophages.[21,22] The early-phase reaction is a non-enzymatic bimolecular reaction of the amino group of protein and glucose. Therefore, it is necessary to increase the concentration of protein or glucose to accelerate the reaction. Increased glucose concentrations in the living body correspond to the state of diabetes. Since proteins in the body vary in life span, it is reasonable to consider that proteins having a longer life proceed to a later stage of the protein glycation reaction than do proteins with a shorter life. On the other hand, Sakurai and Tsuchiya,[23] Smith and Thornalley [24] and Fu *et al.* [25] have reported that no oxidation reaction is involved in the formation of Amadori rearrangement products, whereas oxidation plays a role in the formation of fluorescence and the molecular bridge, which are characteristic features of AGEs. In addition, they noted that Amadori rearrangement products are oxidized to AGEs by active oxygen, and indeed can also serve as a source of active oxygen. Therefore, progression of the protein glycation reaction increases oxidative stress, through generation of active oxygen from Amadori rearrangement products. However, AGEs are considered to be not a single substance, but a group of various substances. In this regard, the present study examined that AGE evaluation system with colorimetry using

the fluorescence characteristic, as proposed by Monnier and Cerami.[11]

Among the 12 Oriental medical prescriptions examined, Ompi-to inhibited the AGEs production to the greatest extent. Tokaku-joki-to, Keishi-bukuryo-gan, Daio-botampi-to, rhubarb prescriptions, and vascular system disorder-eliminating drugs also proved to have strong activity. In contrast, bupleurum root prescriptions such as Saiko-keishi-to, Sho-saiko-to, Sairei-to and Hochu-ekki-to showed only weak activity. Hikiami *et al.*[26] have previously reported the efficacy of Oriental medicines in 141 patients who had complicated diabetes. They investigated the frequency of use of Oriental medical prescriptions for diabetic nephropathy in relation to the disease stage which was determined according to the method prescribed by the Diabetes Research Group of the Ministry of Health and Welfare. Their findings showed that vascular system disorder-eliminating drugs and rehmannia root prescriptions are used at a similar frequency over all the disease stages, whereas the use of bupleurum root prescriptions tended to be less frequent as the stage of advanced nephropathy. In relation to the duration of illness, bupleurum root prescriptions tended to be used more frequently in patients with a shorter history of illness, whereas the drugs for eliminating disturbances of the vascular system were used constantly regardless of the length of the illness. In the present study, we used two types of rehmannia root prescription, i.e., Hachimi-jio-gan and Gosha-jinki-gan. The activity of these medical prescriptions was lower than that of rhubarb prescriptions, but higher than that of bupleurum root prescriptions. Thus, our results with these rehmannia root prescriptions are not consistent with those obtained in the aforementioned patients with complicated diabetes. On the other hand, when the effects of 21 galenicals constituting these Oriental medical prescriptions were tested on the formation of AGEs, Rhei Rhizoma was found to have the highest activity, suggesting that Rhei Rhizoma is responsible for the activity found in rhubarb prescriptions. Tannin-containing galenicals such as Cinnamomi Cortex, Moutan Cortex and Paeoniae Radix all proved to have high activity, suggesting that tannin is involved in the inhibition of the protein glycation reaction. Sakurai and Tsuchiya[23] and Bucala *et al.*[27] stated that superoxide (O_2^-) is produced in the process of AGEs formation from the Amadori products, and that O_2^- reacts with nitric oxide (NO) to produce peroxynitrite ($ONOO^-$) and consequently the hydroxyl radical (·OH). Since tannin eliminates these radicals, as has been demonstrated in our previous study,[28-32] it seems reasonable that tannin has an influence on the inhibition of AGEs formation. In contrast, Rehmanniae Radix and Bupleuri Radix, the major ingredients of rehmannia root prescriptions and bupleurum root prescriptions, respectively, proved to have no inhibitory action on AGEs formation. Therefore, how the activity of rehmannia root prescriptions and bupleurum root prescriptions is produced remains to be clarified by further studies.

The use of aminoguanidine as a protein glycation inhibitor is proceeding to the development stage,[33] and a large-scale double blind clinical study is now underway in patients with diabetic nephropathy. However, careful studies of the safety, as well as the efficacy, of this agent are particularly important. Under these circumstances, new protein glycation inhibitors in addition to aminoguanidine are awaited. Methylguanidine and aminoguanidine derivatives have been examined in diabetic animals.[34] However, there have been few relevant reports on Oriental medicines. The results of the present study revealed that some Oriental medical prescriptions and component galenicals had a more potent inhibitory action on AGEs formation than aminoguanidine, suggesting the possibility of developing remedies for diabetic nephropathy from Oriental medicines. This possibility is also supported by our previous study, in which rhubarb extract was shown to improve the blood glucose levels and parameters of hyperlipidemia as well as renal function in rats with diabetic nephropathy.[35]

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和文抄録

advanced glycation endproducts (AGE) は糖尿病性

腎症の成因に大きく関与しており，AGE生成の抑制が腎症発症・進展防止に重要であることが知られている。このことから，本研究ではAGE阻害薬の可能性を和漢薬に求め，*in vitro* の評価系で検討した。使用した漢方方剤12種類のうち，最もAGE生成を抑制した漢方方剤は温脾湯で，次いで桃核承気湯，桂枝茯苓丸，大黄牡丹皮湯の順であった。実験に供した21種類の構成生薬では大黄，桂皮，牡丹皮，芍薬に強い抑制作用を認め，大黄剤，駆瘀血剤ならびにタンニン生薬がAGEの生成を抑制しているものと考えられた。またこれらは陽性対照物質のアミノグアニジンより強い活性を示した。

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