Effects of (dihydro) caffeic acid tetramer isolated from Salviae Miltiorrhizae Radix on renal function

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Abstract

The effect of (dihydro) caffeic acid tetramer isolated from Salviae Miltiorrhizae Radix on renal function parameters was examined. After intraperitoneal administration of (dihydro) caffeic acid tetramer (2.5, 5 or 10 mg/kg body weight) to rats, the levels of glomerular filtration rate, renal plasma flow and renal blood flow were increased dose-dependently, indicating the excretion-facilitating action of this substance through activation of renal function.

Key words (dihydro) caffeic acid tetramer, glomerular filtration rate, renal plasma flow, renal blood flow, renal failure, rat.

Introduction

(Dihydro) caffeic acid tetramer was previously isolated from Salviae Miltiorrhizae Radix, as a new substance which induces a decrease in the blood levels of urea nitrogen, creatinine, methylguanidine and guanidinosuccinic acid in rats with renal failure.[1] The present study was carried out to investigate the effects of the substance of renal function parameters and to elucidate its characteristic features.

Materials and Methods

Animals and treatment : Male rats of the LWH : Wistar strain with a body weight of 200-210 g, were placed in metabolic cages and kept at a temperature of 23±1℃ under a 12-hr dark-light cycle. They were allowed an adaptation period of several days, during which they were fed on a commercial feed (type CE-2, CLEA Japan Inc., Tokyo, Japan). They were then fed *ad libitum* on an 18% casein diet containing 0.75% adenine, which produced experimental renal failure in the animals. In rats with adenine-induced renal failure, renal impairment becomes aggravated as the period of adenine feeding increases. It was previously confirmed by histological and biochemical procedures that renal failure was present after 6 days of adenine ingestion.[2-5] (Dihydro) caffeic acid tetramer dissolved in saline was administered intraperitoneally (2.5, 5 or 10 mg/kg body weight) to rats after they had been fed an adenine diet for 6 days. Control rats were treated with an equal volume of saline. The blood urea nitrogen level of the rats used in this experiment was significantly increased to 2.7 times (46.1±3.5 mg/dl) that of normal rats. Six rats were used for each experimental group. Values were expressed as means±S.E.

Purification of (dihydro) caffeic acid tetramer from Salviae Miltiorrhizae Radix : As reported previously,[1] an aqueous extract of Salviae Miltiorrhizae Radix (*Salvia Miltiorrhiza* BUNGE) powder was eluted with an H_2O-MeOH-acetone solvent

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system in an MCI-GEL CHP-20P column, to obtain fractions I (H_2O eluate), II (50% MeOH eluate) and III (MeOH-acetone eluate). Fraction II was further fractionated by column chromatography using MCI-GEL CHP-20P and Sephadex LH-20, and after drying, a pale yellowish-brown amorphous powder was obtained. This powder was identified as (dihydro) caffeic acid tetramer in terms of carbon-13 nuclear magnetic resonance (^{13}C-NMR), proton nuclear magnetic resonance (^1H-NMR) spectra, and other data (Fig. 1). The main components were found to be sugar and organic acid in fraction I, and nonpolar phenol and quinone in fraction III.

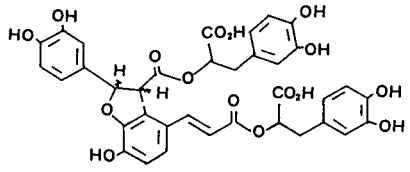

Fig. 1

Examination of renal function : Glomerular filtration rate (GFR), renal plasma flow (RPF), hematocrit (Ht) and renal blood flow (RBF) values were obtained at 5.5－6.0 hr after intraperitoneal administration of the (dihydro) caffeic acid tetramer, since the levels of urea and creatinine excretion were increased about 6 hr after administration. GFR and RPF were measured by renal clearance test using a single intravenous adminstration of sodium thiosulfate or sodium *para*-aminohippurate, respectively, as an indicator.[6,7] At 25 min after intravenous administration of either of these agents, the bladder was reflexly emptied by having each rat inhale ether for 3－5 sec. The urine thus voided was discarded. During the next 30 min, the urine was collected, and collection was terminated after the bladders had again been emptied reflexly by ether inhalation. Blood samples were taken from conscious rats by heart puncture in the middle of the period used for the clearance test. Thiosulfate and *para*-aminohippurate were determined by titrimetry and colorimetry, respectively. RBF was calculated on the basis of RPF and Ht using the equation shown below. Ht was determined with a hematocrit measurement apparatus, model KH-120A (Kubota Co., Ltd., Tokyo, Japan).

$$RBF=\frac{RPF}{1-Ht}\ (ml/min)$$

Statistics : The significance of differences between the control and (dihydro) caffeic acid-treated groups was tested by Student's t test. A p value greater than 0.05 was considered to be statistically insignificant.

Results

The GFR progressively dropped as kidney damage increased due to extended administration of adenine. The rats of the (dihydro) caffeic acid tetramer-treated group showed a dose-dependent increase in the GFR ; as shown in Table I, intraperitoneal administration of 2.5 mg (dihydro) caffeic acid tetramer caused a 9% increase in GFR as compared with the control rats. A further increase in the dose to 5 mg produced a further increase of 33% in the GFR value. (Dihydro) caffeic acid tetramer at a dosage level of 10 mg produced a significant rise in GFR from 2.75 ml/min/kg to 3.90 ml/min/kg (a 42% change, $p<0.05$). In an examination of the effect of intraperitoneal administration of (dihydro) caffeic acid tetramer on RPF, significant increases were observed at the 5 mg and 10 mg levels. As shown in Table I, the RPF value was increased from 12.08 ml/min/kg to 15.90 ml/min/kg at the 5 mg level (a 32% change, $p<0.05$) and from 12.08 ml/min/kg to 17.82 ml/min/kg at the 10 mg level (a 48% change, $p<0.05$). However, there was no significant difference in RPF between the control and (dihydro) caffeic acid tetramer-treated groups at the 2.5 mg dosage level. Changes in RBF calculated from RPF and Ht are shown in Table I. The administration of (dihydro) caffeic acid tetramer at a dose of 2.5 mg caused an increase in RBF (this variation was not statistically significant). However, the RBF value showed a direct correlation with the amount of (dihydro) caffeic acid tetramer administered. The intraperitoneal administration of 5 mg (dihydro) caffeic acid

Table I Effect of (dihydro) caffeic acid tetramer on renal function.

Material	Dose (mg/kg B.W.)	GFR (ml/min/kg)	RPF (ml/min/kg)	RBF (ml/min/kg)
Control	—	2.75±0.22 (100)	12.08±1.57 (100)	24.67±3.16 (100)
(Dihydro) caffeic acid tetramer	2.5	3.01±0.33 (109)	13.30±1.96 (110)	26.92±2.80 (109)
(Dihydro) caffeic acid tetramer	5	3.66±0.40* (133)	15.90±0.96* (132)	30.54±1.73* (124)
(Dihydro) caffeic acid tetramer	10	3.90±0.29* (142)	17.82±2.34* (148)	35.01±4.56* (142)

Abbreviations : GFR, glomerular filtration rate ; RPF, renal plasma flow ; RBF, renal blood flow. Figures in parentheses are percentages of the control value. *Significantly different from the control value, $p<0.05$.

tetramer increased RBF from 24.67 ml/min/kg to 30.54 ml/min/kg, a 24% change. (Dihydro) caffeic acid tetramer at a dose of 10 mg produced a significant rise in RBF from 24.67 ml/min/kg to 35.01 ml/min/kg (a 42% change, $p<0.05$).

Discussion

Constituents of Salviae Miltiorrhizae Radix so far reported include naphthoquinone (phenanthrene quinone) derivatives, such as tanshinone I, tanshinone II-A, tanshinone II-B, cryptotanshinone, isotanshinone I, isotanshinone II, isocryptotanshinone, tanshinonic acid, hydroxytanshinone and miltirone.[8] The substance examined in the present study, (dihydro) caffeic acid tetramer, is a newly found constituent of Salviae Miltiorrhizae Radix. We have previously reported the biological activity of this substance, specifically its suppressive effect on blood urea nitrogen in rats with adenine-induced renal failure.[1] The present study demonstrated the dose-dependent action of this substance of GFR, RPF and RBF, and therefore it appears to have the same action as that previously reported for an aqueous extract from Salviae Miltiorrhizae Radix.[9] The stereoisomer and geometric isomer of (dihydro) caffeic acid tetramer and caffeic acid were found to have no renal function-activating action. The sugar, organic acid, nonpolar phenol and quinone fractions (fractions I and III) showed no such activity either (data not shown). On the other hand, the action of (dihydro) caffeic acid tetramer at a dose of 5 mg/kg body weight corresponds to that of aqueous extract at a dose of 100 mg/kg body weight. Thus, the comparable action at a dose 1/20 that of the aqueous extract indicates that (dihydro) caffeic acid tetramer is the constituent responsible for the activation of renal function by Salviae Miltiorrhizae Radix. In a previous paper, we have reported the improvement of uremia by Rhei Rhizoma, the action being attributed to a proanthocyanidin oligomer of low-molecular-weight tannin.[10] As shown in the present study, the active component of Rhei Rhizoma is different from that of Salviae Miltiorrhizae Radix. The action mechanism of these two components should be further studied in detail. At present, however, it is speculated that Salviae Miltiorrhizae Radix facilitates excretion mainly through activation of renal function, since it has a potent action on GFR, RPF and RBF, whereas Rhei Rhizoma improves the state of renal failure by suppressing the production in the body of uremic toxins such as methylguanidine and guanidinosuccinic acid. Both these active components exhibit peculiar effects in comparison with other constituents of conventional herb medicines.

和文抄録

丹参より単離した新規物質 (Dihydro) caffeic acid tetramer の腎機能パラメーターに及ぼす影響を検討した。(Dihydro) caffeic acid tetramer 2.5, 5, 10 mg/kg 体重の腹腔内投与により糸球体濾過値 (GFR), 腎血漿流量 (RPF), 腎血流量 (RBF) が

用量依存的に増加し，腎機能亢進作用を有することが明らかとなった。

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