Antianoxic Action and Active Constituents of Atractylodis Lanceae Rhizoma

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The screening test was carried out to identify new drugs from natural products for the KCN-induced anoxia model in mice. Acetone extract of Atractylodis Lanceae Rhizoma (*Atractylodes lancea* DC. var. *Chinensis* KITAMURA) had a significant effect in the KCN-induced anoxia model and therefore the extract was selected for further study in order to identify the active principles. The result showed that β-eudesmol was the active component in Atractylodis Lanceae Rhizoma.

Keywords potassiumcyanide-induced anoxia; antianoxic action; Atractylodis Lanceae Rhizoma; sesquiterpenoid; β-eudesmol

Introduction

Atractylodis Lanceae Rhizoma is used therapeutically in China and Japan[1] for rheumatism, abdominal distension, poor appetite with nausea, retention of fluid and phlegm, edema and mild diarrhea. We have already reported the antianoxic action of Evodiae Fructus.[2] The screening test was carried out to identify new drugs from natural products for the KCN-induced anoxia model in mice, and an antianoxic effect was observed for an acetone extract of Atractylodis Lanceae Rhizoma, sesquiterpenoids containing fraction and β-eudesmol. In a preliminary experiment in this laboratory using 50% methanol extract and an acetone extract of Atractylodis Lanceae Rhizoma, the acetone extract showed significantly greater effects than the 50% methanol extract on the KCN-induced anoxia model. Therefore, an analysis of the active constituents of Atractylodis Lanceae Rhizoma was carried out using the acetone extract.

Materials and Methods

Experimental Materials and Procedure Atractylodis Lanceae Rhizoma (*Atractylodes lancea* DC., Compositae) was obtained from a local market in Osaka. It was a standard Japanese pharmacopeia and was powdered, soaked in 5 volumes of acetone or 50% methanol for 2 d, and filtered. This procedure was repeated 3 times. The filtrate was concentrated under reduced pressure below 40 °C, and the solvent was completely eliminated. The yields of the acetone extract and 50% methanol extract were 9.8% and 18.5%, respectively. Fractionation of the active constituents and purification of β-eudesmol were carried out as shown in Fig. 1 and identified based on mass (MS), nuclear magnetic resonance (NMR) and infrared (IR) spectra.[3]

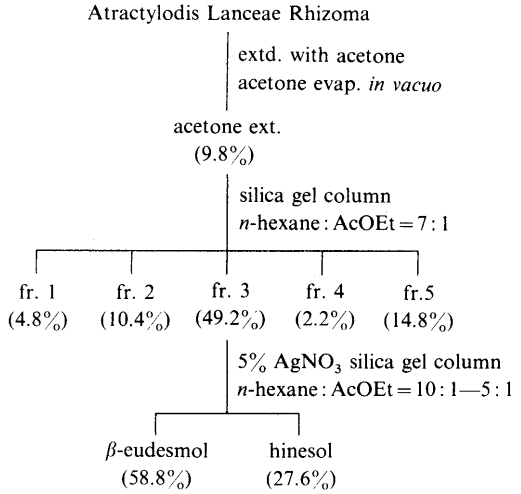

Fig. 1. Flow Diagram of Extraction and Fractionation of an Acetone Extract of Atractylodis Lanceae Rhizoma

KCN-Induced Anoxia Model in Mice Male ddy mice (Kitayama Labes) weighing 18—20 g were injected with a fatal dose of KCN (3.0 mg/kg) through the tail vein. The time taken to reach respiratory arrest was recorded as the survival duration and was measured for 180 s following the KCN injection. Mice which did not show respiratory arrest during the 180 s were counted as survivors, and their survival duration was recorded as 180 s for the calculation of the mean survival duration. Test drugs were suspended in purified water containing 5% arabic gum and administered orally 1 h before the KCN administration. The dosage of fraction was determined based on the rate of recovery for each of the fractions. The control groups were orally administered a mixture of arabic gum and purified water 1 h before the KCN injection. Vinpocetine and flunarizine, used here as reference drugs, were suspended in the same manner as described for the test drugs, and were orally administered as were the test drugs. The effects of the test drugs were evaluated using Student's *t*-test for the survival duration and Fisher's exact test method for the death rate. The dosage was determined based on the rate of recovery for each of the fractions.

Results

Effect of Extracts All of the mice in the control group, which received the KCN administration, had about 30 s of repeated convulsive attacks followed by respiratory arrest leading to death. The mean survival duration was 30.9 ± 1.8 s. As shown in Table I, 9 out of 10 mice survived in the presence of Atractylodis Lanceae Rhizoma acetone extract at 1500 mg/kg, *p.o.*, and 6 out of 10 mice survived at 750 mg/kg, *p.o.* At these concentrations, the extract significantly lowered the death rate and prolonged the mean survival duration as compared to the control group. Flunarizine at 25 mg/kg, *p.o.*, used here as a reference drug, caused 9 out of 10 mice to survive.

Effect of Fraction 1 to 5 As shown in Table II, the mean

TABLE I. Effect of Atractylodes Lancea Acetone Extract on KCN-Induced Anoxia in Mice

Treatment	Dose (mg/kg)	Survival time (s)	Prolongation (%)	No. of mice surviving/ No. used (mortality %)
Control	—	30.9 ± 1.8	—	0/10 (100)
Acetone ext.	750	125.7 ± 22.3[a]	306.4	6/10 (40)[a]
	1500	168.7 ± 11.3[a]	445.5	9/10 (10)[a]
50% MeOH ext.	1500	46.1 ± 3.9	49.2	0/ 9 (100)
Flunarizine	25	166.6 ± 13.4[a]	438.6	9/10 (10)[a]

Test drugs were administered to mice 1 h (*p.o.*) before KCN (3.0 mg/kg, i.v.). Each value represents the mean ± S.E. *a*) $p < 0.01$, compared with the value of control group.

TABLE II. Effect of Fractions 1—5 on KCN-Induced Anoxia in Mice

Treatment	Dose (mg/kg)	Survival time (s)	Prolongation (%)	No. of mice surviving/ No. used (mortality %)
Control	—	32.7± 2.1	—	0/10 (100)
Fraction 1	75	35.8± 1.9	9.4	0/10 (100)
Fraction 2	150	33.7± 2.5	2.9	0/10 (100)
Fraction 3	750	138.8±21.0[a)]	324.7	7/10 (30)[a)]
Fraction 4	30	33.0± 2.0	0.9	0/10 (100)
Fraction 5	250	31.9± 1.4	−2.6	0/10 (100)
Vinpocetine	100	127.3±21.5[a)]	289.6	6/10 (40)[a)]

Test drugs were administered to mice 1 h (*p.o.*) before KCN (3.0 mg/kg, i.v.).Each value represents the mean±S.E. *a*) $p<0.01$, compared with the value of control group.

TABLE III. Effect of β-Eudesmol on KCN-Induced Anoxia in Mice

Treatment	Dose (mg/kg)	Survival time (s)	Prolongation (%)	No. of mice surviving/ No. used (mortality %)
Control	—	31.0± 1.6	—	0/10 (100)
β-Eudesmol	150	34.7± 2.0	11.7	0/10 (100)
	300	124.9±22.5[a)]	302.3	6/10 (40)[a)]
Flunarizine	25	167.3±12.7[a)]	438.9	9/10 (10)[a)]

Test drugs were administered to mice 1 h (*p.o.*) before KCN (3.0 mg/kg, i.v.). Each value represents the mean±S.E. *a*) $p<0.01$, compared with the value of control group.

survival duration of mice in the control group was 32.7±2.1 s and all 10 mice died. In mice treated with fraction 3 at 750 mg/kg, *p.o.*, it was 138.8±21.0 s and 7 out of 10 mice survived. Fraction 3, a sesquiterpenoids containing fraction, was significantly effective in prolonging life as compared to the control. The other fractions were not significantly effective.

Effect of β-Eudesmol In mice treated with β-eudesmol, sesquiterpenoid obtained from fraction 3 as described above, at 300 mg/kg, *p.o.*, there was a significant life-prolonging effect as compared to the control group. The mean survival duration was 124.9±22.5 s with the survival rate of 6 out of 10 mice for β-eudesmol as compared to the control group, whose mean survival duration was 31.0±1.6 s with a 0 out of 10 survival rate (Table III).

Discussion

In order to substantiate the stomachic effect of Atractylodis Lanceae Rhizoma, pharmacological effects have been reported as having an anti-ulcer effect,[4] increasing the secretion of bile[5] and the gastrointestinal motility activity.[6] The present study demonstrated that the acetone extract, and the fraction which was found to be β-eudesmol, exhibited an antianoxic effect. β-Eudesmol also showed strong antianoxic action. Although the mode of action is not yet entirely understood, these results suggest that the antianoxic action of Atractylodis Lanceae Rhizoma extract is due mainly to β-eudesmol. Further investigation of fraction 3 and β-eudesmol are currently in progress.

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