D．考察

本研究より、釣藤鉤はNOの関与による内皮依存性とカルシウム拮抗作用による内皮非依存性血管弛緩作用を有し、各々釣藤鉤含有タンニンと釣藤鉤含有アルカロイドが活性成分であることが明らかになった。In vitroにおいては、その含有量と弛緩率から、釣藤鉤含有タンニンがより強く作用していると考えられた。

さらに、近年、フリーラジカルが血管内皮でのNO作用を減弱させることやthromboxane A_2の産生を増加させるという報告があり、フリーラジカルが血管作動性に関与していることが知られている。そこで、xanthine/xanthine oxidaseのラジカル産生による血管収縮作用を用い、釣藤鉤の収縮抑制作用を検討した。その結果、釣藤鉤はSODと同様にフリーラジカルによる血管収縮を抑制する作用を有し、その活性成分は釣藤鉤含有タンニンとアルカロイドであると考えられた。

以上のことから、釣藤鉤は血管拡張作用並びにフリーラジカルによる血管収縮の抑制作用を有し、高血圧症や動脈硬化症の基ずく脳血管障害の予防効果を有する可能性が示唆された。

E．結論

一酸化窒素（NO）が関与する内皮依存性血管弛緩作用には釣藤鉤含有タンニンがSOD様作用を介して、内皮非依存性血管弛緩作用には釣藤鉤含有アルカロイドがカルシウム拮抗作用を介して関与する。

F．健康危険情報

なし

G．研究発表

1．論文発表

なし

2．学会発表

なし

H．知的財産権の出願・登録状況

1．特許取得

なし

2．実用新案登録

なし

3．その他

なし

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雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yutaka Shimada, Hirozo Goto, Toshiaki Kogure, Kazufumi Kohta, Takahiro Shintani, Takashi Itoh and Katsutoshi Terasawa	Extract prepared from the bark of Cinnamomum cassia Blume prevents glutamate-induced neuronal death in cultured cerebellar granule cells.	Phytotherapy Research	14	466-468	2000
Hirozo Goto, Iwao Sakakibara, Yutaka Shimada, Yuji Kasahara, Katsutoshi Terasawa	Vasodilator effect of extract prepared from Uncariae ramulus on isolated rat aorta.	American Journal of Chinese Medicine	28	197-203	2000

PHYTOTHERAPY RESEARCH
Phytother. Res. **14**, 466–468 (2000)

SHORT COMMUNICATION

Extract Prepared from the Bark of *Cinnamomum cassia* Blume Prevents Glutamate-induced Neuronal Death in Cultured Cerebellar Granule Cells

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We studied the protective effect of a water extract from the bark of *Cinnamomum cassia* Blume on glutamate-induced neuronal death by MTT assay and its action on $^{45}Ca^{2+}$ influx using cultured rat cerebellar granule cells. In a dose-dependent manner, this extract (10^{-5}–10^{-4} g/mL) significantly protected against glutamate-induced cell death and also inhibited glutamate-induced $^{45}Ca^{2+}$ influx. These results suggest that the bark of *Cinnamomum cassia* has a protective effect on glutamate-induced neuronal death through the inhibition of Ca^{2+} influx.

Keywords: *Cinnamomum cassia* Blume; Cinnamomi cortex; glutamate; calcium; cultured cerebellar granule cells.

INTRODUCTION

In traditional Japanese Oriental (Kampo) medicine, the bark of *Cinnamomum cassia* Blume, Cinnamomi cortex, is one of the medicinal plants that have been used for improving various diseases caused by insufficient blood microcirculation. This medicinal plant has also been often administered to patients suffering from ischaemic brain diseases.

Glutamate is known to be one of the mediators of neuronal death caused by ischaemic-hypoxic injury in the brain (Choi, 1990). The extracellular concentration of glutamate elevates in the brain during ischaemia (Benveniste *et al.*, 1984). Excessive release of glutamate resulting from ischaemia overstimulates glutamate receptors, especially NMDA receptors, and induces neuronal death through Ca^{2+} influx (Rothman and Olney, 1986).

In this study, we found that a water extract from the bark of *Cinnamomum cassia* provided a marked protective effect against glutamate-induced neuronal death and also an inhibitory effect on Ca^{2+} influx *in vitro*.

MATERIALS AND METHODS

Cell culture. Cerebellar granule cells were cultured in poly-L-lysine (Sigma) coated 96-well culture plates at a density of 10^6 cells/mL from the brains of 7–8 day old Wistar rats as described previously (Gallo *et al.*, 1982; Shimada *et al.*, 1998). The cells were used at 7–8 days *in vitro* for the experiments.

Preparation of *Cinnamomum cassia* extract. The extract of *Cinnamomum cassia* (CCE) was prepared from the bark of *Cinnamomum cassia* Blume (CC) purchased commercially (China origin, Tochimoto Pharmaceuticals, Osaka, Japan). The herbal material (lot no. 10896) was authenticated by Dr Y. Yamamoto (Tochimoto Pharmaceuticals). The extract (5.1 g) was obtained by boiling the bark (100 g) in water (500 mL) for 50 min and then freeze-drying into a resultant powder.

Cell viability. Cell viability was assessed by MTT staining as previously described (Mosmann, 1983; Shimada *et al.*, 1998). Cultured cells were washed with Mg^{2+}-free Locke's solution (in mM: 154 NaCl, 5.6 KCl, 3.6 $NaHCO_3$, 5.0 HEPES, 2.3 $CaCl_2$, 5.6 glucose, pH 7.4), then incubated in this solution with or without (control) 100 μM-glutamic acid (glutamate) (Sigma), or various concentrations (10^{-6}–10^{-4} g/mL) of CCE with glutamate (100 μM). For a positive control, AP5 (RBI, Natick, MA, USA), a specific NMDA receptor antagonist was used. After 1 h incubation at 37 °C, MTT (Sigma) (500 μg/mL) was applied and incubated for 30 min at 37 °C. Cells were then washed and lysed in isopropanol with 0.04 N HCl to dissolve the blue formazan products. Optical density was read at 570 nm with a spectrophotometer and expressed as a percentage of the control.

$^{45}Ca^{2+}$ influx. Influx of $^{45}Ca^{2+}$ was measured as

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