Induction of apoptosis by rhapontin having stilbene moiety, a component of rhubarb (*Rheum officinale* Baillon) in human stomach cancer KATO III cells

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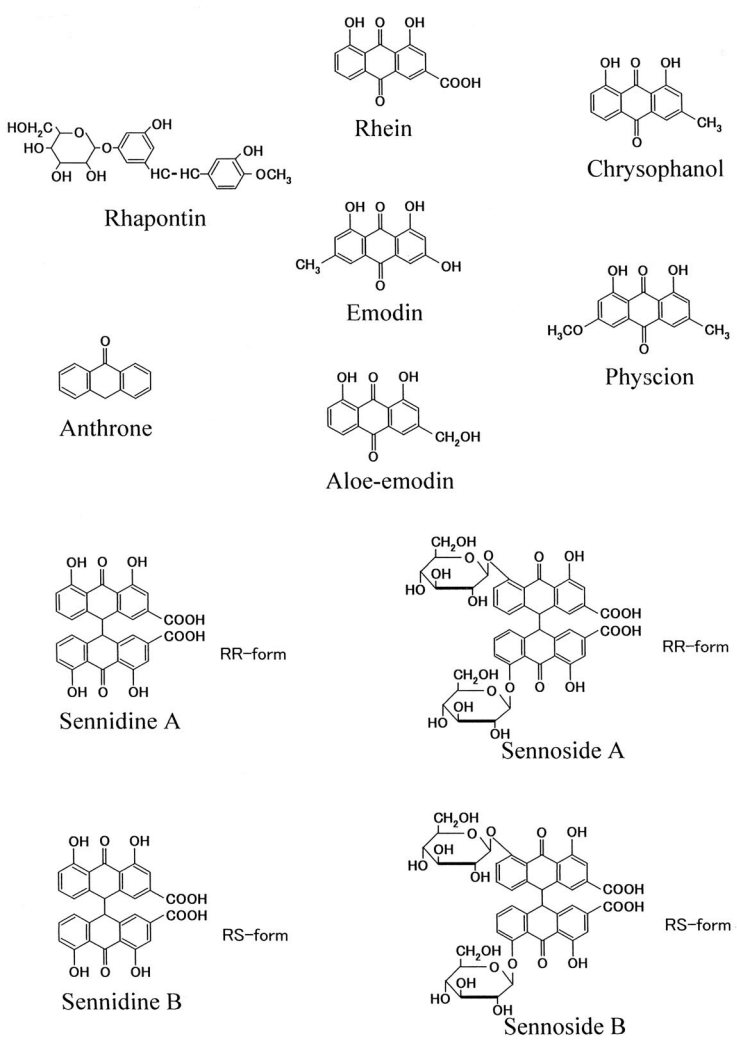

Various components of rhubard (*Rheum officinale* Baillon)

Introduction

Rhubarb (*Rheum officinale* Baillon) is a hardy perennial plants, growing mainly at the higher land of more than 3000 m in China. Rootstock of this plant is traditionally used as a purgative. Many compounds such as physcion, sennidine A and B, sennoside A and B, chrysophanol, emodin, anthrone, aloe-emodin, rhein and rhapontin were reported to be isolate from rhubarb. Recently, considerable attention has been focussed on the sequence of events referred to as apoptosis, and the role of this process in mediating the lethal effects of antineoplastic agents in leukemic cells. Apoptosis is a highly regulated process that is characterized by cell shrinkage, membrane blebbing, chromatin condensation and formation of a DNA ladder with multiple fragments of 180-200 bp caused by inter-nucleosomal DNA cleavage. In this report, we demonstrate for the first time that rhapontin, a component of rhubarb (*Rheum officinale* Baillon), induce apoptosis resulting in suppression of proliferation of human stomach cancer KATO III cells, and that stilbene moiety in the molecule is essential for the induction of apoptosis.

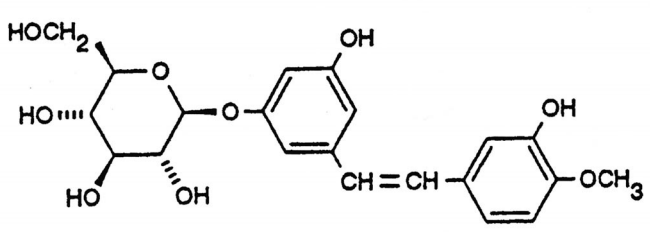

Figure 1. Chemical structure of rhapontin

Table 1. Effects of rhapontin on the growth of KATO III cells

	Concentration (μ M)	Inhibition (%)
Vehicle (50% ethanol)	0	0
rhapontin	50	29.3
	100	26.8
	200	98.6

KATO III cells were exposed to rhapontin at the indicated concentrations for 3 days before the viable cell number was estimated by Trypan blue dye exclusion method. Each value is the mean of triplicate experiments.

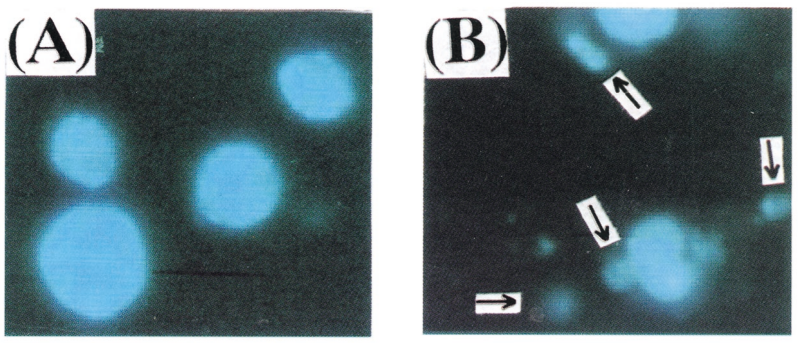

Figure 2.
Morphological changes of KATO III cells.
(A) Non-treated KATO III cells.
(B) KATO III cells treated with 200 μM rhapontin for 3 days. Arrows indicate apoptotic cells.

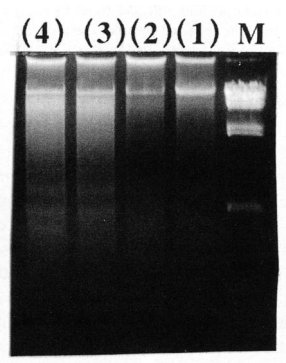

Figure 3.
Dose-dependency of DNA fragmentation by rhapontin in the KATO III cells. Cells were cultivated in the presence of a vehicle (lane 1), 50 μM (lane 2), 100 μM (lane 3) and 200 μM (lane 4) rhapontin for 3 days, then washed and harvested. Isolation of DNA from the cells and electrophoresis on 2% agarose gel were carried out as described in Materials & Methods. M: λDNA digested with Hind III.

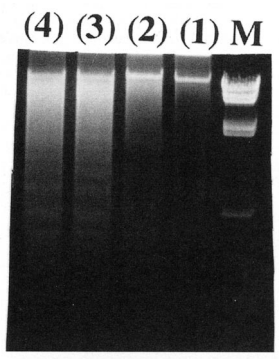

Figure 4.
Time-course of DNA fragmentation by rhapontin in the in the KATO III cells. Cells were cultivated in the presence of a vehicle (lane 1), or 200 μM rhapontin (lanes 2 ~ 4) for 1 day (lane 2), 2 days (lane 3) and 3 days (lane 4), then washed and harvested. Isolation of DNA from the cells and electrophoresis on 2% agarose gel were carried out as described in Materials & Methods. M: λDNA digested with Hind III.

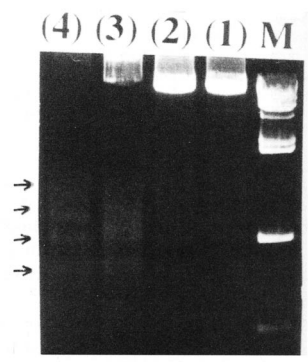

Figure 5.
Dose-dependency of DNA fragmentation by resveratrol in the KATO III cells. Cells were cultivated in the presence of a vehicle (lane 1), 50 μM (lane 2), 100 μM (lane 3) and 200 μM (lane 4) resveratrol for 3 days. Isolation of DNA from the cells and electrophoresis on 2% agarose gel were carried out as described in Materials & Methods. M: λDNA digested with Hind III.

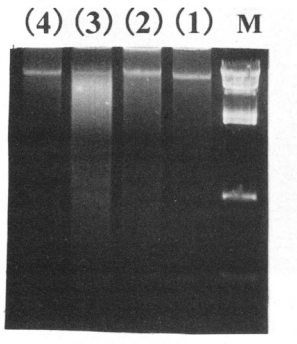

Figure 6.
Effect of N-acetyl-L-cysteine, an antioxidant drug, on DNA fragmentation caused by the addition of 200 μM rhapontin to the KATO III cells pre-cultivated with 5 mM N-acetyl-L-cysteine for 2 h. The cells were cultivated in the presence of a vehicle (lane 1), 5 mM N-acetyl-L-cysteine (lanes 2 and 4) and 200 μM rhapontin (lanes 3 and 4) for 3 days. Isolation of DNA from the cells and electrophoresis on 2% agarose gel were carried out as described in Materials & Methods. M: λ DNA digested with Hind III.

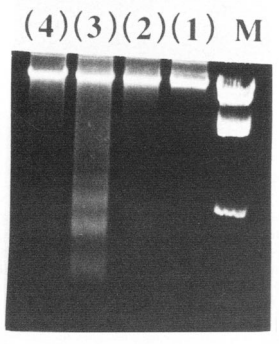

Figure 7.
Effect of N-acetyl-L-cysteine, an antioxidant drug, on DNA fragmentation caused by the addition of 200 μM resveratrol to the KATO III cells pre-cultivated with 5 mM N-acetyl-L-cysteine for 2 h. The cells were cultivated in the presence of a vehicle (lane 1), 5 mM N-acetyl-L-cysteine (lanes 2 and 4) and 200 μM resveratrol (lanes 3 and 4) for 3 days. Isolation of DNA from the cells and electrophoresis on 2% agarose gel were carried out as described in Materials & Methods. M: λ DNA digested with Hind III.

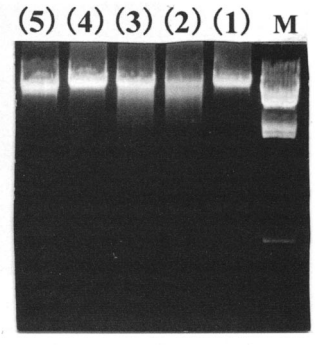

Figure 8.
Effects of rhapontin on normal lymphocyte cells. The cells were cultivated in the presence of a vehicle (lane 1), 50 μM (lane 2), 100 μM (lane 3), 200 μM (lane 4) and 300 μM (lane 5) rhapontin for 3 days. Isolation of DNA from the cells and electrophoresis on 2% agarose gel were carried out as described in Materials and Methods. M: (DNA digested with Hind III.

Results and discussion

Induction of apoptosis by rhapontin. The significant growth-inhibitory activity of rhapontin led us to investigate whether part of the antitumor effect of rhapontin was a result of apoptosis induction. Morphological change showing apoptotic bodies and fragmentation of genomic DNA into oligonucleosomal-sized fragments are characteristics of the occurrence of apoptosis. The morphology of the treated KATO III cells shows apoptotic bodies after 3 days of treatment with 200 μM rhapontin (Fig. 2). Rhapontin was specific for the induction of apoptosis in KATO III cells, because other compounds from rhubarb such as physcion, sennidine A and B, sennoside A and B, chrysophanol, emodin, anthrone and rhein did not induce apoptosis. In the present study, apoptosis by rhapontin in the KATO III cells was observed for the first time.

Mechanism of apoptosis induced by rhapontin and resveratrol. The mechanism of apoptosis inductions by rhapontin and resveratrol was investigated. Active oxidants are reported to be a factor responsible for altering the characteristics of mitochondria membrane. Shimura et al reported on the emittance of apoptosis-inducing factors. They showed that active oxidant was an important factor in apoptosis induction through the non-dependent caspase cascade. In this study it was believed that the active oxidants would work as an apoptosis-inducing signal transfer substance. The active oxidants were attacked by the addition of N-acetyl-L-cysteine, an antioxidant, for 3 days. It was found that N-acetyl-L-cysteine suppressed the DNA fragmentations induced by rhapontin (Fig. 6) and resveratrol (Fig. 7). These findings suggest that growth inhibition of KATO III cells caused by rhapontin or resveratrol result from the induction of apoptosis caused by these compounds as well as the involvement of active oxygen in these inductions.