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Antitumor and Apoptosis-inducing Activities of Naphthoquinone : Esters Isolated from Thai Medicinal Plant : Rhinacanthus nasutus Kurz

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Antitumor and Apoptosis-inducing Activities of Naphthoquinone Esters Isolated from Thai Medicinal Plant: *Rhinacanthus nasutus* Kurz.

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[INTRODUCTION]

Rhinacanthus nasutus Kurz. (family Acanthaceae), has been used as Thai traditional medicine for the treatment of various cancers. The main bioactive components of the plant are known to be naphthoquinones. However, the action targets and anticancer mechanism of these potential natural compounds are poorly understood. In here, we investigated the growth inhibitory effect of rhinacanthins-C, -N and -Q, three main naphthoquinone esters isolated from the roots of *R. nasutus* Kurz., against HeLaS3 cells, and observed that these compounds induced apoptosis of the cells. Next, we examined the actual antitumor activity of the compounds. Since rhinacanthins are hydrophobic compounds, they are not able to inject in bloodstream as soluble form. Therefore, we attempted to solubilize them in liposomes. Liposomal rhinacanthins were injectable and brought therapeutic efficacy in Meth-A sarcoma-bearing BALB/c mice.

[METHODS]

In vitro assay Three main naphthoquinone esters, rhinacanthins-C, -N and -Q (Fig. 1), were isolated and purified from the roots of *R. nasutus* Kurz. HeLaS3 cell proliferation was determined by Tetracolor ONE. Rhinacanthins-induced apoptosis was determined with TUNEL assay, DNA fragmentation, and flow cytometry. The activity of caspase-3 was determined by a Caspase-3/CPP32 colorimetric assay kit.

Therapeutic experiment Liposomes composed of eggPC and eggPG with rhinacanthin-C, -N or -Q (6:3:1 as a molar ratio) were prepared in 0.3 M trehalose solution and sized by extrusion through 100-nm pores. Five week-old BALB/c male mice were implanted *s.c.* into the left posterior flank with $1X10^6$ cells/0.2 ml of Meth-A sarcoma cells. Liposomes containing rhinacanthins at the dose of 5.0 mg/kg/day as the drug were administered *i.p.* into Meth-A sarcoma-bearing mice daily from day 1 to day 10. The tumor volume of each mouse was monitored daily thereafter.

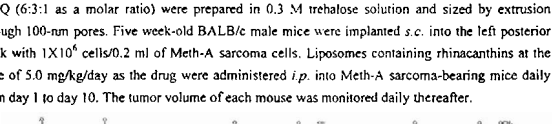

Fig.1. Structure of rhinacanthins RN-C (left), RN-N (center), and RN-Q (right)

[RESULTS AND DISCUSSION]

Induction of Apoptosis by rhinacanthins-C, -N and -Q in HeLaS3 cells The proliferation of HeLaS3 cells was significantly inhibited by rhinacanthins when the concentration of these drugs was increased (3-100 μM) as well as the exposure time was prolonged (24-72 h). The IC_{50} values of free rhinacanthins-C, -N and -Q were 80.0, 65.0 and 73.0 μM of 24 h, and 1.5, 1.5 and 5.0 μM for 72 h, respectively. These results suggested that three main naphthoquinone esters could inhibit the growth of HeLaS3 cells in dose- and time-dependent manners. To determine whether cells death induced by rhinacanthins is associated with apoptosis, we examined the apoptotic characteristics in HeLaS3 cells by morphological changes, DNA fragmentation and cell cycle arrest, detecting by TUNEL staining assay, agarose gel electrophoresis and FACScan. All of these results indicated that rhinacanthins induced apoptosis of the cells. Moreover, rhinacanthin–N activated caspase-3 in the cells.

In vivo antitumor activity of liposomal rhinacanthins Since rhinacanthins showed limited solubility in water, we attempted to prepare liposomal rhinacanthins for *in vivo* use. Rhinacanthins were liposomalized with encapsulation efficiencies of about 100 %. The average sizes of liposomes were 99-106 nm, and the ζ-potentials for them were about -40 mV. Then, the antitumor activities of liposomal rhinacanthins were evaluated. As shown in Fig.2, liposomal rhinacanthins suppressed tumor growth. The mean survival time of untreated and liposomal rhinacanthins-C, -N or -Q treated groups were 32, 43, 45 and 41 days, respectively. These data indicate that the treatment by liposomal rhinacanthin drugs as injectable formulation markedly suppressed the tumor growth and enhanced the survival time of the Meth-A sarcoma-bearing BALB/c mice.

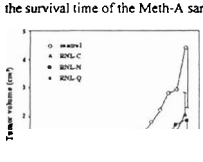

Fig. 2. Suppression of tumor growth by liposomal rhinacanthins-C, -N and -Q in Meth-A sarcoma-bearing mice. Five week-old BALB/c male mice (n=6) were implanted subcutaneously with Meth-A sarcoma cells into their left posterior flank. At 1-10 days after tumor implantation, they were injected intraperitoneally with 0.3 M trehalose (open circle), 5.0 mg/kg/day of liposomal rhinacanthin-C, -N, or -Q. Tumor volume was monitored every day. Data are presented as mean tumor volume and S.D. The S.D. bars are shown only for the last points for the sake of graphic clarity. Significant difference from control is indicated as $*p< 0.05$. Similar results were obtained in separate experiments.

[CONCLUSIONS]

In conclusion, our findings for the first time demonstrate that three main naphthoquinone esters; rhinacanthins-C, -N and -Q isolated from *R. nasutus* Kurz. are capable to inhibit proliferation and induce apoptosis in HeLS3 cells in a dose- and time-dependent manners. It can be considered that the antitumor efficacy of rhinacanthin drugs results from multiple of actions, such as cell cycle arrest, caspase-3-mediated apoptosis as well as targeting DNA topoisomerase II of tumor cells. Correspondingly, liposomal rhinacanthins effectively suppressed tumor growth in Meth-A-bearing mice. Finally, we conclude that liposomes are useful for injectable formulation of hydrophobic drugs.