Natural Product Sciences
7(2) : 60~62 (2001)

Radiosensitizing and Topoisomerase I Inhibitory Effects of *Aloe vera*, *Formitella fraxinea*, and *Ulmus davidiana* Extracts

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Abstract – *Ulmus davidiana*, *Formitella fraxinea*, and *Aloe vera* extracts were detected to have inhibitory effects against topoisomerase I at treatment of 5μg. *Ulmus davidiana* and *Aloe vera* extracts were found to show inhibitory effect similar to camptothecin, *Formitella fraxinea* extract was found to have weak activity. We also found the potential use of those extracts as a radiation sensitizer. Radiosensitizing effect at combination treatment was increased more than 2 times at single treatment of radiation, *Ulmus davidiana* or *Formitella fraxinea* extracts. *Ulmus davidiana* and *Formitella fraxinea* extracts were found to have significant radiosensitizing effect on test tumor cell line. But, *Aloe vera* extract was not detected to have activity as a radiosensitizer. *Ulmus davidiana* and *Formitella fraxinea* extracts are potent radiosensitizers on tumor cell lines and should be considered for further study of active compounds.

Key words – *Ulmus davidiana*, *Formitella fraxinea*, *Aloe vera*, topoisomerase I, radiosensitizer.

Introduction

DNA topoisomerase I has become an important target for chemotherapy since the discovery that camptothecin is a specific enzyme inhibitor, and that this effect is probably responsible for the potent antitumor activity of the drug. DNA topoisomerase I relaxes DNA supercoiling and is required for DNA synthesis, RNA transcription, and perhaps DNA repair and genetic rearrangements (Campoux, 1990; Wang, 1985), and have also been proposed as intracellular targets for cancer chemotherapy (Liu, 1989).

Topoisomerase I inhibitors are very known camptothecin, actinomycin D, and saintopin etc. Among these topoisomerase I inhibitors, camptothecin, representative topoisomerase I inhibitor, is isolated from wood of *Camptotheca accuminata* (Wall *et al.*, 1966). Other inhibitors also are isolated from natural products. While there are many potent antitumor agents acting on topoisomerase II, few agents have been found to act on topoisomerase I. Thus, it is very important to screen or detect biological effect of natural products. It is high possibility to screen noble antitumor agents for target of topoisomerase I inhibition from natural products.

For the evaluation of antitumor effect of *Aloe vera*, *Formitella fraxinea*, and *Ulmus davidiana* extract, the present study was detected inhibitory effect of topoisomerase I, and also exerted radiosensitizing effect at the combination with its three extract.

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Materials and Methods

Preparation of samples – Each 50 g of *Aloe vera*, *Formitella fraxinea*, and *Ulmus davidiana* was extracted with 1L of 75% methanol at room temperature for 3 days, and then evaporated to yield extracts.

Cytotoxicity assay – MTT assay was used to cytotoxic effect against the A549, KB, and HeLa tumor cell lines (Monks *et al.*, 1991).

Topoisomerase I inhibition assay – Topoisomerase I assay was performed according to the protocol provided by TopoGen, Inc. (Columbus, USA).

Radiosensitization assay – The combination effect of radiation and each extract is evaluated by MTT assay. Experimental design was used to modify Steren's method (Steren *et al.*, 1993). Each cell line was trypsinized separately and planted at a density of

5×10^3 cells/well in 4-well plate. Following 24 hours incubations at 37°C in CO_2 incubator, the extract was dispensed the appropriated wells and then incubated for 48 hours. The extract was removed after 48 hours and replaced with fresh media. After replacement with fresh media, each plate was irradiated at 10 Gy. After radiation exposure, all plates were incubated for 4 days. On day 4 after radiation, cytotoxicity was detected by MTT assay.

Results and Discussion

Cytotoxicity – *Aloe vera*, *Formitella fraxinea*, and *Ulmus davidiana* crude extracts exerted growth-inhibitory effects against A549, KB, and HeLa human tumor cell lines. IC_{50} values of *Aloe vera* extract were examined as 730, 820, and 817 μg/ml on A549, KB, and HeLa cell lines, respectively. In case of *Formitella fraxinea* extract, IC_{50} values were 620, 719, and 837 μg/ml on A549, KB, and HeLa, respectively. In case of *Ulmus davidiana* extract, IC_{50} values were 307, 202, and 125 μg/ml on A549, KB, and HeLa, respectively. *Ulmus davidiana* extract exerted stronger cytotoxic effect than *Aloe vera* and *Formitella fraxinea* extracts.

Topoisomerase I inhibition – *Aloe vera*, *Formitella fraxinea*, and *Ulmus davidiana* crude extracts were detected to show inhibitory activity against topoisomerase I at treatment of 5 μg. *Ulmus davidiana* and *Aloe vera* extract were found to have inhibitory effect similar to camptothecin 100 μM, but *Formitella fraxinea* extract was found to have weak activity than camptothecin 100 μM.

Radiosensitivity – Three extracts with radiation showed little cytotoxic effects on A549, KB, and HeLa cell lines. Cytotoxic effects on irradiation of 10 Gy were 80, 93, and 88% as survival rate on A549, KB, and HeLa, respectively. On treatment of *Aloe vera* extract alone, cytotoxic effect was 84, 87, and 83% as survival rate on A549, KB, and HeLa,

Table 1. IC_{50} of *Aloe vera*, *Formitella fraxinea*, and *Ulmus davidiana* extract on tumor cell lines.

Cell line	IC_{50} (μg/ml)		
	Aloe vera	Formitella fraxinea	Ulmus davidiana
A549	730	620	307
KB	820	719	202
HeLa	817	837	125

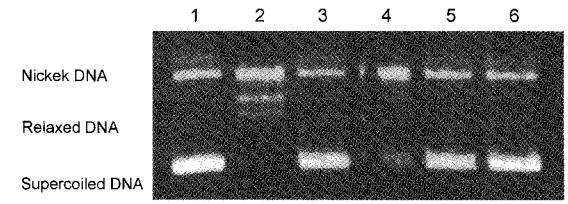

Fig. 1. Topoiomerase I inhibitory effect of 5 *Aloe vera*, *Formitella fraxinea*, and *Ulmus davidiana* extracts.
Lane 1: Supercolid DNA, Lane 2: Supercolid DNA + topoisomerase I, Lane 3: Supercolid DNA + topoisomerase I + camphtothecin (100 μM), Lane 4: Supercolid DNA + topoisomerase I + *Formitella fraxinea*, Lane 5: Supercolid DNA + topoisomerase I + *Ulmus davidiana*, Lane 6: Supercolid DNA + topoisomerase I + *Aloe vera*.

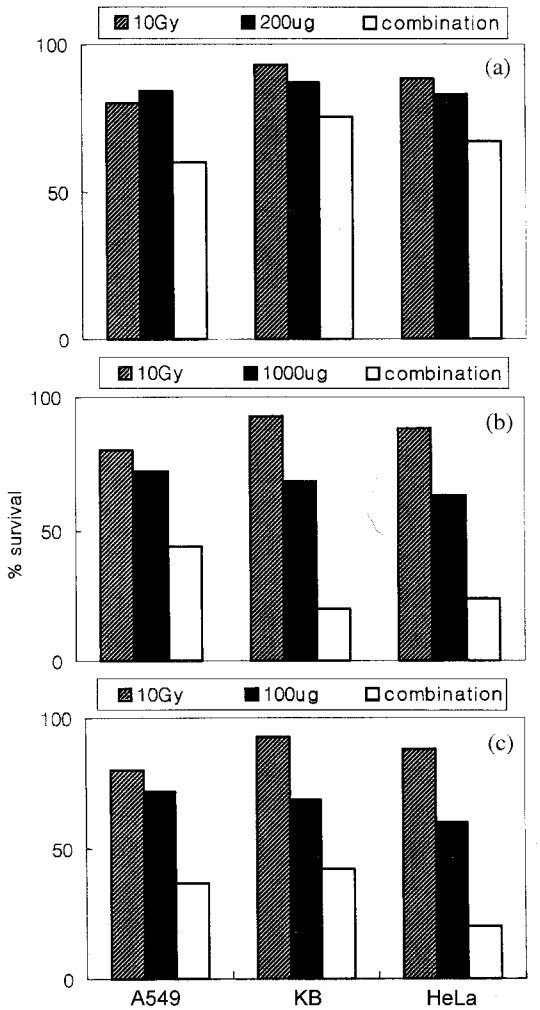

Fig. 2. Combination effect of radiation with *Aloe vera* (a), *Formitella fraxinea* (b), and *Ulmus davidiana* extract (c).

respectively. On treatment of *Formitella fraxinea* extract alone, cytotoxic effect was 72, 68, and 63% as survival rate on A549, KB, and HeLa, respectively. On treatment of *Ulmus davidiana* extract alone, cytotoxic effect was 72, 69, and 60% as survival rate on A549, KB, and HeLa, respectively. Among three extracts, the combination with radiation of *Ulmus davidiana* extract or *Formitella fraxinea* extract was observed to be 2 times more radiosensitive than its combination with *Aloe vera* extract. In combination of *Formitella fraxinea* extract and radiation, cytotoxic effect was 44, 20, and 24% as survival rate on A549, KB, and HeLa, respectively. In combination of *Ulmus davidiana* extract and radiation, cytotoxic effect was 37, 42, and 20% as survival rate on A549, KB, and HeLa, respectively. We found potential of the use of *Ulmus davidiana* and *Formitella fraxinea* as radiation sensitizer.

In this study, we found the following aspects. First, three extracts were examined to have effect of topoisomerase inhibition, and also have cytotoxicity against tumor cell lines. Second, in combination with radiation, *Aloe vera* extract was examined to have no synergistic effect. *Ulmus davidiana* and *Formitella fraxinea* extracts had potential radiosensitization effects.

Topoisomerase I inhibition of *Formitella fraxinea* is known by action of fomitellic acid and ursolic acid (Mizushina *et al.*, 2000). But, it is not yet known on the topoisomerase I inhibition of *Aloe vera* and *Ulmus davidiana*, and on the radiosensitizing effect of *Ulmus davidiana* and *Formitella fraxinea*. Thus, *Ulmus davidiana* and *Formitella fraxinea* should be considered for further study on their active compounds as topoisomerase inhibitor and radiosensitizer.

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(Accepted May 30, 2001)