Abstract: Asiatic acid is a pentacyclic triterpene contained in medicinal plants. The cytotoxic effect of this compound and its augmentative effect on the anticancer drug irinotecan hydrochloride (CPT-11) were investigated in the human colon adenocarcinoma cell line HT-29. Asiatic acid dose-dependently showed cytotoxicity in HT-29 cells. DNA fragmentation, annexin-positive apoptotic cells, and caspase-3 activation were observed in a dose-dependent manner. A caspase-3 inhibitor suppressed the DNA ladder formation in a concentration-dependent manner. Bcl-2 and Bcl-xL proteins were decreased by asiatic acid treatment. These results indicate that asiatic acid induced apoptosis in HT-29 cells via caspase-3 activation. Cytotoxic effects of combined treatment with CPT-11 and asiatic acid on HT-29 cells were further examined. Simultaneous treatment or sequential exposure first to asiatic acid and then to CPT-11 showed an additive effect. Synergism was observed when cells were first exposed to CPT-11 and then to asiatic acid. These results suggest that asiatic acid can be used as an agent for increasing sensitivity of colon cancer cells to treatment with CPT-11 or as an agent for reducing adverse effects of CPT-11. J. Med. Invest. 52: 65-73, February, 2005

Keywords: asiatic acid, apoptosis, CPT-11, combination, cytotoxicity
Chemicals

Cell lines and culture conditions

Cytotoxicity
DNA fragmentation analysis

Assessment of cell death using flow cytometry

Western blot analysis

Assay of caspase-3 activity

Drug interaction analysis
DNA fragmentation

Detection of early and late apoptotic cells by flow cytometric analysis

Bcl-2 and Bcl-xL expression in HT-29 cells

A. Asiatric acid (µg/ml)

B. Inhibitor (µM)

Cytotoxicity of asiatic acid

Detection of early and late apoptotic cells by flow cytometric analysis

Bcl-2 and Bcl-xL expression in HT-29 cells
A. 

\[ \text{\textbeta-actin} \]

Bcl-2

\[ \text{\textbeta-actin} \]

Bcl-x_L

Time (h)

0 6 12 24

B.

C. 

\[ \text{Caspase-3 activity (fold of increase)} \]

Asiatic acid (\(\mu g/ml\))

0 25 50 100

Growth % of control

Asiatic acid concentration (\(\mu g/ml\))

0 10 20 30 40 50

Growth % of control

Asiatic acid concentration (\(\mu g/ml\))

0 10 20 30 40 50

Growth % of control

Asiatic acid concentration (\(\mu g/ml\))

0 10 20 30 40 50
Activation of caspase-3 in HT-29 cells

Cytotoxicity of the asiatic acid/CPT-11 combination
et al
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