분류번호	Ⅱ-P-2

Induction of Apoptosis and Single Strand Breaks by Extract of Pulsatilla Koreana (SB-31).

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Extract of Pulsatilla Koreana (SB-31) showed promising antitumor activity in vitro (J. Kor Cancer Asso 26:959-963, 1994). We studied the mechanism of cytotoxicity of SB-31. HL-60 cells were cocultivated with various concentrations of SB-31 for 5 hours. The DNAs from HL-60 cells exposed to SB-31 showed the ladder pattern typical of apoptosis. Effect of SB-31 on topoisomerase I activity was determined by slight modification of the method by E.Aflalo(1994). The pBR322 DNA showed dose-dependent increase of R-Form DNA upon incubation with SB-31. The topoisomerase I-like activity (Increase of R-Form DNA) was accentuated with higher dose of SB-31. It is postulated that SB-31, which is a fermentation product of Pulsatilla Koreana and which loses its activity when kept in ambient temperature for more than 96 hours, may contain topoisomerase I-like activity and the enhanced excessive single strand breaks induced by SB-31 may result in apoptosis.

METHODS

Cell Lines: Gastric cancer cell line SNU-1, SNU-5, SNU-16 and HL-60 donated from Dr. J. G. Park of Seoul National Univercity Hospital was used.
Orther cell lines tested included A549, Hela, CasKi, MCF-7, HepG2 and Hep3B.
Drug tested: SB-31 is an extract of Pulsatilla Koreana specially prepared and provided by Hanbo Pharm. Co, Korea. It was kept at 4oC until use.
Various concentrations of SB-31 was used at 10 vol % of culture medium. The final concentrations of SB-31 were 0.3, 0.15, 0.075, 0.038, 0.019 mg/ml.
Test of cytotoxicity: A colorimetric assay using MTT, modified from Mosman, Carmichael and alley were used.
We exposed 7 wells of equal numbers of cancer cells and normal MNC to anti cancer agents for 96hrs before measuring the survival or fractional absorbance. Each experiment was performed for 3 times and the mean values are presented.
Test of Apoptosis: HL-60 cells were cocultivated with various concentrations of SB-31 for 5 hours. DNA was extracted from the cells and electrophorosed on 1% agarose gel, at 50v for 1 hour.
Test of Topo I Activity: Effect of SB-31 on topoisomerase I activity was determined by slight modification of the method by E. Aflalo(1994). pBR322 supercoiled DNA was incubated with various concentrations of SB-31 in the presence or in the absence of topoisomerase I for 1 hour. After stopping the reaction, the pBR322 DNA was electrophorosed on 0.8% agarose gel, at 50v for 45 minutes.

RESULTS

1. Gastric cancer cell lines SNU-1, SNU-5 and SNU-16 were exposed to 10 concentrations of SB-31 at culture time of 24, 48, 72, 92 and 120 hours. The ID_{50} value were minimal at 96hr of culture duration for 3 cell lines; 0.34, 0.16 and 0.53mg/ml for SNU-1, SNU-5 and SNU-16 respectively. Most fo the tested cell lines were about 3-4 fold more sensitive to SB-31 than normal MNC.
2. The DNAs from HL-60 cells exposed to SB-31 showed the ladder pattern typical of apoptosis.
3. pBR322 DNA showed dose-dependent increase of R-Form DNA upon incubation with SB-31. This increase of single strand break was accentuated at higher dose of SB-31.
4. The exact mechanism of action of *Pulsatilla Koreana* on the molecular level need further elucidation.