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Enhanced Cytotoxicity of Berberine and Some Anticancer Nucleotides Against Tumor Cell-lines.

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Berberine is one of the most widely distributed protoberberine type alkaloids containing a quaternary nitrogen with pka of about 15. It is known as one of the medicinally important alkaloids displaying a wide spectrum of antibacterial, antiprotozoal and antifungal activities. It was reported that berberine has considerable anticancer activity against Elrichand S-180 tumor cell-lines (Suffness *et al.*, 1985). Ara-C, a most common approved nucleoside anticancer drug, is increasingly used in clinical trials due to the effective remission of various acute leukemias and isoguanosine, a naturally occuring nucleoside analog of guanosine, is shown to have a strong anti-cancer activity *in vitro* and*in vivo* (Kim *et al.*, 1994). It is generally accepted that after nucleosides enter the cells by simple diffusion or with the help of membrane bound transport proteins, phosphorylation in the cytoplasm produces active nucleotide, which can inhibit tumor growth. However, due to charge density nucleotide analogs are unable to cross the cell membrane. Therefore, the first requirment for drug activity is the transport of these nucleotides into target organs through the lipophilic membranes. Many studies have concentrated on developing the means for reducing the charge and increasing the lipophilicity for the transport of anticancer nucleotides and have met some success with various lipophilic esters including long chain fatty acids (Maccoss *et al.*, 1978). Recently, for the selective transport of biologically active nucleotides, artificial quaternary amine salts including lipophilic groups have been employed as carriers of various nucleotides (Fruta *et al.*, 1991, Li *et al.*, 1992). Similar to these studies, we suppose the possibility of ion pair interaction between two anticancer drugs, that is the positive charge of berberine nitrogen and the anion charge of the nucleotides phosphate (Fig. 1), would enhance the membrane permeability. If two anticancer compounds can form an ion pairing complex in the aqueous phase, it can enhance cell membrane transport and finally increase anticancer activity against various tumor cell-lines (*in vitro and in vivo*). In this study, we confirmed that a mixture of berberine and anticancer nucleotides enhances cytotoxicity against some tumor cell-lines *in vitro*.

Berberine, arabinose cytidine(ara-C) and arabinosecytidine monophosphate (ara-CMP) were purchased from Sigma Co. and tumor cell-lines for in vitro studies from ATCC (American Tissure Culture Collection); P-388 (murine leukemia), Molt-4 cell-lines (human leukemia). Isoguanosine was synthesised in 5 steps from guanosine (Sigma Co.) using Divarker's-method (Divarkar *et al.*, 1991, Lee *et al*, 1994) and isoguanosine monophosphate (IGMP) was obtained easily with a kuown procedure (Eckstein *et al.*, 1978). For the preparation of ara-CMP, IGMP and their berberine mixture drugs, respectively, each 10 μmol-drug was dissolved in 10 ml distilled water for a 1mmol stock solution (in case of berberine-nucleotide mixture, 1mmol stock solution means 5mM berberine + 5mM nucletide). The stock solutions were filtered through Acrodisc (0.5 μM pore) and stored at 0°C. Aliquots were diluted in distilled water to produce the desired drug concentrations. For the study of growth inhibitory effect of drugs, tumor cell-lines were grown in RPMI-1640 medium supplemented with 10% FBS, streptomycin 0.1mg/ml and penicillin 100 units/ml at 37°C in 5% carbon dioxide, and the number of remaining cells were counted with a cell counter by MTT method. Each of the two agents, ara-CMP and IGMP was studied in combination with berberine against some tumor cell-lines *in vitro*.

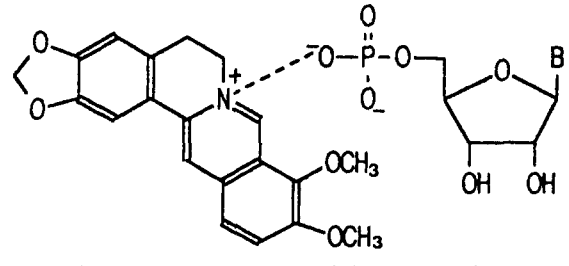

Fig. 1. Schematic representation of the proposed 1:1 complex formed between berberineand nucleoside monophosphate. B means purine or pyrimidine base.

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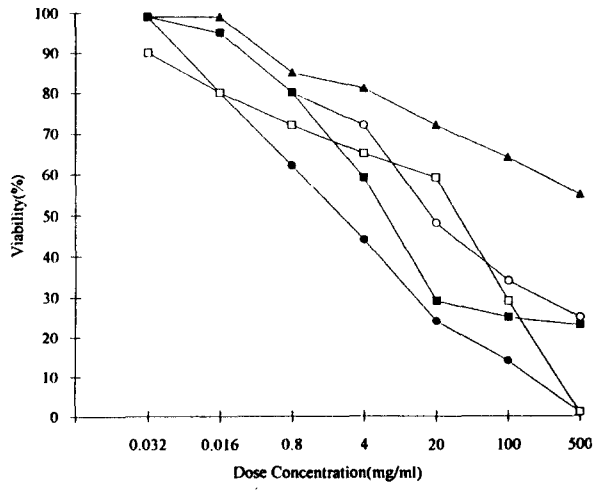

Fig. 2. Cytotoxicity of berberine and ara-CMP, Isoguanosine monophosphate (IGMP) either alone or used in combination against P-388. Berberine (□), ara-CMP (○), IGMP (▲), berberine+ara-CMP (■), berberine+ IGMP (●). In the combination experiments, the berberine: nucleotide molar ratio was 1:1. Data was derived from two independant experiments.

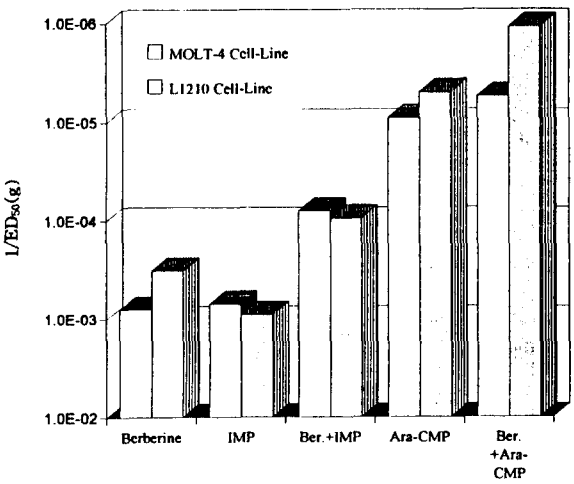

Fig. 3. Cytotoxicity of some nucleotides, and their berberine-mixture against Molt-4 cell-line and L1210. In the combination-experiments, the berberine: nucleotides molar ratio was 1:1. Ber.: berberine, IGMP: isoguanosine monophosphate, data-derived from three independant experiments

The results of these studies are summarized in Fig. 2 and 3. Fig. 2 shows that ara CMP, IGMP and their berberine mixture exhibited cytotoxicity against P-388. Two drugs used in combination, at a berberine:nucleotide molar ratio of 1:1, produced an enhanced cytotoxicity greater than either berberine or anticancer nucleotides given alone at all ranges of dosage.

Ara-CMP, IGMP and berberine, when individually tested, exhibited ED_{50} values of about 1.7×10^{-5}g/ml, above 5×10^{-4}g/ml and 3×10^{-5}g/ml, respectively, but ara CMP-berberine and IGMP-berberine mixtures show about 10-fold and 100-fold ED_{50} values of ara-CMP and IGMP, respectively. And berberine:anticancer nucleotide concentration ratio eliciting the maximum effect was 1:1. However, increased actions were observed also for other combination ratios (1:3, 1:5) (data not shown). Fig. 3 illustrates the effectiveness of drugs evaluated in terms of ED_{50} values against tumor cell-lines. All the nucleotide anticancer drugs in combination with berberine used in these experiments also gave a clearly superior cytotoxicity than the anticancer agents used alone.

The extent of cytotoxicity enhancement was in the range of from 2 to 20-times, The data obtained in this study represent a futher step toward elucidation of the synergistic mechanisms of berberine-anticancer nucleotide complex. However they can also offer new clues that enhanced cytotoxicity of berberine-nucleotide mixtures is due to the increased cell-membrane transport rates through the ion pairing complex of the two drugs.

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