similar terminal half-lives, $t_{1/2,\beta} = 0.693/\lambda$ (2.5 *versus* 3.2 h). These minor differences were also noted in the volumes of distribution of the central compartment [139 *versus* 160 mL/kg for the (+)- and (−)-enantiomer, respectively]. Although these pharmacokinetic parameter values illustrate differences between the enantiomers of I, the data do not establish a definitive pharmacokinetic profile in view of the limited sample number.

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Cytotoxic Effects of *Eleutherococcus senticosus* Aqueous Extracts in Combination with N^6-(Δ^2-Isopentenyl)-adenosine and 1-β-D-Arabinofuranosylcytosine Against L1210 Leukemia Cells

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Abstract □ The use of the aqueous extracts of *Eleutherococcus senticosus* in combination with either cytarabine or N^6-(Δ^2-isopentenyl)-adenosine gave additive antiproliferative effects against L1210 murine leukemia. The ED_{50} for *E. senticosus* root extracts against L1210 cells was ~75 μg/mL. *E. senticosus* appears to be potentially useful for reducing the concentration of conventional antimetabolites used for their antiproliferative effects on tumor cells.

Keyphrases □ Cytarabine—*Eleutherococcus senticosus*, N^6-(Δ^2-isopentenyl) adenosine, L1210 leukemia □ *Eleutherococcus senticosus*—cytarabine, N^6-(Δ^2-isopentenyl) adenosine, L1210 leukemia

The Far Eastern plant *Eleutherococcus senticosus* (Rup. + Maxim.) Maxim. (Araliaceae or Ginseng family), formerly known as *Hedera senticosa* and *Acanthopanax senticosus*, is commonly known as "Siberian Ginseng," "Touch-me-not," "Devil's shrub," "Eleutherococc," and "Wild Pepper" (1). This plant, which has recently become an item for export from the People's Republic of China, is most abundant in the Khabarovsk and Primorsk Districts of the Soviet Union, with a distribution extending to the middle Amur region in the North, Sakhalin Island and Japan in the East, and South Korea and the Chinese Provinces of Shansi and Hopei in the South (1, 2). *E. senticosus* has been used extensively in the Soviet Union as an "adaptogen" (3), defined by these authors (4) as a nontoxic substance with so-called "normalizing" actions on a wide range of physical, chemical, and biochemical parameters. The effects of *E. senticosus*, as well as those of other natural products for alleviating numerous pathological changes when administered on a chronic basis, have been the subject of several recent reviews (2, 5–7).

There have been numerous reports suggesting that crude, unfractionated *E. senticosus per se* has cytostatic activity as well as metastasis-preventing effects against the following systems: Walker 256 and Ehrlich ascites tumor cells (8), and SSK sarcoma (9), spontaneous tumors in AKR mice (10), indole oil-induced leukemia (11), mammary tumors in C3H mice (12), as well as urethane-induced pulmonary tumors in CC57 mice (13). Moreover, the literature also suggested that *E. senticosus* extracts when administered in combination with thiotepa (14, 15), cyclophosphamide (14, 16), hydrocortisone (17), 6-mercaptopurine (18), or rubomycin-C (19) had a potentiating or enhancing effect on the parent agent. These data suggested the importance in evaluating the possible effects of *E. senticosus per se*, and its ability to enhance the antiproliferative property of two established cytotoxic agents: cytarabine hydrochloride (I) and N^6-(Δ^2-isopentenyl)-adenosine hemihydrate (II).

BACKGROUND

Cytarabine (I), an important pyrimidine antimetabolite used in combination chemotherapy for the treatment of acute myelocytic leukemia (20–22), has the potential for the treatment of chronic myelocytic leukemia as well, when given with the deaminase-inhibitor, tetrahydrouri-

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Table I—Antiproliferative Effects of *E. senticosus* Extracts Against L1210 Leukemia Cells [a]

Concentration[c], μg/mL	Time, h[b] 24 Cells/mL × 10^{-5}	24 Inhibition[d], %	48 Cells/mL × 10^{-5}	48 Inhibition, %	72 Cells/mL × 10^{-5}	72 Inhibition, %
0	18 ± 1.0	0	38 ± 2.0	0	44 ± 2.0	0
25	16 ± 1.0	11	31 ± 1.0	18	37 ± 1.0	16
50	9 ± 1.5	50	23 ± 2.0	39	24 ± 2.0	39
100	6 ± 2.0	67	16 ± 4.0	58	22 ± 2.5	50
200	7 ± 1.0	61	18 ± 2.0	53	23 ± 1.0	48
400	6 ± 2.0	67	16 ± 1.0	58	22 ± 1.5	49

[a] Each T-flask contained 5 mL of growth medium (RPMI-1640 plus 10% fetal calf serum) plus 1 × 10^5 L1210 leukemia cells. Incubations were conducted at 37°C in a 5% carbon dioxide environment as described previously (43). [b] After added to culture medium. Aliquots (0.20 mL) of each cell suspension were aseptically removed at the designated time for the determination of total and viable cell count (43). [c] Experiments conducted in triplicate were initiated (zero time) when lyophilized *E. senticosus* in RPMI 1640 without serum was added (0.1–0.2 mL) to each culture flask at the final designated concentrations. [d] Inhibition of L1210 proliferation was based on a comparison of treated cultures *versus* untreated cells in control cultures.

dine (23). In addition, cytarabine can be used in combination with other agents for the palliative treatment of large bowel and stomach cancer (22).

The primary biochemical site of action for cytarabine following its enzymatic phosphorylation to cytosine arabinoside-5′-triphosphate is thought to occur as an inhibitor of DNA synthesis during the S-phase of mitosis (24, 25). It inhibits formation of new DNA replicons as well as chain elongation in cultured human lymphoblasts (26), and can be lethally incorporated into low molecular weight species of RNA in L5178Y mouse leukemia cells (27).

Resistance to the antileukemic property of cytarabine has been attributed to diminished phosphokinase (28, 29) and/or enhanced deaminase activity (30–33).

N^6-(Δ^2-Isopentenyl)adenosine (II), a nucleoside previously shown to be both an inhibitor of growth and cytotoxic to human leukemic myeloblast and Sarcoma-180 cells (34), and also found by Hare and Hacker (35) and also in several isoaccepting species of tRNA, is capable of interfering with the transport of unmodified nucleosides through the cytoplasmic membranes of mouse embryo cells at the level of the transmembrane translocation function. This inhibition of membrane transport is believed to be responsible for the ability of nucleoside II to alter RNA synthesis in phytohemagglutinin-stimulated mouse spleen lymphocytes as well as to be immunosuppressive in its nature (36). In the latter context, it has been possible to prepare an antibody with serologic specificity for II (37). Previous studies by Hacker (38) have demonstrated that L1210 mouse leukemia cells do possess the necessary enzyme systems to phosphorylate I to the nucleotide level *in vitro*. It is not yet certain whether this biotransformation is a prerequisite for its antileukemic property.

Although II has antineoplastic and cytotoxic effects against leukemia cells when administered to humans, it is known to be susceptible to enzyme degradation. Results obtained by Chassy and Suhadolnik (39) indicated that adenosine deaminase catalyzes conversion of II to inosine. Hall and Mintsioulis (40) reported that elevated adenosine deaminase activity in human blood significantly facilitates the degradation of II. Chheda and Mittelman (41) have shown that the biological half-life of II after intravenous administration was 4 h. Compound II has also been prepared entrapped in a controlled-release polymeric delivery system using a silicone polymer monolithic disk to evaluate its relatively greater antineoplastic properties (42, 43). More recent studies by Hacker and Chang (44) using the adenosine deaminase inhibitor pentostatin, in combination with a silicone polymeric delivery system (45, 46) have led to a system for potentiating and prolonging the cytotoxic effects of II against L1210 leukemia cells in culture.

EXPERIMENTAL

The preparation of N^6-(Δ^2-isopentenyl)adenosine (i^6A) (II) was conducted as described previously (35, 36, 43). 1-β-D-Arabinofuranosylcytosine (cytarabine; Ara-C; NSC-63878) was supplied[1]. Aqueous extracts were prepared from *E. senticosus* roots by stirring for 10 min at 60°C followed by filtration, freezing, and lyophilization, as previously described (47).

L1210 lymphocytic leukemia cells were cultured to various densities as suspension cultures in fresh medium (RPMI-1640 plus L-glutamine and 10% fetal calf serum)[2] in plastic tissue culture flasks[3] at 37°C in a 5% carbon dioxide atmosphere (38, 42, 43). The technique for monitoring the progress of cell cultures by an inverted microscope–video system and the determination of total cell count and cell viability have been described earlier (42–44). The number of viable and/or total cell number monitored at various time periods before and during treatment was used as a measure of cytostatic or cytotoxic activity and is reported as percent inhibition in Tables I and II.

RESULTS AND DISCUSSION

This investigation sought to establish whether an extract of *E. senticosus* [with so-called "adaptogen" (3) and broad antitumor properties (4–19)] has cytotoxic effects on L1210 murine leukemia cells alone and in combination with two nucleoside antimetabolites (I and II) (20–44).

Inhibition of cellular proliferation by *E. senticosus* extract is seen to be concentration dependant between 25–100 μg/mL for a period up to 72 h following its addition to cultured L1210 leukemia cells (Table I). The magnitude of inhibition is greatest during the logarithmic or replicative phase of cellular growth, with diminished effects during the late-logarithmic and/or stationary stage. The ED_{50} is ~75 μg/mL. The maximal level of inhibition by *E. senticosus* extract alone is seen to occur at higher concentrations (200–400 μg/mL). The possibility exists that the crude extract of *E. senticosus* may contain multiple components which act to antagonize or negate the antiproliferative effects that have been observed against L1210 cells (Table I). This may account in part for the plateauing effect observed.

In those experiments where *E. senticosus* extracts were combined with I or II in varying concentrations, several salient features resulted (Table II). Nucleoside I (1 μg/mL) is equivalent in its antileukemic effect to nucleoside II (25 μg/mL) resulting in a 40% level of inhibition at those respective concentrations (Table II). A combination of *E. senticosus*

Table II—Antiproliferative Effects of *E. senticosus* Extract in Combination with N^6-(Δ^2-Isopentenyl)adenosine (I) and 1-β-D-Arabinofuranosylcytosine (II) Against L1210 Cells

Addition at Time Zero[a]	Cytotoxicity Level[b] (48 h) Cells/mL × 10^{-5}	Change in Total[c] Cell Number, %
None	38 ± 2.0	+53
E. senticosus (100 μg/mL)	16 ± 4.0	−58
E. senticosus (200 μg/mL)	18 ± 2.0	−53
I (1 μg/mL)	23 ± 1.0	−40
E. senticosus (200 μg/mL) + I (1 μg/mL)	10 ± 2.0	−74
II (10 μg/ml)	32 ± 1.0	−16
E. senticosus (100 μg/mL) + II (10 μg/mL)	8 ± 1.0	−78
II (25 μg/ml)	23 ± 1.0	−40
E. senticosus (200 μg/mL) + II (25 μg/mL)	0	−100

[a] Each T-flask contained 5 mL of growth medium (RPMI-1640 plus 10% fetal calf serum) plus 1 × 10^5 L1210 leukemia cells. The experiments were initiated (time zero) by the addition of each agent shown in RPMI-1650 without serum (0.05–0.10 mL) with prior sterile filtration before introduction into the cell culture. [b] Aliquots (0.20 mL) of each cell suspension were removed 72 h after the introduction of each agent for the determination of cell number, viability, and HPLC analyses for N^6-(Δ^2-isopentenyl)adenosine (I) and 1-β-D-arabinofuranosylcytosine (II). Values reflect 3–5 individual determinations using duplicate cultures. [c] Change in total cell number (%) for control cultures (no addition) represents increase in cell number (+ sign) compared with zero time. Inhibition in cell number (− sign) reflects cytotoxicity compared with value for 48-h control cultures.

[1] Natural. Products Br., Div. of Cancer Treatment, National Cancer Institute, Bethesda, Md.; or Cytosar-U or cytarabine, The Upjohn Co., Kalamazoo, Mich.

[2] Grand Island Biological Co., Grand Island, N.Y.

[3] Corning; no. 25100.

extract (100 μg/mL) and II (10 μg/mL) gave a similar level of inhibition (74–78%) compared with the arithmetic sum of their individual effects when used separately. Again, the most effective concentrations of *E. senticosus* extract appear to be in the 50–100 μg/mL range. Doubling the concentration of *E. senticosus* extract (200 μg/mL) in the presence of nucleoside II (25 μg/mL) resulted in an overall level of inhibition diminished by 20% when compared with the sum of their individual effects against L1210 cells. Again, this confirms our earlier suggestion that crude extracts of *E. senticosus* could contain components that interfere with its antiproliferative and "adaptogen" types of activity.

A combination of nucleoside I (1 μg/mL) plus *E. senticosus* extract (200 μg/mL), in contrast to the previous results, clearly resulted in a mutually additive antiproliferative effect on cultured L1210 cells. No synergistic effect was observed in the present investigation.

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