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Report

Effect of Helenalin and Bis(helenalinyl)malonate on Nucleic Acid and Protein Synthesis in Human KB Carcinoma Cells

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Helenalin and bis(helenalinyl)malonate were shown to be cytotoxic against the growth of human KB carcinoma cells. DNA synthesis was inhibited significantly. This inhibition was afforded because of the drugs' effects on a number of enzyme activities. The inhibition of IMP dehydrogenase and ribonucleotide reductase complex activities correlated positively with the inhibition of DNA synthesis of the KB cells. DNA polymerase activity was inhibited by the drugs to a lesser degree. The deoxyribonucleotide pools were markedly reduced in the presence of the drug, which would be consistent with a blockage of the enzyme ribonucleotide reductase as well as suppression of DNA synthesis. XMP levels were also reduced, which is consistent with suppression of IMP dehydrogenase activity by the drugs. Ribonucleoside phosphate pools, particularly CDP and GDP, were elevated after drug treatment, which would be expected with a blockage at ribonucleotide reductase. Thus DNA alkylation is not the mechanism of action of the antineoplastic sesquiterpene lactones; rather, the cell-killing effect is related to DNA synthesis inhibition by the drug.

KEY WORDS: antineoplastic agents; helenalin; bis(helenalinyl)malonate; KB carcinoma; nucleic acid inhibition.

INTRODUCTION

Helenalin is a pseudoguaianolide sesquiterpene lactone isolated from plants of the family, *Compositae*. Kupchan (1) suggested that the cytotoxicity of the sesquiterpene lactones was related to the presence of the α, β-methylene-γ-lactone functionality. Lee *et al.* (2) observed in H.Ep-2 cells that the α,β-unsaturated cyclopentenone moiety also contributed to the pharmacological activity. The sesquiterpene lactones form a thiol adduct with the thiol group of amino acids via a rapid Michael-type addition (3). There appears to be no evidence of DNA nucleotide strand scission or drug binding (4). Subsequently, helenalin was shown to be active against the growth of Walker 256 carcinosarcoma, Ehrlich carcinoma ascites, Lewis lung carcinoma, and P-388 lymphocytic leukemia cells (5,6) *in vivo*, and cytotoxic activity has been demonstrated in human KB or H.Ep-2 carcinoma cells (5). To date, the sesquiterpene lactones have not been advanced into human studies because of the lack of large quantities of the natural product for clinical studies and because of the sparsity of studies regarding its mode of action in cancer cells.

Helenalin, a stereotype sesquiterpene lactone, has been evaluated for acute toxicity in mice (manuscript in preparation). At the therapeutic dose, 8 mg/kg, little toxicity has been noted; however, as the dose is increased, i.e., at 25 mg/kg day, cardiac toxicity has been demonstrated. A synthetic bis ester of helenalin, bis(helenalinyl)malonate (Fig. 1), was shown to be more potent than helenalin in suppressing P-388 lymphocytic leukemia, i.e., T/C% = 261, compared to the value for helenalin, T/C% = 162. In this cell line, both agents suppressed DNA synthesis preferentially, with moderate inhibition of protein and RNA synthesis (6). Further studies in P-388 cells on the mode of action of sesquiterpene lactones as a protein synthesis inhibitor showed that the agents were initiation inhibitors blocking the conversion of the ternary initiation complex to the 48S and 80S initiation complex (7). In the rabbit reticulocyte system, the eIF-3 initiation factor was shown to be the most sensitive to helenalin (8). On the other hand, the mode of action of sesquiterpene lactones with regard to DNA synthesis suppression is essentially unknown. In Ehrlich ascites carcinoma cells, some evidence exists that sesquiterpene lactones suppress the activities of nuclear DNA polymerase and thymidylate synthetase (5). However, the concentration of drugs used in these studies was unusually high. In P-388 lymphocytic leukemia cells, little inhibitory action was observed against DNA polymerase or thymidylate synthetase activities (6). Since the Ehrlich ascites is a carcinoma, it was decided to investigate the effects of helenalin and bis(helenalinyl)malonate on nucleic acid and protein synthesis of human KB epidermoid carcinoma of the

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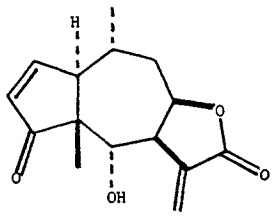
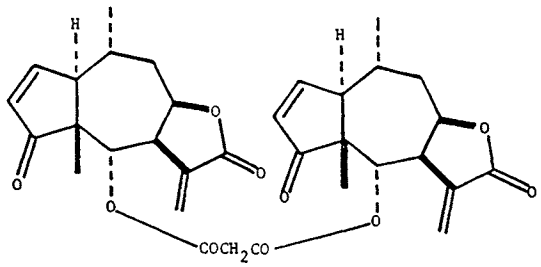


HELENALIN



BIS(HELENALINYL) MALONATE

Fig. 1. Structures of helenalin and bis(helenalinyl) malonate.

pharynx. Further, it was known that helenalin was active against the growth of this cell line (5). The present study was undertaken to determine the effects of cytotoxic helenalin concentrations on enzymes involved in DNA synthesis that require sulfhydryl groups for catalytic reactivity.

MATERIALS AND METHODS

All radioisotopes were purchased from New England Nuclear. All other chemicals were obtained from Sigma Chemical Company. GF/F filters and PEI plates were purchased from Fisher Scientific.

Helenalin was isolated from the plant *Bauduina augustifolia* (9). Bis(helenalinyl)malonate was chemically synthesized from helenalin by treatment with malonyl dichloride in dry benzene, as described previously (4). Purity of compounds was determined by nuclear magnetic resonance (NMR) and elemental analysis (C,H).

The human KB epidermoid carcinoma cells, obtained from the American Type Culture collection, were maintained in minimum essential medium (MEM), penicillin/streptomycin, and 10% fetal calf serum. Drugs were suspended in an aqueous solution of 0.05% Tween 80(polysorbate 80)–water by homogenization and sterilized by filtration. The growth inhibition by the sesquiterpene lactones was determined at concentrations of 10, 50, and 100 μM for a 3-day period with 5×10^4 cells in 1 ml of medium according to the NIH protocol (10). Cell number per milliliter was determined with a hemocytometer by counting the viable cells after staining with 0.4% trypan blue. Day 3 was used to calculate the ED_{50} value, i.e., the concentration of drug required to inhibit 50% of the cell growth, since the log growth was exponential at this time.

In vitro incorporation of labeled precursors into DNA, [*methyl*-^{3}H]thymidine (84 Ci/mmol), RNA [6-^{3}H]uridine (22.4 Ci/mmol), and protein [4.5-^{3}H(N)]leucine (56.3 Ci/mmol) of 10^6 cells was determined for 60 min by the method of Liao *et al.* (11). Drugs were present at 0.25, 0.50, 1, and 2 times the concentration of their respective ED_{50} values in the tissue culture cells. The reaction mixtures were inactivated with acid. The DNA acid-insoluble precipitate was collected by vacuum suction on GF/F glass-fiber disks which were washed with cold 10% perchloric acid containing 1% sodium pyrophosphate (6). Acid-insoluble precipitates from the RNA and protein experiments were collected on Millipore nitrocellulose filters washed with 10% trichloroacetic acid. The filter disks were placed in Scintiverse, dried, and counted in a Packard scintillation counter (6). The following enzymatic activities were determined in cells at multiples of the ED_{50} values for each drug. DNA polymerase α activity was determined on a cytoplasmic fraction (12) using the incubation medium of Sawada *et al.* (13), with [*methyl*-^{3}H]dTTP (82.4 Ci/mmol), dCTP, dGTP, and dATP. Incubation was for 60 min at 37°C. The acid-insoluble precipitate was collected on glass-fiber disks and counted. Formate incorporation into purines for 40 min at 37°C was determined by the method of Spassova *et al.* (14), with 0.5 μCi [^{14}C]formic acid (52 mCi/mmol). Purines were separated by silica gel thin-layer chromatography (TLC) eluted with *N*-butanol:acetic acid:water (4:1:5). Using standards for guanine and adenine, the appropriate spots were scraped and counted. Inosinic acid dehydrogenase activity was determined by the method of Becker and Lohr (15) using 30 min of incubation at 37°C with [8-^{14}C]inosine-5′-monophosphate (61 mCi/mmol). XMP was separated from IMP by TLC on PEI plates eluted with 0.5 *M* $(NH_4)_2SO_4$. The appropriate spot (standard, XMP) was scraped and counted. Thymidylate synthetase activity was determined using a supernatant ($9000g \times 10$ min) fraction by the method of Kampf *et al.* (16) with [^{3}H]dUMP (11 Ci/mmol) incubated for 30 min. The nucleotides were absorbed on charcoal and filtered on Whatman No. 1 filters, and a sample of the aqueous filtrate was counted. *N*-Ethylmaleimide, a known thiol alkylating agent, at a concentration from 0 to 200 μM, was utilized to assess inhibition of the enzyme activities employed in this study.

Ribonucleoside diphosphate reductase activity was measured by a modification of the method of Moore and Hurlbert (17). An aliquot of the $5000g$ supernatant was incubated for 30 min at 37°C with reaction medium containing 0.1 μCi [5-^{3}H]CDP (16.2 Ci/mmol). The reaction was stopped by boiling; samples were incubated with calf intestine alkaline phosphatase, spotted on PEI plates, and eluted with ethanol/saturated sodium borate/ammonium acetate/EDTA. Plates were scraped at the R_f of the standard, deoxycytidine, and counted. The modified method of Holmgren (18) was used to measure the activity of thioredoxin. An aliquot of the ($5000g \times 10$ min) supernatant was added to cuvettes containing insulin (1 mg/ml in potassium phosphate buffer). Dithiothreitol solution (0.1 *M*) was added, and the change in absorbance at 650 nm was determined for 90 min. The glutaredoxin system was evaluated by measured GSH-disulfide oxidoreductase (transhydrogenase) activity of KB cells by the literature method of Holmgren (19). Drugs were incubated with the $5000g \times 10$ min supernatant and the reoxidation of NADPH was measured for 1 min at 340 nm at 25°C.

After KB cells had been incubated with the drugs for 3 hr, ribonucleoside phosphates and deoxyribonucleoside triphosphates were extracted with perchloric acid by the method of Bagnara and Finch (20). After neutralization with 5 *N* KOH and 1 *M* KH_2PO_4, the levels of ribonucleoside phosphates were measured by the HLPC method of McKeaf and Brown (21). The neutralized extract was applied to a Whatman partisil-10 SAX ion-exchange column and eluted by a linear gradient of a 7.0 m*M* to 0.5 *M* KCl/KH_2PO_4 buffer system. Nucleoside phosphates were detected and quantitated by absorbance at 254 nm with a plotter integrator. Deoxyribonucleoside triphosphate pool levels were determined by the method of Hunting and Henderson (22). The neutralized extract was incubated for 30 min at 37°C with reaction medium containing calf thymus DNA, *Escherichia coli* DNA polymerase I, and nonlimiting amounts of three deoxyribonucleoside triphosphates not being assayed, including 0.04 μCi [methyl-^{3}H]dTTP (80 Ci/mmol) or [5-^{3}H]dCTP (15–30 Ci/mmol). The samples were spotted on Whatman No. 3 filters, which were rinsed in 5% trichloroacetic acid/40 m*M* sodium pyrophosphate and in 95% ethanol, after which they were dried and counted for radioactivity.

RESULTS AND DISCUSSION

Helenalin and bis(helenalinyl)malonate significantly inhibited the growth of human KB epidermoid carcinoma cells. The ED_{50} values (concentrations for 50% inhibition of cell growth) were 83 μ*M* for helenalin and 13.2 μ*M* for bis(helenalinyl)malonate. Helenalin at 332 μ*M* and bis(helenalinyl)malonate at 158 μ*M* in KB cells inhibited DNA synthesis more than RNA ($P \leq 0.05$). The ID_{50} values for inhibition of DNA synthesis in KB cells was 262 μ*M* for helenalin and 102 μ*M* for bis(helenalinyl)malonate (Fig. 2). Both drugs inhibited RNA synthesis marginally, but the inhibition was not of sufficient magnitude to calculate an ID_{50} value. The suppression in RNA synthesis (i.e., 30%) may be due to the reduction of ribonucleotide levels by the sesquiterpene lactones (Table I). Both helenalin and bis(helenalinyl)malonate significantly reduce the level of CTP (Table I). Bis(helenalinyl)malonate also significantly suppressed ATP, GTP, and UTP levels in KB cells. Previous studies performed in P-388 lymphocytic leukemic cells have demonstrated that sesquiterpene lactones helenalin and bis(helenalinyl)malonate did not affect mRNA polymerase activity (23).

KB cell protein synthesis was also inhibited by both drugs, but the suppression of protein synthesis generally was also less than the observed DNA synthesis suppression. Helenalin demonstrated an ID_{50} value of 270 μ*M* and bis(helenalinyl)malonate a value of 144 μ*M* for the inhibition of protein synthesis of KB cells (Fig. 2). Since the mode of action of sesquiterpene lactones as protein synthesis inhibitors has been investigated in P-388 cells and rabbit reticulocytes, this study concentrated on the inhibition of DNA synthesis by these agents. It is interesting to note that prior studies have indicated that the reactive functional groups of the sesquiterpene lactones are the α-methylene-γ-lactone moiety and the α,β-unsaturated cyclopentenone ring (1,2). Since bis(helenalinyl)malonate has twice as many potential functional groups for Michael-type addition than helenalin, a number of enzymes involved in DNA synthesis were examined which contain thiol groups within the active site of the enzyme to determine if there was a quantitative difference afforded by the two agents. Preliminary studies showed that *N*-ethylmaleimide, a thiol-specific agent, indeed inhibited these enzyme activities of KB cells.

Nuclear DNA polymerase activity was marginally inhibited by both sesquiterpene lactones; an ID_{50} value of 442 μ*M* was obtained for helenalin and a value of 900 μ*M* for bis(helenalinyl)malonate (data not shown). Cytoplasmic DNA polymerase α of KB cells was inhibited 35% by helenalin at 332 μ*M* and 38% by bis(helenalinyl)malonate at 158 μ*M* (Table II). These data suggest that sesquiterpene lactones were more specific for the α isozyme, which contains

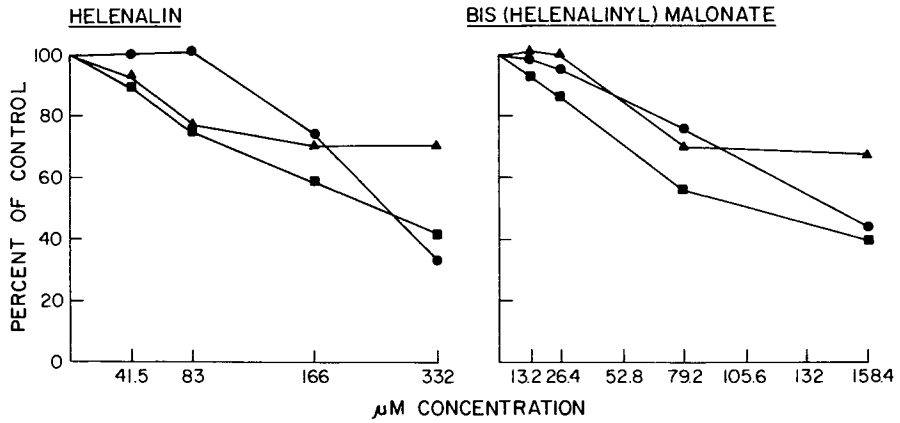


Fig. 2. Effects of helenalin and bis(helenalinyl)malonate on DNA, RNA, and protein synthesis in human KB cells. *In vitro* incorporation studies were conducted with 10^6 KB cells and 1 Ci of [*methyl*-^{3}H]thymidine (84 Ci/mmol), [6-^{3}H]uridine (22.4 Ci/mmol), or [4,5-^{3}H(N)]leucine (56.5 Ci/mmol). The control value for the thymidine incorporation (■--■) was 31,900 dpm/10^6 cells, that for uridine incorporation (●--●) was 43,300 dpm/10^6 cells, and that for leucine incorporation (▲--▲) was 18,400 dpm/10^6 cells. $N = 6$ and standard deviations do not exceed 7% for all values in the table. Incubations were conducted for 60 min.

Table I. The Effects of Helenalin and Bis(helenalinyl)malonate on Ribonucleoside Phosphate Pool Levels of KB Cells After a 3-hr Incubation ($N = 6$)

	Percentage of control[m]											
	XMP	AMP	ADP	ATP	GMP	GDP	GTP	UDP	UTP	CMP	CDP	CTP
Control	100[a]	100[b]	100[c]	100[d]	100[e]	100[f]	100[g]	100[h]	100[i]	100[j]	100[k]	100[l]
Helenalin												
41.5 μ*M*	113	—	90	102	112	135*	100	99	99	93	63*	110
83 μ*M*	40*	80	123	101	118	176*	106	98	98	83	220*	176*
166 μ*M*	67*	92	89	116	161*	120	107	80	80	92	121	89
332 μ*M*	45*	87	120	93	154*	135*	95	90	90	—	157	51*
BHM												
39.6 μ*M*	77*	—	67*	79*	77*	80	95	79	79	77	163*	101
79.2 μ*M*	67*	56*	93	87	80	142*	95	82	69*	87	163*	96
158.4 μ*M*	56*	54*	64*	62*	68	115	66*	61*	61*	58*	102	82

[a] 22 nmol XMP/10^8 cells.
[b] 23 nmol AMP/10^8 cell.s
[c] 44 nmol ADP/10^8 cells.
[d] 180 nmol ATP/10^8 cells.
[e] 40 nmol GMP/10^8 cells.
[f] 31 nmol GDP/10^8 cells.
[g] 60 nmol GTP/10^8 cells.
[h] 34 nmol UDP/10^8 cells.
[i] 170 nmol UTP/10^8 cells.
[j] 30 nmol CMP/10^8 cells.
[k] 13 nmol CDP/10^8 cells.
[l] 120 nmol CTP/10^8 cells.
[m] Standard deviations were less than 15% for all values.
* $P \leq 0.05$.

a thiol group, than the β isozyme. The inhibition of thymidylate synthetase was not of sufficient magnitude to obtain an ID_{50} value for either drug (Table II); helenalin, at 332 μM, inhibited the enzyme activity by 48% and bis(helenalinyl)malonate, at 158 μM, inhibited the enzyme activity by 34% in KB cells. The suppression of the activity rate-limiting enzyme of purine synthesis, IMP dehydrogenase, by helenalin or bis(helenalinyl)malonate in KB cells paralleled the inhibition of DNA synthesis at the same drug concentrations (Table III). A similar decrease in the ribonucleotide pool level of XMP was afforded by both drugs, with bis(helenalinyl)malonate affording a dose-dependent reduction of XMP whereas helenalin did not. IMP concentrations were elevated ~44% after treatment with the drugs.

Guanine (0.1 to 10 μg/ml) or GMP (100–300 μ*M*) was added to the culture medium with the sesquiterpene lactone

Table II. *In Vitro* Effects of Helenalin and Bis(helenalinyl)malonate on Purine Synthesis, IMP Dehydrogenase, DNA Polymerase α, and Thymidylate Synthetase Activities of KB Cells ($N = 6$)

	Percentage of control ($X \pm SD$)			
Drug	Formate incorporation into purine	IMP dehydrogenase activity	Thymidylate synthetase activity	DNA polymerase activity
Helenalin	100 ± 6[a]	100 ± 7[b]	100 ± 9[c,*]	100 ± 8[d]
41.5 μ*M*	86 ± 6	79 ± 6	93 ± 8	98 ± 7
83 μ*M*	71 ± 3*	51 ± 7*	80 ± 9*	77 ± 7*
166 μ*M*	52 ± 4*	50 ± 7*	67 ± 6*	67 ± 6
332 μ*M*	49 ± 5*	37 ± 4*	52 ± 6*	65 ± 5*
Bis(helenalinyl)malonate				
13.2 μ*M*	106 ± 4	94 ± 6	98 ± 7	98 ± 6
39.6 μ*M*	105 ± 6	89 ± 5	77 ± 8*	97 ± 8
79.2 μ*M*	66 ± 7*	62 ± 5*	83 ± 6	85 ± 6
158.4 μ*M*	39 ± 4*	47 ± 7*	66 ± 7*	67 ± 7*

[a] 23,000 dpm/mg protein/40 min.
[b] 39,900 dpm/mg protein/30 min.
[c] 31,900 dpm/mg protein/30 min.
[d] 1910 dpm/mg protein/60 min.
* $P \leq 0.001$.

Table III. The Effects of Helenalin and Bis(helenalinyl)malonate on Deoxyribonucleoside Triphosphate Pool Levels of KB Cells After a 3-hr Incubation ($N = 4$)

	Percentage of control ± SD			
	dATP	dGTP	dTTP	dCTP
Control	100 ± 5[a]	100 ± 5[b]	100 ± 8[c]	100 ± 3[d]
Helenalin				
20.7 μ*M*	66 ± 2*	90 ± 4	88 ± 2	90 ± 10
41.5 μ*M*	25 ± 3*	66 ± 5*	50 ± 3*	46 ± 6*
83.0 μ*M*	14 ± 2*	21 ± 5*	30 ± 1*	22 ± 3*
166.0 μ*M*	10 ± 7	11 ± 4*	14 ± 2*	19 ± 5*
332.0 μ*M*	13 ± 2*	20 ± 4*	30 ± 3*	16 ± 2*
BHM				
39.6 μ*M*	59 ± 14*	100 ± 27	54 ± 16*	80 ± 9*
79.2 μ*M*	31 ± 5*	58 ± 5*	33 ± 2*	54 ± 5*
158.4 μ*M*	4 ± 2*	26 ± 2*	35 ± 1*	10 ± 2*

[a] 13.9 pmol dATP/10^6 cells.
[b] 11.7 pmol dGTP/10^6 cells.
[c] 45.3 pmol dTTP/10^6 cells.
[d] 16.2 pmol dCTP/10^6 cells.
* $P \leq 0.001$.

at the ED_{50} value for 3 hr, and thymidine incorporation into DNA was determined. These studies demonstrated that the simple addition of guanine did not bypass the blockage afforded by the drugs and that IMP was not the only site in synthesis of d(NTP) that was affected by the sesquiterpene lactones.

Both helenalin and bis(helenalinyl)malonate treatment resulted in slight to moderate increases in ribonucleotide diphosphate levels (Table I). These data suggested that the sesquiterpene lactones might also inactivate ribonucleotide reductase activity. Thus, the effect of the sesquiterpene lactones on ribonucleotide reductase activity was evaluated as well as the hydrogen generation system of thioredoxin and glutaredoxin enzymes. Ribonucleotide reductase activity in KB cells was reduced more than 35% by helenalin and bis(helenalinyl)malonate at their respective ED_{50} values. Reduction of this enzyme activity should lower the deoxyribonucleotide pool levels in the KB cells. Indeed at the ED_{50} values for helenalin (83 μ*M*), the four deoxyribonucleotide pool levels were reduced by 70% or more (Table III). Bis(helenalinyl)malonate required a concentration of 158 μ*M* to achieve greater than 65% reduction of all four deoxyribonucleotides pools. The reduction of the deoxyribonucleotide pools was sufficient to explain most of the DNA synthesis inhibition in KB cells exhibited by these drugs. At the ID_{50} value for inhibition of DNA synthesis, the deoxyribonucleotide pools were reduced to less than 50% of control values in all cases. In fact, most of the deoxyribonucleotide pools were reduced to 10–15% of control levels. At higher levels of helenalin (332 μ*M*), a recovery in the deoxyribonucleotide pool was noted in dGTP and dTTP. This limited increase in the deoxyribonucleotide pools may be the result of the drug's inhibition of DNA polymerase α activity, which would reduce the incorporation of deoxyribonucleotide into DNA and allow d(NTP) to accumulate in the cells.

The inhibition of ribonucleotide reductase activity itself by the sesquiterpene lactones was not concentration dependent, nor would the inhibition of this enzyme fully account for all of the observed DNA synthesis inhibition (Table IV). However, measurement of the thioredoxin and glutaredoxin activities (Table IV) demonstrated the ability of the sesquiterpene lactones to block significantly the hydrogen transfer from both the thioredoxin and the glutaredoxin donor systems in a dose-dependent manner. This, in combination with the observed inhibition of ribonucleotide reductase activity, should be enough to cause a severe metabolic block for the synthesis of d(NTP). A metabolic block at this enzyme site should cause an increase in the ribonucleotide pool levels. Significant increases in GDP and CDP pools were noted after 3 hr of incubation with either sesquiterpene lactones in KB cells.

In conclusion even though thiol specific enzymes, i.e., DNA polymerase α and thymidylate synthetase, were inhibited by sesquiterpene lactones, the inhibition of the activity

Table IV. *In Vitro* Effects of Helenalin and Bis(helenalinyl)malonate on Ribonucleoside Diphosphate Reductase, Thioredoxin, and Glutaredoxin Activities in KB Cells ($N = 6$)

	Percentage of control (X ± SD)		
Drug	Ribonucleotide reductase activity	Thioredoxin activity	GSH disulfide oxide activity
Control	100 ± 5[a]	100 ± 6[b]	100 ± 4[c]
Helenalin			
41.5 μ*M*	61 ± 6*	85 ± 5*	51 ± 3*
83 μ*M*	58 ± 5*	61 ± 6*	45 ± 3*
166 μ*M*	53 ± 6*	48 ± 5*	41 ± 4*
332 μ*M*	59 ± 6*	21 ± 4*	31 ± 3*
Bis(helenalinyl)malonate			
36.6 μ*M*	69 ± 7*	50 ± 6*	41 ± 4*
79.2 μ*M*	68 ± 6*	47 ± 6*	27 ± 3*
158.4 μ*M*	64 ± 6*	16 ± 4*	21 ± 3*

[a] 2855 dpm/hr/mg protein.
[b] 0.004 optical density unit/min/mg protein.
[c] 0.028 optical density unit/min/mg protein.
* $P \leq 0.001$.

was not to a degree sufficient to explain the full inhibition of DNA synthesis observed in KB cells. However, the inhibition of IMP dehydrogenase and ribonucleotide reductase activities together would appear sufficient to explain the DNA synthesis inhibition of KB cells. Incubation of KB cells with guanine or GMP showed that DNA synthesis did not recover, indicating that blockage by the drug was above IMP dehydrogenase in the purine pathway, probably at the ribonucleotide reductase site. Thus the sesquiterpene lactones do appear to afford some selectivity with regard to thiol-bearing enzymes involved in DNA synthesis, similar to the finding observed with helenalin and bis(helenalinyl)malonate as initiation protein synthesis inhibitors (8).

However, the ID_{50} values for inhibition of DNA synthesis did not show an exact correlation with the ED_{50} values for inhibition of cell growth. The ID_{50} for DNA synthesis inhibition was approximately 3 times the ED_{50} values for helenalin, but 7.7 times those for bis(helenalinyl)malonate. These findings suggest that factors other than inhibition of DNA synthesis may also contribute to the cytotoxicity of sesquiterpene lactones in the KB cell line.

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