Clinical research

Chelidonium and *Sanguinaria* alkaloids as anti-HIV therapy

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Several plant products (including glycyrrhizin, hypericin and pseudohypericin) possess value in the management of HIV (1). Two traditional phytomedicinals, *Chelidonium majus* (Celandine) and *Sanguinaria canadensis* (Bloodroot) were evaluated clinically for anti-HIV activity. Both plants contain aromatic benzo-[c]phenanthridine alkaloids known to inhibit HIV reverse transcriptase (2). The agents were compounded into capsules of freeze-dried material of various proportions [RETRO-ZIP] and supplied at modest cost to a small number of AIDS and HIV-exposed individuals manifesting a broad spectrum of clinical progression.

Although confounded by small cohort size (n=13) and use of multiple anti-virals, this agent appears to exhibit anti-HIV activity at an appropriate dosage. Particularly striking resolution of persistent generalized lymphadenopathy (PGL) was observed in most subjects. Further studies should now seek to assess the controlled use of these agents as low-dose, long-term therapy in appropriately staged patient populations.

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HYPOTHESIS

The report of Kakiuchi, et al. (2) on the property of benzo[c] phenanthridine alkaloids in binding to viral nucleic acid gave impetus to this study, as this class of alkaloid is found in many Papaveraceous plants including *Chelidonium* (chelilutine and chelirubine) and *Sanguinaria* (sanguilutine and sanguirubine). Chelidonine, the principal alkaloid of *Chelidonium*, has also been reported to "moderately" inhibit HIV reverse transcriptase (3,4).

As both of these species occupy a prominent place in the naturopathic pharmacy and have been subjected to prior toxicological and clinical evaluation [*Chelidonium* as a cholagogue (5), *Sanguinaria* as a topical anti-neoplastic(6)] the hypothesis that these plants might serve as a readily available and low-cost alternative to the conventional nucleoside analogues (with different or novel pharmacokinetics) prompted examination of their clinical properties in the small cohort (n=13, 8 male [M1-M8], 5 female [F1-F5]) of HIV+ and AIDS patients currently under care.

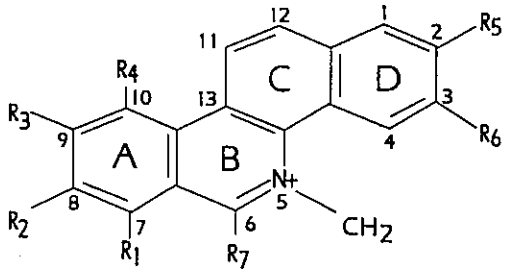

FIGURE 1. Basic structure of benzo[c]phenanthridine alkaloid, as found in phytomedicinals *Chelidonium majus* and *Sanguinaria canadensis*. Compounds which are 8,9 oxygenated at the A ring and 2,3 oxygenated at the D ring are the most effective reverse transcriptase inhibitors.

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MATERIALS AND METHODS

A 75/25% mixture of tinctures of *Chelidonium* and *Sanguinaria* (20 gtts TID) was initally employed. This proved undesirable due to the unpalatibility of the plant components in their native state. This form was eventually superceded by encapsulated freeze-dried *Chelidonium* and *Sanguinaria* (350mg/ capsule: 175mg freeze-dried *Chelidonium* tops, 175mg *Sanguinaria* root).

This formula, Retro-ZIP I, 350mg (RZ_1, manufactured by *Eclectic Institute*, Portland OR), was prescribed on the following dosage schedule: 1 capsule TID for the first week; 2 capsules TID for the second week; 3 capsules TID thereafter. The formula exhibited significant adverse actions; these were mainly nausea and vomiting (4 patients: F4, F5, M7,M8), dizziness and vertigo (2 patients: F4, M8) and an allergic reaction characterized by angioedema (1 patient: M8) requiring treatment with an H2 antagonist (Cimetidine). Untoward response was quite variable: Several patients experienced no side-effects regardless of dosage (3 patients: M3, M4, F3); others exhibited profound nausea and emesis with less than 2 capsules per day (F3), ameliorated somewhat by taking the formula with meals.

The majority of the side effects resulting from the use of RZ_1 appear to result from the high doses of *Sanguinaria* used in the formula. This agent is a known gastric irritant and contains emetine-like principles. When the patients reporting nausea and hyperemesis were switched to a an exclusively *Chelidonium* formula (freeze-dried, 175mg) and given the identical dose, none reported nausea or vomiting, nor any other side effects.

This led to a modification of the formula by decreasing *Sanguinaria* component to 5mg, increasing *Chelidonium* to 175mg and adding 20mg of *Ulmus fulva*, a plant with a mild demulcent effect on the gastric membranes. This formula was given the name Retro-ZIP II, 200mg (RZ_2) which eliminated the hyperemesis problem, except for 1 patient (F4) who can a tolerate 1/2 capsule TID dose. One patient (M3) reported "putty-like" stools, which are probably the result of increased hepatobiliary activity (3). All patients were switched to (RZ_2) and all results reported herewith are with this formula.

RESULTS

In addition to the small number (n=13) and uncontrolled design of this study, a evaluation of the effectiveness of RZ_2 was also confounded by the number of patients using multiple anti-viral agents or procedures. These included AZT, ddI, ddC, plasmapheresis, passive immunotherapy and the concurrent use of *Glycyrrhiza glabra* solid extract *(Table 1)*. Four patients did not receive any conventional antiviral therapy (CAVT), though all used the *Glycyrrhiza* concurrently with RZ_2. However, 6 patients had been on AZT for 1 year or longer, or had been shifted to other nucleosides due to drug resistance. None of the patients commenced conventional anti-viral therapy at the same time to starting RZ_2 therapy. Thus the patient pool can be divided into two subgroups: a healthier group not yet employing CAVT (CD4: mean 281.6, SD [n-1 unbiased] $\pm$ 102.33; variance [n method, biased] 8377.4; maximum value 459, minimum value 212) and a more progressed group using long-term CAVT prior to begining RZ_2 therapy. This second group is characterized by more frequent opportunistic infections (>1) and lower CD4 counts (mean 68.2, SD [n-1 unbiased] $\pm$ 72.68; variance [n method, biased] 4622.73; maximum value 222, minimum value 5). Interestingly, both groups had essentially similar responses to RZ_2 *(Table 1)*.

Thus, although it would be impossible to "filter out" the effects of CAVT in the second group, it is not unreasonable to suppose that the changes observed after commencing RZ_2 are the effects of RZ_2 and not CAVT, as the average time on CAVT prior to initiating RZ_2 therapy was >1 year.

The effect of *Glycyrrhiza glabra* (GG) supplementation also complicates a complete analysis of RZ_2 effectiveness. Glycyrhizzin, the sulphated polysaccharide of GG has been reported to inhibit HIV reverse transcriptase (3). However, in the 2 patients who had been on GG for greater than 1 year prior to initating RZ_2 therapy, neither showed decrease in persistent generalized lymphadenopathy (PGL) or CD8 levels which were observed when RZ_2 therapy was initiated.

PGL, CD4 and CD8 levels were evaluated. A percent threshold ($\leq$ 7%= "no change"; > 7%= "minimal change"; $\geq$10%= "moderate change") rather than absolute cell count was used in an attempt to offset variations in this data, such as use of multiple laboratories, collection spacing, etc.

1. *Lymphadenopathy*. All (n=8) of the patients experiencing PGL reported diminished node size and tenderness which was verified clinically. Three of the 6 patients (M1, M2, M8) reported dramatic resolution of lymphadenopathy (defined as complete or near complete resolution of lymphadenopathy in 2 or more non-contiguous node sites within 3 weeks of initiating RZ_2).

2. *CD8 counts*. Four of 11 patients (36.3%) showed a slight decrease in CD8 counts, defined as a drop of $\leq$7% from the most recent test prior to initiating RZ_2. One subject, M8, had a more pronounced decrease (>7%), while 5 patients showed no change and one patient had a mild increase.

3. *CD4 counts*. Four patients (36.3%) of eleven showed a slight increase in CD4 counts, defined as $\leq$7% as compared to the most recent test prior to initiating RZ_2. Four patients (36.3%) showed essentially no change in CD4 counts, while 3 (27.2%) patients had decreases. Two of these three patients were receiving chemotherapy for lymphoma.

4. *Self-assessment*. A questionnaire followup was sent to each patient receiving RZ_2 for a period of at least 2 months prior to the publication of this study. Of the eight patients who chose to rate the overall importance of continuing RZ_2, 6 (75%) rated their continuing RZ_2 as "very important", and 2 (25%) as "unsure." Six of 8 patients (75%) responding chose to rate their energy levels as "increased", while 2 (25%) reported no improvement. Side effects have been described previously.

DISCUSSION

Although only a preliminary study, initial results recommend the further evaluation of RZ_2 for possible anti-retroviral activity. This small study addresses questions of dosimetry and toxicology. Efficacy and long-term effects must await suitably designed Phase II type studies.

The heterogenicity of the patient population, coupled with the multitude of therapeutic interventions, makes the qualification of clinical effectiveness difficult. Further testing is necessary to determine exactly the nature of this possible anti-viral effect.

Both *Chelidonium* and *Sanguinaria* are complex phytomedicinals, with several powerful alkaloids. Although chelidonine has garnered the majority of attention as a potental anti-viral reverse transcriptase inhibitor, other alkaloids (chelilutine, chelirubine, sanguilutine and sanguirubine), all aromatic benzo[c]phenanthridines, also exhibit anti-viral activity, which probably confirms the empiric wisdom of using the plants in their native state. Further work is needed to standardize growing conditions to allow for optimal alkaloid content, as variability has been a reported problem (5).

It is axiomatic that no new work in alkaloid research has occurred in over twenty years -especially with regard to anti-cancer screening. Yet several characteristics of the alkaloids make them ideal candidates for anti-viral research. Alkaloids are fat-soluble, have a protracted half-life, pass through the blood-brain barrier and also resist acid hydrolysis.

Benzo[c]phenanthridines apparently work by interfering with the viral template (7). Previous studies have qualified the reverse transcriptase activity of chelidonine as "moderate" (3,4) when compared to nitidine and fagaronine, two similar benzo[c]phenanthridine class alkaloids of highly unpredictable toxicity. Yet is is important to note that the rate of HIV-reverse transcriptase inhibition by chelidonine parallels nitidine at low concentration and continues to increase at high concentration*(Figure 2)*. This implies that of the plants containing benzo[c]phenanthridines, *Chelidonium* alkaloids possess "equivalent" anti-HIV activity at low dose *(Figure 2, Box A)* while continuing to show unimodal and increasing activity at higher dose *(Figure 2, Box B)*. Thus although only a "moderate" reverse transcriptase inhibitor, *Chelidonium* and *Sanguinaria* (at appropriate dosage) are relatively non-toxic and possess a positive therapeutic index, commending their utilization as both low-dose and long-term agents.

Benzo[c]phenanthridine alkaloids also appear to exert a direct effect upon the immune system. In this study a decrease in CD8 levels and resolution of PGL, sometimes

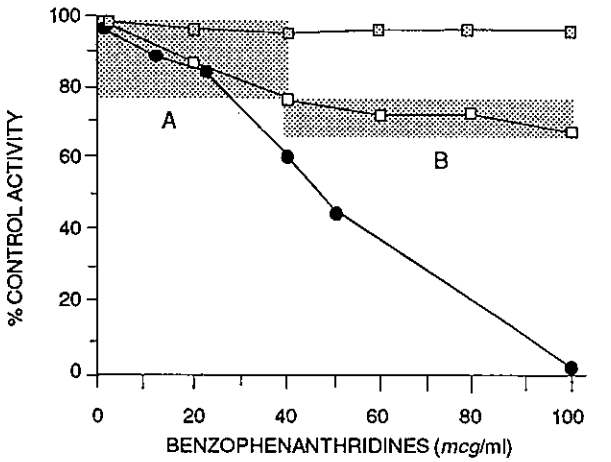

FIGURE 2. Effect of benzophenanthridine concentration on HIV-1 reverse transcriptase activity. Five serial dilutions of the samples were made in DMSO (10micro liter) and added to enzyme reaction mixture just prior to incubation. Control assays were performed without the compounds or extracts but contained an equivalent amount of DMSO. Results are expressed as % control activity. *From:* J. Natural Products, 1991;54(1):148.

Slope A shows a comparable rate of reverse transcriptase inhibition between chelidonine and nitidine chloride in low dose concentrations. Slope B shows that the rate of reverse transcriptase inhibition by chelidonine is unimodal and continues to increase at higher doses (% control activity: Approx. 78% at 40mcg/ml, 63% at 100mcl/ml)

quite dramatic, was observed. Previous studies have also shown CD8 lowering and CD4 enhancement (8).These two phenomena may be linked in some manner, as *Chelidonium* has been a traditional medicine with a history of being employed in leukemia (9).

Other researchers have investigated the potential anti-HIV activity of *Chelidonium*. UKRAIN, a proprietary product, purportedly a thiophosphoric acid derivative from *Chelidonium majus* (10), reportedly increased T4 cell levels in an ARC patient treated with the "immune-stimulating agent UKRAIN." The private venture of the Ukrainian Cancer Institute of Vienna, Austria, Ukrain has generated negative attention. *AIDS-Forsching* recently described the exploitation of AIDS patients by purveyors of fraudulent medication and claimed that a single injection of the drug sold for more than 400 Austrian schillings -$300 US (11).

RZ_2 is available at reasonable cost, approximately $2.25 per bottle of 45 capsules, a one week supply. A pediatric suspension (RZ*jr*) is now available, and an enhanced RZ_3 derived exclusively from roots grown at neutral pH (to maximize alkaloid content) is also planned. Investigators interested in evaluating these products should contact the author at the address listed.

Gender	ID#	Age	Concurrent treatment					Related conditions	Clinical parameters			Self-asessment		
			AZT	ddI	ddC	Gly	Other		CD4	CD8	PGL	Energy	Rate	Side Effects
Male	M1	61	X			X		Molluscum	+	-	DD	I	4	
	M2	28				X	4	PGL	=	=	DD	I	4	
	M3	41		X		X	8	Molluscum, Herpes	=	=	D			4
	M4	61	P			X	8	KS, PCP	=	-		N	2	
	M5	38	P	X		X	7,8	Zoster, PCP, MAI	=	=				
	M6	30	P	X		X			+	+	N	I	4	
	M7	38	X	P	X	X		KS. Molluscum	++	-	N	N		1
	M8	46				X	1,2,6	Lymphoma, KS, PGL	-(*)	- -	DD	I	4	1,2,3
Female	F1	50	P			X	2,6	Lymphoma	-(*)	-	D	I	4	
	F2	32	I			I								
	F3	34				X			+	=	D		4	
	F4	24	X	X	P	X	3,5,6	Hepatitis, neuropathy			D			1,2
	F5	51	X			I	1,5	Zoster	-	=	D	I	2	1

TABLE 1. Prelimary results of Retro-ZIP usage in 13 HIV and AIDS patients. Shaded rows denote patients with AIDS-defining symptomatology, depressed CD4 counts (<50) or frequent opportunistic infections (>1). **KEY: Concurrent treatment.** *AZT, ddI, ddC, Glycyrrhiza glabra:* X/ currently using medication; P/ past usage; I/ intermittent usage. *Other:* 1/ passive immunotherapy; 2/ chemotherapy for AIDS-related lymphoma (Bleomycin); 3/ plasmapheresis; 4/gp160 vaccine; 5/ Acyclovir; 6/ Prednisone; 7/ Aerosolized Pentamidine; 8/ other prophylactic antimicrobial therapy (Bactrim, Dapsone, etc.). **Clinical parameters.** *CD4, CD8:* =/ no change (<=7%); +/ slight increase (>7%,); ++/ increase (>=10%); -/slight decrease (<=7%); --/decrease (>=10%). *PGL:* D/ decrease; DD/ dramatic decrease; N/ no PGL reported. **Self-Asessment:** *Energy:* I/ increased; N/ no change; D/ decreased. *Rate:* 4/ very important; 3/positive; 2/ unsure; 1/ unimportant, no positive impact observed. *Side effects:* 1/nausea or hyperemesis at high dose; 2/ nausea or hypermesis at low dose; 3/angioedema; 4/ changes in stool consistency. **NOTE**: -(*) signifies that CD4 depression is most likely the result of chemotherapy. Prior to chemotherapy M8 had a decrease in CD4 cells (537 on 11/1/90 to 441 on 7/11/91) .

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