Efficacy of Scopadulcic Acid A against *Plasmodium falciparum* in Vitro

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Scoparia dulcis is a perennial herb widely distributed in many tropical countries. It is used as an herbal remedy for gastrointestinal and many other ailments, and in Nicaragua extracts are used to treat malaria. Phytochemical screening has shown that scopadulcic acid A (SDA), scopadulcic acid B (SDB), and semisynthetic analogues are pharmacologically active compounds from *S. dulcis*. SDB has antiviral activity against *Herpes simplex* virus type 1, antitumor activity in various human cell lines, and direct inhibitory activity against porcine gastric H^+, K^+-ATPase. A methyl ester of scopadulcic acid B showed the most potent inhibitory activity against gastric proton pumps of 30 compounds tested in one study. Compounds with antiviral, antifungal, and antitumor activity often show activity against *Plasmodium falciparum*. In *P. falciparum*, the plasma membrane and food vacuole have H^+-ATPases and the acidocalcisome has an H^+-Ppase. These proton pumps are potential targets for antimalarial therapy and may have their function disrupted by compounds known to inhibit gastric proton pumps. We tested pure SDA and found in vitro activity against *P. falciparum* with an IC_{50} of 27 and 19 μM against the D6 and W2 clones, respectively. The IC_{50} against the multidrug-resistant isolate, TM91C235, was 23 μM.

Malaria is one of the most significant public health concerns in many tropical areas of the world. At least 40% of the world population is exposed to the parasite. Malaria Foundation International estimates that malaria causes up to 500 million clinical cases of disease and is responsible for over one million deaths annually; every 30 seconds, a child somewhere dies of malaria.[1]

Scoparia dulcis L. (Scrophuraliaceae) is a perennial herb growing up to a half-meter in height that is widely distributed in many tropical countries. It is known by many common names including *Typycha Kuratu, Vassourinha,* and *Escobilla*. Numerous reports of using the whole plant, root, and extracts as herbal remedies for a variety of disorders exist.[2−4] Some of its reported properties include analgesic,[5] antiinflammatory,[5] sedative,[5] hypertensive,[5] in vitro and in vivo antiviral activity,[6−9] cytotoxicity,[10] B-glucuronidase inhibition,[11,12] H^+, K^+-ATPase inhibition,[13,14] sympathomimetic activity,[15] muscle relaxant,[15] and antitumor effects.[16] Extracts have shown activity against Gram positive bacteria and fungi.[17] Indigenous tribes of Nicaragua use a hot water infusion and/or decoction of the leaves or whole plant to treat malaria, belly pain, menstrual disorders, insect bites, fevers, heart problems, liver and stomach disorders, and venereal disease, for blood cleansing, and as an aid to child birth or a general tonic.[4] Phytochemical screening determined that many of its pharmacological properties are due to the existence of certain phytochemicals (diterpenes) including scopadulcic acid A (SDA), scopadulcic acid B (SDB), and scopadulciol. Scopadulciol and SDB were found to be inhibitors of gastric H^+, K^+-ATPase,[13,14] and SDA is an inhibitor at high concentrations. A methylester of scopadulcic acid B was the most potent inhibitor of porcine gastric proton pumps among 30 related compounds tested.[13] These compounds may interfere with parasite nutrient transport through a similar mechanism.[18−20]

On the basis of these known pharmacological properties of diterpenes from *S. dulcis* and the availability of pure SDA, we sought to determine its activity against three clones of *P. falciparum*. Larry E. Overman, Ph.D. of the University of California at Irvine, provided this pure synthetic chemical entity. His group recently reported enantiodivergent total synthesis of (+)- and (−)-scopadulcic acid A.[21]

Scopadulcic acid A has activity against *P. falciparum* in vitro with an IC_{50} of 27 μM against the D6 clone and an IC_{50} of 19 μM against the W2 clone. The IC_{50} against the multidrug-resistant, TM91C235, isolate was 23 μM. The IC_{50} values for chloroquine were 9.3, 266, and 24 nM against D6, W2, and TM91C235, respectively. The IC_{50} values for mefloquine were 36, 4.8, and 59 nM against D6, W2, and TM91C235, respectively. The mechanism of action of SDA against *P. falciparum* is unknown. If the in vitro activity against *P. falciparum* is related to inhibition of ATPase pumps, we would anticipate that the methyl ester of SDB, a potent inhibitor of gastric H^+, K^+-ATPase, would have a lower IC_{50} than SDA.

Experimental Section

Parasites and Culture Technique. Three well-characterized *P. falciparum* clones with differing sensitivities to a wide range of antimalarial drugs were used for the drug assays. The W-2 clone was prepared from the Indochina I isolate; it is susceptible to mefloquine but resistant to chloroquine, sulfadoxine, pyrimethamine, and quinine. The D-6 clone was prepared from the African Sierra I/UNC isolate; it is naturally resistant to mefloquine but susceptible to chloroquine, sulfadoxine, pyrimethamine, and quinine. The TM91C235 isolate was derived from a patient in Thailand who failed mefloquine twice; this isolate is also resistant to chloroquine, sulfadoxine, pyrimethamine, and quinine. All isolates were maintained in the laboratory under previously described conditions.[22,23]

Test Compounds. Chloroquine and mefloquine were used as controls. Scopadulcic acid A was provided by Dr. Overman.

Parasite Growth Inhibition Assay in Vitro. The in vitro assays were conducted by using a modification of the semi-automated microdilution technique of Desjardins et al.[24] and Chulay et al.[25] Three *P. falciparum* malaria parasite clones,

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W-2, D-6, and TM91C235, were utilized in susceptibility testing. They were derived by direct visualization and micromanipulation from patient isolates.

Briefly, test compounds were dissolved in DMSO and diluted 400-fold in RPMI 1640 culture medium supplemented with 25 mM $NaHCO_3$ and 10% Albumax I (Gibco BRL, Grand Island, NY). These solutions were subsequently serially diluted 2-fold with a Biomek 1000 (Beckman, Fullerton, CA) over 11 different concentrations from 50 000 to 48.8 ng/mL. The parasites were exposed to serial dilutions of each compound for 24 h and incubated at 37 °C with 5% CO_2 and 90% N_2 prior to the addition of [^{3}H]hypoxanthine. After a further incubation of 18 h, parasite DNA was harvested from each microtiter well using a Packard Filtermate 196 Harvester (Meriden, CT) onto glass filters. The samples were washed to remove any unincorporated labeled hypoxanthine. Uptake of [^{3}H]hypoxanthine was measured with a Packard topcount scintillation counter. Concentration−response data were analyzed by a nonlinear regression logistic dose response model, and the IC_{50} value (50% inhibitory concentration) for each compound was calculated.

Cytotoxicity Assay. Formal cytotoxicity testing was not performed because of a limited quantity of the SDA. The lack of hemolysis in our in vitro testing system would indicate that the toxicity to red blood cells was minimal. A literature search found no SDA cytotoxicity data.

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