In Vivo Antimalarial Activity of Isosungucine, an Indolomonoterpenic Alkaloid from *Strychnos icaja*

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Abstract

Isosungucine (**1**) is a quasi-symetric bisindolomonoterpenoid alkaloid isolated from the roots of *Strychnos icaja*. The *in vivo* antimalarial activity against the *P. vinckei petteri* murine strain was determined. In the Peters 4-days suppressive test, **1** suppressed the parasitemia by almost 50 percent on day 4.

Malaria is one of the major infectious diseases in many tropical and subtropical regions, leading to more than one million deaths (principally young African children) out of 400 million clinical cases each year. Since the end of the 1980s, *Plasmodium falciparum* has developed resistance almost everywhere in the world to chloroquine and other currently used antimalarial drugs [1,2]. Urgent efforts are therefore necessary to identify new classes of antimalarial drugs.

Strychnos icaja Baillon (Loganiaceae) is a liana distributed all throughout central Africa and is the only African *Strychnos* species found to date to contain strychnine. Its roots were formerly used for the preparation of arrow and ordeal poisons [3]. However, some tribes of pygmies from Cameroon have treated malaria with *S. icaja* roots [4,5].

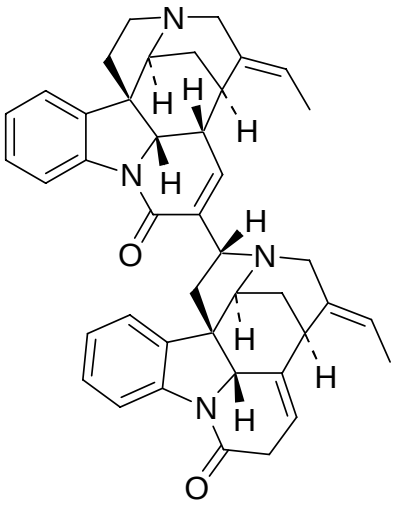

Fig. **1** Chemical structure of isosungucine (**1**).

The *in vitro* antiplasmodial properties of indolomonoterpenic alkaloids were described initially in 1991 by Wright et al., with the most active alkaloid being dihydrousambarensine, a bisindole tertiary alkaloid isolated from *Strychnos usambarensis* roots [6]. Among these bisindole antiplasmodial alkaloids, dihydrousambarensine and strychnopentamine were then tested *in vivo* against *Plasmodium berghei* but were inactive at a dose of 30 mg/kg/day [7]. Nevertheless, it was shown later that dihydrousambarensine was 25 times more active against chloroquine-resistant strains than chloroquine-sensitive strains of *Plasmodium falciparum.* As the *P. berghei* strain used in this study was chloroquine-sensitive, this could explain the inactivity *in vivo*, in addition to the differences between the biology of the two species. Finally, in 2004, for the first time, the *in vivo* antimalarial activity of isostrychnopentamine, another indolomonoterpenic alkaloid, has been demonstrated against *P. berghei* and *P. vinckei* [8].

The *in vitro* antiplasmodial activities of isosungucine (**1,** Fig. **1**) and its derivatives have been described previously [9]. The IC_{50} for isosungucine was 1.32 µM on FCA chloroquine-sensitive strain and 0.27 µM on W2 chloroquine-resistant strain. Compound **1** exhibited 7-35-fold higher activity against *P. falciparum* strains compared with human cancer cell lines (depending on *Plasmodium* strains and human cell lines), thus indicating a good selectivity [9]. Very recently, it was shown that the bisindole alkaloids from *S. icaja* possessing antiplasmodial properties were practically devoid of strychnine-like activity *in vitro*[10]. The objective of the present investigation was to demonstrate the *in vivo* antimalarial activity of isosungucine (**1**, Fig. **1**), a dimeric indolomonoterpenic alkaloid from *S. icaja*.

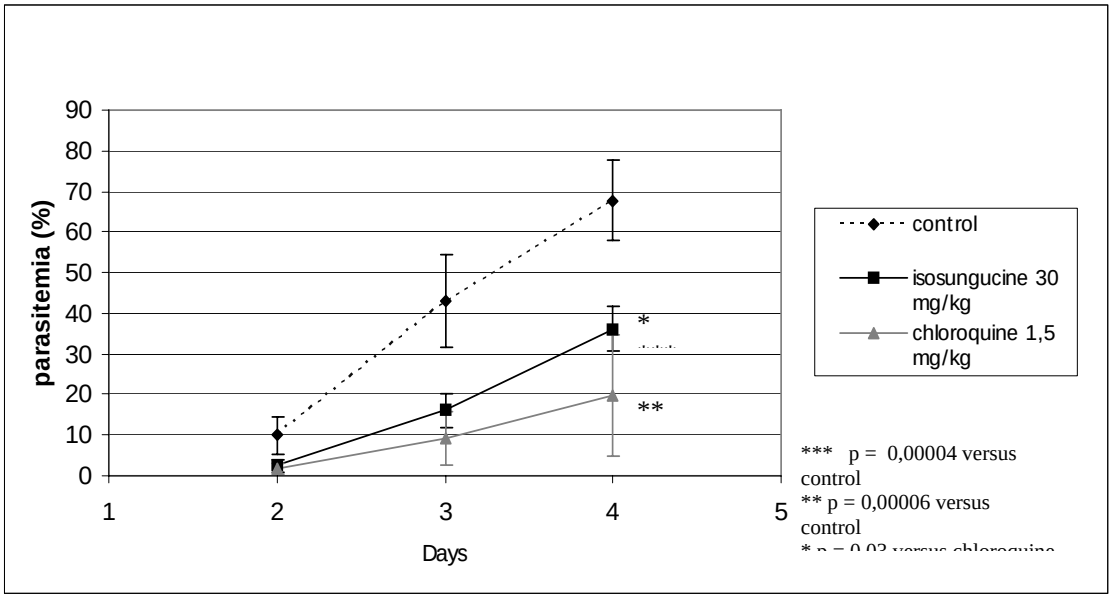

Fig. **2** *In vivo* antimalarial activity of isosungucine (**1**) on mice infected by *Plasmodium vinckei petteri*. Data are shown as mean ± SD of six mice per condition. The 4-days suppressive test was performed as described by Peters[12]. Mice were inoculated with trophozoites and were treated 2h later with drug or vehicle at the indicated dose. The injections were repeated daily for 3 days. The parasitemias were compared at days 2-3-4.

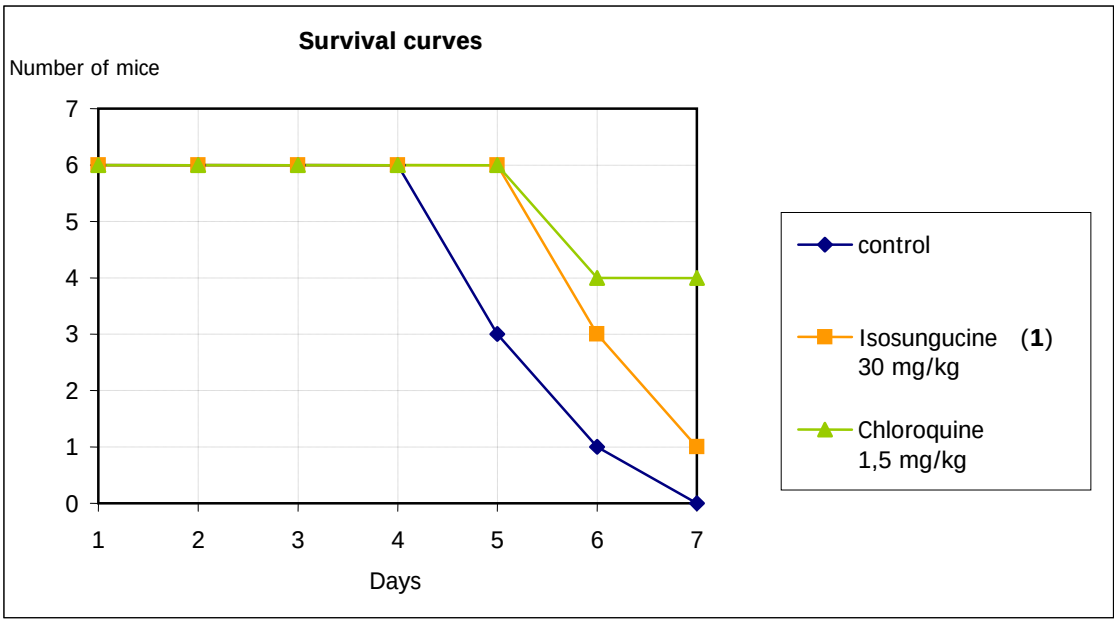

Fig. **3** Survival curves for mice treated with isosungucine(**1**) 30 mg/kg, chloroquine (1,5 mg/kg) and vehicle (control).

Oxygenated derivatives of **1**, 18-hydroxy-isosungucine and strychnogucine B, were more active and more selective than compound **1** [9,11], nevertheless these compounds were isolated in too small quantities to be evaluated *in vivo*.

The *in vivo* antimalarial activity of **1** on *P. vinckei petteri* (chloroquine-sensitive) is presented in Fig. **2**. The procedure followed is the classical 4-days suppressive test of Peters [12] where the test compound is administered for four days to the malaria-infected mice. This procedure is proposed by the WHO as the first line primary screen for *in vivo* testing of potential antiplasmodial compounds[13,14]. At 30 mg/kg/day, parasitemia was reduced on day 2 by 75 % and on day 4 by 47% and survival was increased of at least 24 hours. Nevertheless, at day 7, only one mouse was still alive (0 in control and 4 in chloroquine group) (Fig. **3**). These results are encouraging because the ED_{50} values obtained from other plant extracts or purified constituents when tested for their antimalarial activity are often largely higher than these doses [15],[16].

In conclusion, **1** has an interesting antiplasmodial activity profile, both *in vitro* on various laboratory strains and *in vivo* against a chloroquine sensitive murine strain. Showing selectivity for *Plasmodium* cells compared to human cells [17], and exhibiting no cross resistance with chloroquine, **1** shows an original profile, clearly different from isostrychnopentamine [8] and could be a good lead for antimalarial therapy. It is now important to investigate the molecular targets affected by **1** to trigger *Plasmodium* death. Moreover, it would be very interesting to evaluate the potentialities of the oxygenated derivatives of **1** in animal models. This will need reisolation or (semi)synthesis of additional quantities of these compounds.

Materials and Methods

Chloroquine diphosphate (97%, Sigma C6628, Bornem, Belgium), was used as antimalarial reference. Isosungucine (**1**) was isolated from *Strychnos icaja* as previously described[11] and its purity was assessed by HPLC (> 95%). Stock solutions (1 mM) were prepared with 5% DMSO (final concentration for *in vitro* assays maximum 0.5%). The roots of *S. icaja* were collected near Kasongo-Lunda (RDCongo). Voucher specimen of the plant (Duvigneaud H787) was deposited in the herbarium of the Pharmaceutical Institute, at Liège and in the herbarium of the Belgian National Botanical Garden, at Meise. *Plasmodium vinckei petteri* strain was provided by Prof P. Grellier (Museum National d'Histoire Naturelle, Paris, France). *P. vinckei petteri* was maintained in mice by syringe passage. Female Swiss mice (10 weeks of age, 20±2 g), obtained from Charles River (Charles River Laboratories, Brussels), were maintained under controlled conditions of temperature (22 ± 3 °C, relative humidity 50 - 80 %) and illumination (12 h light, 12 h dark) and were provided with a standard diet and water. All animal husbandry and handling conditions were accorded to the Principles of Laboratory Animal Care and the Belgian Regulation ("*Arrêté Royal du 14 Novembre 1993 Relatif à la Protection des Animaux de Laboratoire*"). The present work was approved by the Ethical Committee for using animals at the University of Liège (No 79). They were inoculated on day 1 intraperitoneally with 20 µl of infected mice blood (parasitemia of about 20%) diluted in physiological saline (50%). The treatment dose was given intraperitoneally 2 h after infection on day 1 and was repeated once daily for 3 days, as a "4-days-blood schizonticidal test" [12]. For this study, isosungucine was dissolved first with DMSO (final concentration: 2%) and Tween 80 (final concentration: 2.5%) then in 0.9% sodium chloride with a volume of injection of 0.1 ml. For each drug, 6 mice were studied, and their parasitemia and mortality were followed. In parallel, 6 mice as control animals received only the vehicle (0.9% sodium chloride, DMSO, Tween, 0.1 ml, I.P.) and 6 mice received chloroquine (1.5 mg/kg). On day 2-3-4, thin blood smears were made from mouse tail blood and stained by Giemsa. At least 2000 cells were counted to determine parasitemia.

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