

Letters to the Editor

Simvastatin Treatment Shows No Effect on the Incidence of Cerebral Malaria or Parasitemia during Experimental Malaria[▽]

Statins, 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, reduce in vitro growth of *Plasmodium falciparum* (5). With great interest, we read the letter of Pradines et al. reporting on a 10-fold-higher growth inhibition of *P. falciparum* by atorvastatin than by simvastatin, suggesting that atorvastatin be considered a candidate drug for malaria treatment (14).

Apart from the cholesterol-lowering activity of statins, immunomodulation and pleiotropic effects of statins may significantly impact infection-related survival (4, 15). In patients with cardiovascular risks and chronic kidney disease, prior statin treatment reduced the incidence of sepsis (1, 2, 6, 7), and in experimental sepsis models, simvastatin prolonged the survival time of mice (13). Cerebral malaria (CM) shares common pathophysiological features with sepsis, especially with regard to pathology of the endothelium (3). Prior to the report of Pradines et al. (14), we hypothesized that statins might be a therapeutic option for CM. Therefore, we tested the effects of simvastatin in *Plasmodium berghei* ANKA-infected C57BL/6 mice, a well-established experimental model of CM. As previously described, groups of 6- to 8-week-old female C57BL/6 mice were infected with *P. berghei* ANKA parasites by intraperitoneal injection on day 0 (12). Oral or intraperitoneal applications with up to 100 mg of simvastatin/kg of body weight were given at different dosages and points in time (Table 1). Much higher doses than used in humans were administered, considering the described 3-hydroxy-3-methylglutaryl-coenzyme A reductase up-regulation in rodents, and no severe toxic effects in mice have been observed in similar experimental settings (9, 10). The primary end point of our study was the incidence of CM. Clinical symptoms of CM in study animals were checked at least twice per day. Parasitemia was assessed by light microscopy and expressed as a percentage of red blood cells. All experiments were conducted in the animal laboratory of the Bernhard Nocht Institute for Tropical Medicine and approved by responsible authorities.

Our results show that there was no difference in the incidence or time to CM or in the level of parasitemia of simvastatin-treated mice and controls (Table 1). We conclude that simvastatin has no relevant effect on in vivo parasite growth inhibition and clinical outcome of *P. berghei* ANKA-infected C57BL/6 mice. Consequently, we aborted further experiments. To our best knowledge, statins have not been given to *P. berghei*-infected mice before, and in vitro data on growth inhibition of this parasite is lacking. Our attempt to reduce the incidence of CM in mice by simvastatin treatment as proof of the principle failed, although our model is sensitive to immunomodulatory strategies (12) and comparable doses of simvastatin induced anti-inflammatory effects in other diseases (9, 13).

Nevertheless, it might still be possible that other statins have beneficial effects on malaria, as there are differences in the chemical, pharmacokinetic, and pharmacodynamic properties of statins. Therefore, further experiments on plasmodial growth inhibition and anti-inflammatory responses are needed. Specifically, it would be useful to reveal the pharmacological

TABLE 1. Incidence of cerebral malaria during *Plasmodium berghei* ANKA infection is not influenced by simvastatin treatment

Treatment ^a	Parasitemia (%) on day 5 postinfection (mean ± SEM)	No. with mice with CM/total no. of mice ^b
Control ^c	2.76 ± 0.57	5/5
Simvastatin		
i.p. (low dose) ^d	2.38 ± 0.66	4/4
i.p. (dose escalation) ^e	3.04 ± 1.20	5/5
i.p. (high dose) ^f	2.04 ± 0.41	5/5
Oral ^g	2.98 ± 1.04	9/10

^a C57BL/6 mice were infected intraperitoneally (i.p.) with 1×10^6 *P. berghei* ANKA-infected red blood cells and treated with simvastatin on the time points indicated in footnotes d to g. For i.p. treatment, simvastatin was activated by alkaline hydrolysis (reconstitution in phosphate-buffered saline and pH adjustment to 7.4).

^b The number of mice showing symptoms of CM per total number of *P. berghei* ANKA-infected mice is indicated. Mice were scored positive when they exhibited early symptoms of CM (reduced locomotion, ataxia), which occurred between day 6 and day 8 postinfection (p.i.). Mice were sacrificed when death was considered to be inevitable. Cumulative Kaplan-Meier survival analysis was calculated by Cox regression and tested by log rank test. Parasitemia was assessed by microscopy on day 5 p.i., and the values were compared by nonparametric (Wilcoxon) test. Also, no differences were observed on days 3 and 6 p.i. (data not shown). A *P* value of <0.05 was considered significant. The different control treatments showed no significant difference in the incidence of CM.

^c i.p. injection of 200 μ l dissolving solution (0.9% NaCl containing 10% ethanol) is shown.

^d i.p. treatment on days 2, 4, and 6 with 2 μ g/g of body weight.

^e i.p. treatment on day 2 with 0.5 μ g/g, on day 4 with 1 μ g/g, and on day 6 with 2 μ g/g of body weight.

^f i.p. treatment on days 2, 4, and 6 with 40 μ g/g of body weight.

^g Oral treatment on days -1, 0, 1, 3, and 5 with 100 μ g/g of body weight.

mechanisms by which statins inhibit *P. falciparum* growth and how neuroprotection in CM could be achieved. Finally, considerable differences of malaria in mice and humans have to be acknowledged when taking statins further in treatment development (8, 11).

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All authors participated in design, animal experiments, and analysis and interpretation of the data. Substantial input came from all investigators. R.K. initiated the study, R.K. and T.J. designed the study, and both were involved in all phases of the study. N.S. was responsible for preparation of simvastatin for application in mice. R.K. wrote the manuscript, and T.J. and J.M. critically reviewed the paper. All authors checked the final manuscript.

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