

Simvastatin treatment shows no effect on the incidence of cerebral malaria or parasitemia during experimental malaria

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1 Statins, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, reduce *in*
2 *vitro* growth of *Plasmodium falciparum* (4). With great interest we read the letter of Pradines
3 *et al.* reporting on a 10-times higher growth inhibition of atorvastatin compared to
4 simvastatin, considering atorvastatin a candidate drug for malaria treatment (14).
5 Apart from cholesterol-lowering activity, immunomodulation and pleiotropic effects of statins
6 may significantly impact infection-related survival (15). In patients with cardiovascular risks
7 and chronic kidney disease prior statin treatment reduced the incidence of sepsis (1, 2, 6, 7)
8 and in experimental sepsis models simvastatin prolonged survival time of mice (13). Cerebral
9 malaria (CM) shares common pathophysiological features with sepsis, especially with regard
10 to pathology of the endothelium (3). Prior to Pradines *et al.* report (14), we hypothesized that
11 statins might be a therapeutic option for CM. Therefore, we tested the effects of simvastatin in
12 *P. berghei* ANKA-infected C57BL/6 mice, a well established experimental model of CM. As
13 previously described, groups of 6-8 weeks old female C57BL/6 mice were infected with *P.*
14 *berghei*-ANKA parasites by intraperitoneal injection on Day 0 (12). Oral or intraperitoneal
15 applications with up to 100 mg/kg bodyweight simvastatin were given at different dosages
16 and points in time (Table I). Much higher doses than used in man were administered
17 considering the described HMG-CoA reductase up-regulation in rodents and no severe toxic
18 effects in mice have been observed in similar experimental settings (9, 10). The primary
19 endpoint of our study was the incidence of CM. Clinical symptoms of CM in study animals
20 were checked at least twice per day. Parasitemia was assessed by light microscopy and
21 expressed in percent of red blood cells. All experiments were conducted in the animal
22 laboratory of the Bernhard Nocht Institute for Tropical Medicine and approved by responsible
23 authorities.

24 Our results show that there was no difference in the incidence or time to CM, nor in the level
25 of parasitemia between simvastatin-treated mice and controls (Table I). We conclude that

1 simvastatin has no relevant effect on *in vivo* parasite growth inhibition and clinical outcome
2 of *P. berghei* ANKA-infected C57BL/6 mice. Consequently, we aborted further experiments.
3 To our best knowledge, statins were not given to *P. berghei*-infected mice before and *in vitro*
4 data on growth inhibition of this parasite is lacking. Our attempt to reduce the incidence of
5 CM in mice by simvastatin treatment as a prove of principle failed, although our model is
6 sensitive to immunomodulatory strategies, as comparable doses of simvastatin induced anti-
7 inflammatory effects in other diseases (9, 12, 13).
8 Nevertheless, it might still be possible that other statins have beneficial effects on malaria as
9 there are differences in chemical, pharmacokinetic and pharmacodynamic properties.
10 Therefore, further experiments on plasmodial growth inhibition and anti-inflammatory
11 responses are needed. Specifically, it would be usefull to reveal the pharmacological
12 mechanisms by which statins inhibit *P. falciparum* growth and how neuroprotection in CM
13 could be achieved. Finally, considerable differences of malaria in mice and humans have to
14 be acknowledged when taking statins further in treatment development (8, 11).

REFERENCES

1. **Almog, Y., Shefer, A., Novack, V., Maimon, N., Barski, L., Eizinger, M., Friger, M., Zeller, L., and Danon, A.** 2004. Prior statin therapy is associated with a decreased rate of severe sepsis. *Circulation* **110**:880–885.
2. **Almog, Y., Novack, V., Eisinger, M., Porath, A., Novack, L., and Gilutz, H.** 2007. The effect of statin therapy on infection-related mortality in patients with atherosclerotic diseases. *Crit. Care Med.* **35**:372–378.
3. **Clark, I.A., Alleva, L.M., Mills, A.C., and Cowden, W.B.** 2004. Pathogenesis of malaria and clinically similar conditions. *Clin. Microbiol. Rev.* **17**:509–539.
4. **Grellier, P., A. Valentin, V. Millerieux, J. Schrevel, and D. Rigomier.** 1994. 3-Hydroxy-methylglutaryl coenzyme A reductase inhibitors lovastatin and simvastatin inhibit in vitro development of *Plasmodium falciparum* and *Babesia divergens* in human erythrocytes. *Antimicrob. Agents Chemother.* **38**: 1144–1148.
5. **Greenwood, J., and Mason, J.C.** 2007. Statins and the vascular endothelial inflammatory response. *Trends Immunol.* **28**:88–98.
6. **Gupta, R., Plantinga, L.C., Fink, N.E., Melamed, M.L., Coresh, J., Fox, C.S., Levin, N.W., and Powe, N.R.** 2007. Statin use and hospitalization for sepsis in patients with chronic kidney disease. *JAMA* **297**:1455–1464.

7. **Hackam, D.G., Mamdani, M., Li, P., and Redelmeier, D.A.** 2006. Statins and sepsis in patients with cardiovascular disease: a population-based cohort analysis. *Lancet* **367**:413–418.
8. **Hunt, N.H., Golenser, J., Chan-Ling, T., Parekh, S., Rae, C., Potter, S., Medana, I.M., Miu, J., and Ball H.J.** 2006. Immunopathogenesis of cerebral malaria. *Int J Parasitol.* **36**:569–582.
9. **Kim, D.Y., Ryu, S.Y., Lim, J.E., Lee, Y.S., and Ro, J.Y.** 2007. Anti-inflammatory mechanism of simvastatin in mouse allergic asthma model. *Eur. J. Pharmacol.* **557**:76–86.
10. **Kita, T., Brown, M.S., and Goldstein, J.L.** 1980. Feedback regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in livers of mice treated with mevinolin, a competitive inhibitor of the reductase. *J. Clin. Invest.* **66**:1094–1100.
11. **Lamikanra, A.A., Brown, D., Potocnik, A., Casals-Pascual, C., Langhorne, J., and Roberts, D.J.** 2007. Malarial anemia: of mice and men. *Blood* **110**:18–28.
12. **Lepenies, B., Pfeffer, K., Hurchla, M.A., Murphy, T.L., Murphy, K.M., Oetzel, J., Fleischer, B., and Jacobs T.** 2007. Ligation of B and T lymphocyte attenuator (BTLA) prevents the genesis of experimental cerebral malaria. *J. Immunol.* **179**:4093–4100.
13. **Merx, M.W., Liehn, E.A., Graf, J., van de Sandt, A., Schaltenbrand, M., Schrader, J., Hanrath, P., and Weber, C.** 2005. Statin treatment after onset of sepsis in a murine model improves survival. *Circulation* **112**:117–124.

14. Pradines, B., Torrentino-Madamet, M., Fontaine, A., Henry, M., Baret, E., Mosnier, J., Briolant, S., Fusai, T., and Rogier, C. 2007. Atorvastatin is ten-fold more active in vitro than other statins against *Plasmodium falciparum*. *Antimicrob. Agents Chemother.* **51**:2654–2655.

15. Terblanche, M., Almog, Y., Rosenson, R.S., Smith, T.S., and Hackam, D.G. 2007. Statins and sepsis: multiple modifications at multiple levels. *Lancet Infect. Dis.* **7**:358–368.

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Conflict of interest statement

All authors and acknowledged individuals: no conflict of interest.

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Contributors

All authors participated in design, animal experiments, analysis and interpretation of the data. Substantial input came from all investigators. RK initiated the study, RK and TJ designed the study and both were involved in all phases of the study. NS was responsible for preparation of simvastatin for application in mice. RK wrote the manuscript and TJ and JM critically reviewed the paper. All authors checked the final manuscript.

Legends to the figures

Table I.

The incidence of cerebral malaria (CM) during *Plasmodium berghei* ANKA (PbA) infection is not influenced by simvastatin treatment.

Treatment ^a	Parasitemia on day 5 post infection [% ± SEM]	No. CM/total ^b
control ^c	2.76 ± 0.57	5/5
simvastatin i.p. (low dose) ^d	2.38 ± 0.66	4/4
simvastatin i.p. (dose escalation) ^e	3.04 ± 1.20	5/5
simvastatin i.p. (high dose) ^f	2.04 ± 0.41	5/5
simvastatin oral ^g	2.98 ± 1.04	9/10

^a C57BL/6 mice were infected intraperitoneal (i.p.) with 1×10^6 PbA-infected red blood cells and treated with simvastatin at the indicated time points. 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 PbA-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 post infection (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 compared by nonparametric (Wilcoxon) test. Also no differences were observed on days 3 and 6 p.i. (data not shown). A value of $p < 0.05$ 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 at day 2, 4, and 6 with 2 µg/g body weight

^e i.p treatment at day 2 with 0.5 µg/g, at day 4 with 1 µg/g and at day 6 with 2 µg/ body weight

^f i.p. treatment at day 2, 4, and 6 with 40 µg/g body weight

^g oral treatment on days -1, 0, 1, 3, 5 with 100 µg/g body weight

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