PHARMACOLOGICAL ASSESSMENT OF THE ROLE OF NITRIC OXIDE IN MOUSE MODELS OF MALARIA *IN VIVO*

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Malaria (*Plasmodium*) infections in mice share many similarities with human malaria and are common laboratory models of the human disease. However, a major species difference is the development of hypothermia, not fever, in murine malaria. Nitric oxide (NO) is implicated in the immune response and the pathophysiology associated with malaria. We have evidence of increased NO production in blood and other tissues of malarial mice, but its value to the host is unclear. In order to understand better the role(s) of NO, a pharmacological investigation was conducted to determine whether increased concentrations of NO *in vivo* have (1) an antimalarial action and/or (2) a hypothermic effect. Male MF1 mice were inoculated intravenously (i.v.) with mouse erythrocytes parasitised by *P. berghei* or *P. chabaudi* on day 0; controls received uninfected erythrocytes. Plasma NO was measured, by Griess assay of nitrites following nitrate reductase treatment, as plasma total nitrite concentrations (PTNC). Plasma was prepared from heparinised blood collected by cardiac puncture under terminal general anaesthesia produced by inhalation of diethyl ether. *P. berghei* produced a rapid, high parasitaemia following an unsustained rise in PTNC, necessitating humane killing on day 4 or 5. *P. chabaudi* produced a self-limiting parasitaemia, which was cleared in association with sustained high PTNC. Six putative NO inducers and three NO donors were tested for their ability to increase PTNC in uninfected mice, the most effective agents then being evaluated for their ability to decrease morbidity caused by *P. berghei*. Lipopolysaccharide (LPS, *Salmonella abortus equi*) was the most effective inducer, as indicated by PTNC 3 h after subcutaneous (s.c.) injection: 0.9% saline (10 ml/kg) 6 ± SD 5 nmol/ml; LPS (0.5 mg/kg) 85 ± 46 nmol/ml, $P < 0.01$; LPS (4 mg/kg) 119 ± 60 nmol/ml, $P < 0.001$, all groups $n = 6$). Increases in PTNC induced by LPS were associated with falls in colonic temperature (minus ~1.5°C). S-Nitrosoglutathione (SNOG, 16.8 mg/kg s.c.) was the most effective NO donor *in vivo*, elevating PTNC for at least 90 min, but having no effect on temperature. LPS (4 mg/kg, once daily) and SNOG (16.8 mg/kg, twice daily) were injected s.c. in *P. berghei* infected mice on days 1, 2 and 3. LPS, but not SNOG, increased PTNC (control 43 ± 24 nmol/ml; LPS 89 ± 37 nmol/ml, $P < 0.05$, $n = 7$), splenomegaly and survival (on days 4, 5 and 6), whilst decreasing parasitaemia and hypothermia compared with infected mice receiving vehicle (4% dimethyl sulfoxide in olive oil, 10 ml/kg). Two NO synthase inhibitors, aminoguanidine hydrochloride (AG) and S-(2-aminoethyl)isothiourea dihydrobromide (100 and 200 mg/kg intraperitoneal injection, i.p.) were evaluated for their ability to reduce the rise in PTNC induced by LPS (0.5 mg/kg i.v.) in uninfected mice. AG proved more effective and was tested for its ability to compromise the natural resolution of *P. chabaudi*. AG (200 mg/kg i.p. once daily on days 5 - 13) inhibited the *P. chabaudi* induced rise in PTNC (day 14 PTNC: 0.9% saline 61 ± 40 nmol/ml, AG 5 ± 4 nmol/ml, $P < 0.05$, $n = 5$) and increased morbidity, but had no effect on parasitaemia or hypothermia measured on alternate days from day 0 to day 28. In summary, no consistent relationship was evident between the ability of the pharmacological agents used here to modify NO activity *in vivo*, monitored as PTNC, and their effects on malaria parasitaemia and colonic temperature in mice.}

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