Title: Absolute Bioavailability of cis-Mirincamycin and trans-Mirincamycin in Healthy Rhesus Monkeys, and ex-vivo Antimalarial Activity against Plasmodium falciparum

Keywords: Bioavailability, Mirincamycin, Primate, Antimalarial, Pharmacokinetics

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Manuscript summary:

Number of text pages: 18
Number of Running title: 52/54
Number of words in abstract: 244/250
Number of references: 24
Number of figures: 3
Number of tables: 3
Abstract:

The pharmacokinetics, oral bioavailability and ex vivo antimalarial activity of mirincamycin isomers in a healthy rhesus monkey model were assessed to support lead optimization of novel non-hemolytic drugs for radical cure and causal prophylaxis of malaria. Fourteen male rhesus monkeys were randomized to four groups, which included cis- and trans- isomers by the oral and intravenous routes, with vehicle-only controls for each dosing route. Concentration-time data were collected for seven days, and were analyzed by non-compartmental analysis. Cis-mirincamycin had an absolute oral bioavailability of 13.6% which was slightly higher than trans-mirincamycin (11.7%), but this difference was not statistically significant. There was a statistically significant difference between AUC$_{0-48h}$ of cis-mirincamycin and trans-mirincamycin after oral dosing. When cultured in vitro with the W2 clone of Plasmodium falciparum, the 50% inhibitory concentration for cis-mirincamycin, trans-mirincamycin, and dihydroartemisinin were 11,300, 12,300 and 2.30 nM, respectively. However, when dosed primate plasma was cultured ex vivo against the W2 clone, both isomers had much greater relative potency compared to their in vitro activity relative to dihydroartemisinin-an increase of approximately 100 fold for cis, and 150 fold for trans. Further, oral ex vivo activity was significantly higher than intravenous activity for both isomers, particularly during the first 90 minutes following dosing, suggesting the first-pass formation of one or more metabolites with blood-stage antimalarial activity. Identification of the metabolic pathways and metabolites may help to further delineate the properties of this class of drugs with previously demonstrated liver stage antimalarial activity.
Introduction:

The US Army antimalarial drug development program is currently focusing on development of drugs able to eliminate the merozoite and hypnozoite forms of liver-stage malaria parasites that could serve respectively as agents for causal (liver-stage) antimalarial chemoprophylaxis or the ‘radical cure’ of blood and liver stage Plasmodium vivax. However, the discovery of novel liver-stage agents without hemolytic liability in patients with G6PD-deficiency lags far behind the development of safe, effective blood stage agents due to limitations in preclinical in vitro and in vivo models (22).

Mirincamycin was investigated as a promising lead candidate as a causal prophylactic and/or radical curative antimalarial drug. Both Powers and Schmidt showed that mirincamycin, a lincosamide antibiotic, administered orally as a single agent had intrinsic radical cure and causal prophylactic activity in the rhesus Plasmodium cynomolgi model (14) (17). When administered orally in a causal prophylaxis model, clindamycin did not completely protect any animal up to a dose of 80 mg/kg/day for 9 days, while mirincamycin protected 3 of 6 animals at 40 mg/kg/day for 9 days. Mirincamycin was likewise superior as a radical cure agent in this model – while clindamycin did not cure any animal up to 80 mg/kg/day for 7 days, mirincamycin cured 2 of 3 animals at 40 mg/kg/day for 7 days. A dose-related delay in parasite patency occurred with both drugs for both indications. In 1985, Schmidt showed that 2.5 mg/kg mirincamycin, 0.375 mg/kg primaquine and 2.5 mg/kg chloroquine for 7 days potentiated the efficacy of primaquine’s antihypnozoite activity following an inoculum of $8.5 \times 10^3$ sporozoites of Plasmodium cynomolgi (16).
Mirincamycin is a mixture of stereoisomers, cis-mirincamycin and trans-mirincamycin (Figure 1). Apparent causal prophylactic activity of both isomers was demonstrated in *Plasmodium berghei* infected mice at subcutaneous doses of 1.1, 3.3, 10 and 40 mg/kg/day for 3 days (3, 4). Mirincamycin isomers were also found to be safe and have acceptable pharmacokinetic parameters in rodent models. The pharmacokinetic and *ex vivo* blood-stage antimalarial pharmacodynamic relationships of the two isomers were evaluated in a healthy Rhesus monkey model. This was one of a series of experiments performed in support of the US Army and the Medicines for Malaria Venture’s shared critical path to develop new treatments for relapsing malaria.

**Materials and Methods:**

**Animals.** Fourteen male Indian-origin rhesus monkeys (*Macaca mulatta*) were studied. Monkeys were randomized to 4 parallel groups of 3 animals (cis- PO and IV, trans- PO and IV) with an additional control animal in each dosing route group (vehicle only). Each animal was dosed and plasma samples were collected into heparin coated syringes at 0, 15, 30, 60 min, 2, 4, 6, 8, 12, 24 hr, 2, and 7 days, with an additional specimen collected at 5 min for animals dosed IV. All plasma samples were frozen at -80°C before analysis by LC/MS. All animal experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committees of AFRIMS and the Walter Reed Army Institute of Research.

**Drugs.** Mirincamycin synthesis has been described previously (8). Mirincamycin isomers were synthesized by Bridge Organics Co. (Vicksburg, MI), and provided to the Walter Reed Army Institute of Research (WRAIR) by MALDEVCO, Inc.
Separation and purity of the isomers was confirmed by SRI International using infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy, HPLC and Mass Spectrometry. Both cis-mirincamycin (optical rotation $+148.69^\circ$; $c = 1.011$, H$_2$O), trans-mirincamycin (optical rotation $+144.99^\circ$; $c = 0.6867$, H$_2$O). Both isomers were dissolved in 0.45% saline at 5 mg/ml for intravenous and oral administration, and 0.45% saline was used as a control for each dosing route. Dihydroartemisinin (DHA) powder for *in vitro* and *in vivo* studies was synthesized by Starks Associates, 1280 Niagara St, Buffalo, NY and provided to WRAIR.

**Sample preparation.** Mirincamycin isomers in plasma samples were extracted by protein precipitation. Briefly, a 100 µL of plasma sample was added into 200 µL of cold acetonitrile, and mixed vigorously for 60 sec. Then, the mixture was centrifuged at 3,000 x g for 10 min, and supernatant was collected into a tube for LC/MS analysis.

**LC/MS analysis of biological samples.** Mirincamycin in extracted samples were separated by a liquid chromatography system (Waters 2695 Separations, Waters Corp., MA) equipped with an XTerra MS C18 column. Chromatographic resolution was obtained by running a 6 min acetonitrile: 0.1% v/v formic acid gradient from 20 to 78% acetonitrile within 3 min and held for 3 min at 0.6 mL/min flow rate. Both isomers eluted at 3.8 min at the same m/z ratio of 439. The mass of both isomers was acquired on a single quadrupole mass spectrometer (Waters Micromass ZQ, Waters Corp., MA) operated using electrospray ionization in positive ion mode. The voltages applied to both isomers for cone, capillary, extractor and RF lens were 30 V, 2 kV, 2 V, and 0.5 V, respectively. The source, cone and desolvation temperature were set at 20 °C, and 350 °C, respectively. Nitrogen gas was produced by a nitrogen generator.
and the gas flows were applied at 40 L/h for cone and 450 L/h for desolvation. The gas cell pirani pressure was < $10^{-4}$ mbar. The data were recorded in the single ion recording mode. The intra-assay accuracy and precision for analyzing cis-mirincamycin at 1.80 (LLOQ), 3.61, and 923 µg/L were within ± 12.1% and the coefficients of variation were less than 14%. For trans-mirincamycin, the intra-assay accuracy and precision at 1.95 (LLOQ), 3.91, and 1000 µg/L were within ± 18.1% and the coefficients of variation were less than 6.4%. The inter-assay accuracy and precision for cis-mirincamycin and trans-mirincamycin at the same three concentrations (15.6, 125, and 500 µg/L) tested were within ± 5.11 and ± 11.2 % error and coefficients of variation were < 4.8% and < 8.5%, respectively. Both cis- and trans-mirincamycin were stable in plasma (15.6 and 500 µg/L) at room temperature (25 °C) for up to 4 and 6 h with the % difference from time 0 within ± 11.2% and ± 12.8%, respectively.

Measurement of Antimalarial Activity- In vitro drug susceptibility was measured by incubating serial dilutions of mirincamycin isomers with a W2 Plasmodium falciparum clone for 72 hours using a non-isotopic histidine rich protein 2 (HRP2) ELISA method (12). The Plasmodium falciparum-based bioassay for the measurement of the antimalarial activity in plasma has been previously described (20). Briefly, by modifying a method for an in vitro drug susceptibility test, the antimalarial activity in plasma containing an unknown concentration of drug can be equated to known concentrations of DHA required to inhibit parasite growth. Primate plasma at each pharmacokinetic time-point from animals dosed with cis- and trans-mirincamycin by each dosing route was incubated with a W2 P. falciparum clone, and growth inhibition was measured using a non-isotopic HRP2 ELISA method (13).
Growth inhibition activity of primate plasma *ex vivo* was compared to a standard curve generated for DHA as a reference control. Relative potency was determined by comparing both *in vitro* and *ex vivo* pharmacodynamic AUC₀₋₁₂h and C_max measurements with corresponding pharmacokinetic measurements.

**Data analysis.** Concentration-time data collected during 48 hours after dosing were analyzed by non-compartmental analysis using PK Solutions 2.0 (Summit Research Services, Montrose, CO). Comparisons of pharmacokinetic data between experimental groups were made by Mann-Whitney U test, with p<0.05 as a criterion for statistical significance. Absolute bioavailability was calculated using the formula

\[
F = \frac{[AUC]_p \times dose_{IV}}{[AUC]_{IV} \times dose_{po}}
\]

for each isomer.
Results:

Pharmacokinetics. It was found that the plasma concentrations of mirincamycin isomers decreased rapidly for the first 30 minutes following IV dosing, while oral plasma concentration increased gradually and peaked at 0.5-1 hours after dosing (Figure 2). Cis-mirincamycin had an absolute oral bioavailability of 13.6% which was slightly higher than trans-mirincamycin (11.7%), but this difference was not statistically significant (Table 1). There was a statistically significant difference between AUC0-48h of cis-mirincamycin and trans-mirincamycin after oral dosing (30% higher for cis), but not Cmax. There was no significant tissue distribution between the two isomers, with mean Vd/F of 10 L/kg by the oral route and 15-20 L/kg in intravenous groups. Clearance was slow with a mean terminal elimination half life of 9-15 hours depending on route and isomer; concentrations were detectable at up to 48 hours. Half life by the oral route was longer for cis-mirincamycin compared to trans-mirincamycin, and cis-mirincamycin had a slower clearance than trans-mirincamycin. No biologically significant differences between isomers were observed in terms of pharmacokinetics or toxicity.

Toxicity. Acute toxicity was limited to loose stools of 1-3 days duration following a single oral dose with cis and 1-2 days with the trans isomer. There were mild transient elevations in alanine transaminase (ALT) – 1 of 6 animals experienced a 30% rise above the upper limit of normal (ULN) in the cis- group while the control animal had a 10% rise. For aspartate transaminase (AST) 3 animals in each of the cis- and trans- groups had a rise of 1.5-2x ULN at 12 hours; however control animals had similar elevations. All elevations resolved within 48 hours after dosing.

Pharmacodynamics. In the in vitro system, the IC50 of cis-mirincamycin, trans-mirincamycin, and DHA against W2-clones expressing HRP-2 were 11,300, 12,300
and 2.30 nM, respectively after 72 hours of incubation (Table 2). In the *ex vivo* system, both isomers showed peak antimalarial activity against W2 clones of *Plasmodium falciparum* 1 hour following oral dosing and 5-15 min following intravenous dosing (Figure 3). There were no statistically significant differences in *ex vivo* antimalarial activity between the two isomers. While DHA was approximately 5,000-fold more potent against *P. falciparum* than mirincamycin in the *in vitro* model, the *ex vivo* potency of both isomers increased dramatically relative to the *in vitro* DHA reference measurements with an approximate 100-fold increase for *cis*, and 150-fold for *trans* (Table 3). Further, oral *ex vivo* activity was significantly higher than intravenous activity for both isomers, particularly during the first 90 minutes following dosing, suggesting the first-pass formation of one or more metabolites with blood-stage antimalarial activity. *cis*-Mirincamycin orally was approximately 2-fold more potent *ex vivo* than *in vitro* against *P. falciparum* compared to the IV route at *C*<sub>max</sub> and AUC<sub>0-12h</sub> comparison. This difference was slightly less pronounced with *trans*-mirincamycin which was only 1.2x more potent at *C*<sub>max</sub> and 1.9x more potent by AUC comparison.

**Discussion:**

Mirincamycin is a member of the lincosamide antibacterial class, and in addition to mirincamycin, other members of this class have been found to have antimalarial properties, particularly clindamycin (15) (19). We demonstrate that in the Rhesus model, both isomers of mirincamycin have low absolute oral bioavailability which is similar to that seen with lincomycin (~20%), but substantially lower than clindamycin which was previously shown to have 90% bioavailability in
humans (10). The $T_{\text{max}}$ of both isomers after oral dosing is approximately 0.5-2 hours, which is not substantially different from other lincosamides. Both isomers of mirincamycin have high apparent volumes of distribution suggesting extensive tissue distribution. Our results correlate well with a previous report where the pharmacokinetic data following a single 150 mg/kg dose of mirincamycin in human subjects could not be fitted to a single compartment model due to the large volume of distribution (11).

We found that the elimination half-life of both isomers varied between 10-15 hours, which is similar to rodent data reported from WRAIR in 2008 (3). In contrast, pharmacokinetic studies of mirincamycin mixtures in the 1970-80’s reported a half-life of 2-3 hours in both mice and monkeys (1). This discrepancy is thought to be due to the availability of more sensitive assay methods incorporating LC/MS analysis. The higher method sensitivity and longer period of sample collection in this study may have resulted in a longer apparent terminal elimination half-life in our pharmacokinetic analysis, and also enabled a better understanding of metabolism in vivo (9). trans-Mirincamycin had a shorter half-life and more rapid clearance than cis-mirincamycin by the oral route. The lower AUC and bioavailability of trans-mirincamycin may have resulted from faster metabolism and/or clearance compared to cis-mirincamycin. Several reports have determined that the major metabolic pathway of lincosamides is via hepatic phase I biotransformation enzymes (6). Wynalda reported that CYP3A4 is the major enzyme responsible for degradation of clindamycin and CYP3A5 is a minor pathway (24). Two major metabolites - clindamycin sulfoxide and n-desmethyl clindamycin - are formed following hepatic transformation with antimicrobial activity against Helicobacter pylori (23). Therefore, it is possible that metabolic transformation of mirincamycin also occurs via this
enzyme superfamily, which may be responsible for generating active metabolites with increased antimalarial activity. However, current understanding is limited and it is thought that all lincosamides are metabolized by multiple enzymatic pathways, so the major routes of metabolism and excretion remain poorly understood.

Among lincosamide derivatives, it is likely that lincomycin is the most potent drug against both the D6 and W2 clones of *Plasmodium falciparum* *in vitro* (11). However, lincomycin does not appear to be active in animal models of malaria (7). Meanwhile, clindamycin demonstrated antimalarial efficacy in the mouse model but not in the primate *P. cynomolgi* model, while mirincamycin showed efficacy in both the mouse and primate models (3). In the present experiment, dosed monkey plasma with both mirincamycin isomers showed antimalarial activity against the W2 *P. falciparum* clone *ex vivo*, with slightly greater pharmacodynamic activity and relative potency observed with cis-mirincamycin. A recent report by Held (5) found that the cis-mirincamycin isomer had a slightly higher median 50% inhibitory concentration (IC$_{50}$) compared to trans against clinical isolates of *Plasmodium falciparum* from Gabon. Of note, both mirincamycin isomers appeared to be much more potent using a prolonged 6 day incubation method. However, this could be explained by differences in technical methods related to the *in vitro* assay. As previously reported (20), the method we used here may not be optimal for drugs with relatively low potencies due to limits of detection. Longer incubations with low-potency drugs against malaria have shown increased potency at 96 hours and beyond when 2 life cycles are covered. A recent study by Wein *et al*. (21), suggested that drug mechanism of action may have a significant influence on the outcomes and reliability of the HRP-2 *in vitro* method. There was greater relative potency by the oral route compared to IV for both cis- and trans- isomers at C$_{\text{max}}$, and over the first 90 minutes after dosing, suggesting
hepatic biotransformation leading to active metabolites. However, this difference diminished over the course of dosing, with only a 2-fold difference by AUC\textsubscript{0-12h} for cis- and trans-. While it is possible that low absorption may contribute to the low apparent oral bioavailability of mirincamycin, it is likely that first pass metabolism with production of metabolites unmeasured in this experiment may also have been an important factor in the apparently low exposure measured. Despite the potential formation of active metabolites and greater \textit{P. falciparum} activity \textit{ex vivo}, it should be noted that mirincamycin’s blood-stage activity is quite modest compared to that of DHA and other antimalarials in the \textit{ex vivo} bioassay. Although the pharmacodynamic experiment here does not provide direct evidence of liver stage antimalarial activity, it supports the likelihood that previously demonstrated antimalarial efficacy of mirincamycin in primates (16) is attributable to liver stage activity given very low observed activity against blood stage \textit{in vitro} and \textit{ex vivo} in this experiment. However, clindamycin is known to have blood stage activity both \textit{in vitro} and \textit{in vivo} (19), so it is unclear whether liver stage activity is predominant across this class. The healthy Rhesus model allows for efficient screening of pharmacokinetic parameters, and efficient preliminary PK-PD assessments to optimize experiments in the Rhesus relapsing \textit{P. cynomolgi} model, which will be reported in a future experiment. The potential for differential toxicities and/or improvements in efficacy of isomers over racemates are of interest, and a proven strategy for improving the therapeutic index of drugs. Schmidt \textit{et al.} previously demonstrated reduced toxicity and a substantial increase in therapeutic index of the d-primaquine isomer over the l-form, with no reduction in therapeutic efficacy compared to the racemate (18). The most common adverse events related to lincosamide derivatives are gastrointestinal disturbances, especially acute diarrhea (6). Both isomers caused transient loose
stools in this experiment after a single dose, with a non-significantly longer duration 
(2-3 days) among animals treated with the cis- isomer. None of the animals suffered 
from prolonged diarrhea, nor was there any evidence of Clostridium difficile associated 
colitis which represents the greatest potential liability with this class. There were no 
clinically significant changes in hematological parameters or kidney function after a 
single dose of either isomer, but there were non-clinically significant elevations in 
AST and ALT which were also observed in vehicle-only control animals. These 
physiologic responses in primates during experimental studies have been observed 
previously (2).

In conclusion, cis-Mirincamycin had a slightly more favorable 
pharmacokinetic profile than trans-mirincamycin, and both compounds had ex vivo P. 
falciparum blood stage activity that, while modest compared to DHA, increased 
dramatically compared to in vitro activity. The longer exposure and smaller clearance 
of cis-mirincamycin may result in higher antimalarial activity in vivo. This will be 
explored in upcoming efficacy experiments in a Rhesus Plasmodium cynomolgi 
relapsing malaria model. The low apparent bioavailability of both isomers may have 
resulted from first pass metabolism and production of active metabolites. 
Identification of metabolic pathways and potential active metabolites will be useful to 
better understand the antimalarial properties of this drug class, and develop analogues 
to support future drug development efforts to further optimize the mirincamycin lead 
compound.

Acknowledgements:
Ms. Raveewan Siripokasupkul, Ms. Roongnapha Apinan, and Ms. Kuntida 
Tangthongchawiiriya of AFRIMS Department of Immunology and Medicine
performed laboratory PK and PD analysis; Dr. Montip Gettyacamin and LTC Yvonne van Gessel of AFRIMS Department of Veterinary Medicine managed the primate colony and developed SOPs. Dr. Ian Bathurst and Dr. Carl Kraft of MMV provided resources, program direction and oversight of this project. Dr. Richard Westerman of Maldevco, Inc. provided mirincamycin isomers synthesized by Bridge Organics, Vicksburg, MS. Compound purity was certified by Dr. Peter Lim, Dr. Ronald Spanggord, Patrick Mcauley, and Jennifer Wang of SRI International. LTC Bryan Smith, Dr. Qiqui Li, LTC Mike Kozar and Dr. Geoff Dow provided consultation as part of the WRAIR Integrated Project Team. This study was supported by the United States Army Research and Materiel Command and the Medicines for Malaria Venture (MMV). The opinions or assertions contained herein are the views of the authors and are not to be construed as official, or as reflecting true views of the Department of the Army, or the Department of Defense.
References

11. MMV. Mirincamycin Collaborative Project, MMV08/0084 2008, Medicines for Malaria Venture: Walter Reed Army Institute of Research.


Legends:

Figure 1. Chemical structures of cis-mirincamycin and trans-mirincamycin.

Figure 2. Plasma concentration-time courses of intravenous (left panel) and oral (right panel) cis-mirincamycin (triangle) and trans-mirincamycin (rectangle) after dosing for 48 hours. Each time point represents the mean value for three animals.

Table 1. Pharmacokinetic parameters of cis-mirincamycin and trans-mirincamycin after intravenous and oral dosing in healthy rhesus monkeys. Time points represent the mean value for three animals.
Table 2. The relative *in vitro* potency of *cis*-mirincamycin and *trans*-mirincamycin compared to DHA against W2-clones of *P. falciparum*.

Figure 3. Pharmacodynamic profiles expressed as DHA equivalents (nM) after subtracting baseline activity of *cis*-mirincamycin (triangle) and *trans*-mirincamycin (rectangle) following intravenous 4 mg/kg (left panel) and oral 20 mg/kg (right panel) dosing. Each time point represents the mean value for three animals.

Table 3. The *ex vivo* pharmacodynamic activities of *cis*-mirincamycin and *trans*-mirincamycin against W2-clones of *Plasmodium falciparum*. Time points represent the mean value for three animals.
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Table 1. Pharmacokinetic parameters of *cis*-mirincamycin and *trans*-mirincamycin after intravenous and oral dosing in healthy rhesus monkeys. Data were presented as mean value for three animals.

<table>
<thead>
<tr>
<th>Parameters</th>
<th><em>cis</em>-mirincamycin</th>
<th><em>trans</em>-mirincamycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IV (4 mg/kg)</td>
<td>PO (20 mg/kg)</td>
</tr>
<tr>
<td>AUC_{0-4h} (ug.h/L)^a</td>
<td>4.597±0.666</td>
<td>3.050±0.198*</td>
</tr>
<tr>
<td>AUC_{0-inf} (ug.h/L)^b</td>
<td>4.705±0.684</td>
<td>3.193±0.222*</td>
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<tr>
<td>Cmax (ug/L)^c</td>
<td>9.693±1.690</td>
<td>554±1.61</td>
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<tr>
<td>Tmax (h)^d</td>
<td>N/A</td>
<td>2.08±1.88</td>
</tr>
<tr>
<td>Vd/F (L/kg)^e</td>
<td>15.0±3.30</td>
<td>10.1±1.77</td>
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<tr>
<td>T_{1/2} (h)^f</td>
<td>12.3±1.41</td>
<td>15.4±2.13*</td>
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<tr>
<td>Cl/F (L/h/kg)^g</td>
<td>0.84±0.12</td>
<td>0.46±0.03*</td>
</tr>
<tr>
<td>Absolute Bioavailability (%)</td>
<td>100</td>
<td>13.6</td>
</tr>
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</table>

^a Area under curve from time 0 to 48 h  
^b Maximum concentration  
^c Adjusted volume of distribution  
^d Time to maximum concentration  
^e Adjusted clearance  
^f Terminal elimination half-life  
^g Statistically significance between isomers (p<0.05)
Table 2. The relative *in vitro* potency of *cis*-mirincamycin and *trans*-mirincamycin compared to DHA against W2-clones of *P. falciparum*.

<table>
<thead>
<tr>
<th>Agents</th>
<th>IC$_{50}$ (nM)</th>
<th>Relative potency$^a$</th>
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</thead>
<tbody>
<tr>
<td>Dihydroartemisinin</td>
<td>2.30</td>
<td>1.00</td>
</tr>
<tr>
<td><em>cis</em>-mirincamycin</td>
<td>11,300</td>
<td>2.03 x 10$^{-4}$</td>
</tr>
<tr>
<td><em>trans</em>-mirincamycin</td>
<td>12,300</td>
<td>1.87 x 10$^{-4}$</td>
</tr>
</tbody>
</table>

$^a$Relative potency was reported as DHA equivalent values.
Figure 3. Pharmacodynamic profiles expressed as DHA equivalents (nM) after subtracting baseline activity of *cis*-mirincamycin (triangle) and *trans*-mirincamycin (rectangle) following intravenous 4 mg/kg (left panel) and oral 20 mg/kg (right panel) dosing. Each time point represents the mean value for three animals.
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<table>
<thead>
<tr>
<th>Agents</th>
<th>$E_{\text{max}}$&lt;sup&gt;a&lt;/sup&gt; (nM)</th>
<th>$C_{\text{max}}$&lt;sup&gt;b&lt;/sup&gt; (nM)</th>
<th>PD/PK relative potency&lt;sup&gt;c&lt;/sup&gt;</th>
<th><em>ex vivo</em> / <em>in vitro</em> potency by Emax</th>
<th>AUC&lt;sub&gt;PB&lt;/sub&gt;&lt;sup&gt;d&lt;/sup&gt; 0-12h (nM.h)</th>
<th>AUC&lt;sub&gt;PK&lt;/sub&gt;&lt;sup&gt;e&lt;/sup&gt; 0-12h (nM.h)</th>
<th>PD/PK relative potency&lt;sup&gt;f&lt;/sup&gt;</th>
<th><em>ex vivo</em> / <em>in vitro</em> potency by AUC</th>
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<tr>
<td><em>cis</em>-mirincamycin</td>
<td>178</td>
<td>22,100</td>
<td>8.06x10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>39.7</td>
<td>145</td>
<td>8,530</td>
<td>1.70x10&lt;sup&gt;-2&lt;/sup&gt;</td>
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<td>(intravenous)</td>
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<td><em>cis</em>-mirincamycin</td>
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<td>1,260</td>
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<td>108</td>
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<td>5,300</td>
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<td><em>trans</em>-mirincamycin</td>
<td>406</td>
<td>15,700</td>
<td>2.59x10&lt;sup&gt;-2&lt;/sup&gt;</td>
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<td>7,410</td>
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<td><em>trans</em>-mirincamycin</td>
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<td>3.04x10&lt;sup&gt;-2&lt;/sup&gt;</td>
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<td>4,010</td>
<td>3.72x10&lt;sup&gt;-2&lt;/sup&gt;</td>
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<td>(oral)</td>
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*a. Maximum *ex vivo* antimalarial activity expressed in DHA equivalents  
b. Maximum concentration of mirincamycin from pharmacokinetic study  
c. $E_{\text{max}}$ divided by $C_{\text{max}}$  
d. Area under curve of *ex vivo* antimalarial activity expressed as DHA equivalents  
e. Area under curve of mirincamycin from pharmacokinetic study  
f. AUC<sub>PB</sub> divided by AUC<sub>PK</sub>*