Artemisinin estimated in malaria tea trial patient blood.

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Objective:

To measure amount of artemisinin the serum of a healthy human adult after consumption of dried leaf *Artemisia annua* (DLA). This was important for determining the amount of artemisinin in the blood after artemisinin was delivered as DLA and to measure length of persistence. The results were used to estimate the amount of artemisinin in the serum of adult humans who were treated with *A. annua* and *A. afra* in the Munyangi et al. malaria clinical trial.

Method:

One adult human female subject (age 71, 140 lb [63.6 kg]) consumed 3 g powdered, encapsulated DLA (*A. annua* SAM, 2018 garden crop) had 3 blood draws: just prior to consumption of DLA; at 2 hr post consumption of DLA; and at 5 hr post consumption of DLA (taken on a different day at least a week later). Serum was isolated from the blood and analyzed for artemisinin using GCMS per the standard method.

Results:

In a healthy human subject: Artemisinin (MW = 282.33) amount in the DLA was about 1.5% (15mg/g), so amount consumed (delivered) was 45 mg artemisinin. Estimating this human subject had about 4.13 L blood (https://reference.medscape.com/calculator/estimated-blood-volume), the amount of delivered artemisinin/mL blood could not exceed 10.90 mg/L, or 10.90 µg/mL. Human serum is 50-70% red blood cells (solids), so for this human subject, we estimated 55% serum (2.3 L), so the highest serum concentration of artemisinin would actually be about 20 mg/L or 20 µg/mL.

Artemisinin EC50 concentration required to kill *P. falciparum* is 10 nM, so 2.82 ng/mL (WHO 2016; Hassan et al. 1990) also cited by Alin and Bjorkman (1994) at 9-10 ng/mL, which seems to be the generally cited blood level needed for killing. For this human subject, the amount of measured artemisinin per mL serum and amount relative to the minimum dose required to kill *P. falciparum* are in Table 1.

Table 1. Artemisinin levels in human blood or serum vs. minimum killing concentration against *P. falciparum* using an *in vitro* assay.

Calculated or measured component	Artemisinin				
	Total mg	mg/L	µg/mL	% 0 hr delivery	Change vs. 10 ng/mL[2]
Highest possible blood concentration at consumption (0 hr)	45	10.9	10.9	100	na
Highest possible serum concentration at consumption (0 hr)	45	19.62	19.62	100	na
Blood concentration at 2 hr	16.15	3.91	3.91	38	~ 400 fold
Serum concentration at 2 hr	16.19	7.04	7.04	36	>700 fold
Blood concentration at 5 hr	0.39	0.09	0.09	0.8	~9 fold
Serum concentration at 5 hr	0.37	0.16	0.16	0.8	16 fold

na, not applicable.

[1] Hypothetical based on delivered amount of artemisinin in DLA.

[2] Based on the minimum of ~10 ng artemisinin/mL serum required to kill *P. falciparum* in serum.

Conclusions:
We already validated that >99% of DLA artemisinin is released in the tea infusion preparation protocol. Based on that value, at 2 hr post ingestion of encapsulated DLA 36-38% of the initial DLA artemisinin level enters the blood/serum. That demonstrates that DLA-delivered artemisinin is much more bioavailable than delivery of the pure drug. At both 2 and 5 hr post ingestion, artemisinin concentration is, respectively, >700 and 17 fold greater than the minimum required to kill *P. falciparum*.

In malaria-infected subjects treat with *A. annua* or *A. afra* tea infusions and their placebo infusions:
These estimates refer to the *A. annua* and *A. afra* tea infusions that were used to treat malaria patients in the Munyangi et al. 2019 malaria clinical trial. Artemisinin amounts were also calculated for the 25-fold dilutions used to prepare the placebo tea infusions. Results are in Table 2:

Table 2. Estimated amounts of artemisinin delivered in tea infusions of *A. annua* and *A. afra* from the 2019 malaria tea infusion clinical trial.

Artemisinin in samples	*A. afra* 1:4 blend	*A. annua* LUX	*A. annua* BUR
mg/g DW	0.036	1.34	1.70
mg in 5 L tea	0.18	6.70	8.50
mg in 0.330 L dose[1]	0.06	2.23	2.83
ng/mL patient blood	12	450[2]	566[2]
In the 1/25 tea infusion placebos			
ng/mL patient blood	0.48	18[2]	22.6[2]

[1] Dose given each of three times a day.
[2] These doses all exceed the minimum blood concentration of artemisinin that would induce *Plasmodium* killing.

Conclusions:
Only the *A. afra* placebo had an artemisinin blood concentration that was below the killing threshold against *P. falciparum*. If once further considers the concentration in the serum, the liquid (excluding the red blood cells) portion of the blood, the concentration would be about twice that in Table 2, perhaps higher if the patient were anemic. Again only the *A. afra* placebo has an artemisinin blood concentration that was below the killing threshold against *P. falciparum.*

References:

Alin M.H., Bjorkman A., 1994. Concentration and time dependency of artemisinin efficacy against *Plasmodium falciparum* in vitro. Am J Trop Med Hyg 50:771-776

Hassan A.M., Björkman A., Ashton M., 1990. In vitro activity of artemisinin, its derivatives, and pyronaridine against different strains of *Plasmodium falciparum*. Transactions of the Royal Society of Tropical Medicine and Hygiene, 84, 635-637

WHO 2016 UPDATE: Artemisinin Derivatives: Summary of Nonclinical Safety Data - Introductory Remarks https://extranet.who.int/prequal/sites/default/files/documents/55%20Nonclinical%20overview%20artemisinin%20derivatives_Jan2016_0.pdf Accessed May 28, 2019.