SUPPLEMENTARY APPENDIX

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to:

Ivermectin as a potential COVID-19 treatment from the pharmacokinetic point of view

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Supplemental data

Biodistribution considerations

The data-processing in this work is an oversimplification of the pharmacokinetic processes because it is based solely on total C_{max} as a drug exposure surrogate. To address this issue, we have compiled this supplemental data file to analyze the influence of the tissue accumulation and protein binding of ivermectin.

Ivermectin has a large volume of distribution of ca. 10 L/kg, albeit not comparable to that of the antimalarial drugs (e.g. 132-261 L/kg for chloroquine) [1]. Clinically relevant dosing of ivermectin is associated with significant tissue distribution, whereby different organs and tissues attain higher levels relative to plasma (there are no human data for lung concentrations) (**Table S1**). Nevertheless, the main depot of the drug is invariably fat tissue. In rats and domestic ruminant animals, the plasma levels are ca. 4 times lower relative to those in the lung, and eventually the lung levels are ca. 4 times lower than those in fat tissue. With oral and itraruminal application the lung/plasma ratio is lower: approximately 3.14. This firmly indicates that, in the target tissue for COVID-19 treatment, the levels achievable through clinically relevant dosing are intermediate between those in the central compartment and those in fat tissue. As there are no human data for lung accumulation of ivermectin, but fat-distribution levels have been described [2], the latter could be used as a surrogate marker for lung exposure estimation. The maximal fat concentrations are 3 times higher than those in plasma for the same post-ingestion period. Hence a tissue accumulation factor of **≤ 3** could be assumed as a plausible correction estimate for the maximal lung levels of the drug on the basis of the plasma C_{max}.

Another important consideration is the protein binding, which cannot be mimicked precisely *in vitro* [3]. Ivermectin is **93%** bound to plasma proteins [1], and considering the large volume of distribution, significantly bound in tissues. Considering a free fraction of approximately **10%**, the non-bound and hence biologically active concentrations in humans are very low.

Eventually if the pooled biodistribution factor of $\left(\frac{3}{10}\right)$ is applied to the pharmacokinetic data in the main-article to account for both lung accumulation and protein binding, then the known dosage regimens of ivermectin could be considered as completely incompatible with achieving the SARS-CoV-2 inhibitory levels in the target tissue: IC_{50} of *ca.* 2.5 μmol/L and the level causing almost total viral eradication of 5 μmol/L reported in the study of Caly et al.[4].

Table S1 Tissue distribution and plasma levels data in Sprague-Dawley rats, ruminant domestic animals and patients with *Onchocerca volvulus* [2,5-7].

Species	Dose (μg/kg)	Route	Days/hours post application	Fat tissue levels (ng/g)	Lung concentration (ng/g tissue)	Plasma concentration (ng/mL)	Lung/plasma ratio	Fat/lung ratio
Rats [6]	300	Subcutaneous	1 Day	493	166*	23*	**7.22**	**2.97**
Rats [6]	300	Subcutaneous	4 Days	110	27*	4*	**6.75**	**4.07**
Goat [5]	200	Subcutaneous	2 Days	281.12	87.09	24.45	**3.59**	**3.23**
Goat [5]	200	Subcutaneous	7 Days	35.21	12.17	2.69	**4.52**	**2.89**
Goat [5]	200	Oral	2 Days	62.19	18.15	6.03	**3.00**	**3.43**
Goat [5]	200	Oral	7 Days	4.81	1.05	0.35	**3.00**	**4.58**
Sheep [6]	300	Intraruminal	7 Days	32*	4*	1.3*	**3.08**	**8**
Cattle [6]	300	Intraruminal	7 Days	84*	29*	8.3*	**3.49**	**2.89**
Cattle [6]	300	Subcutaneous	7 Days	220*	66*	45*	**1.47**	**3.33**
Cattle [7]	200	Subcutaneous	1 Day	-	97	40	**2.37**	**-**
Humans [2]	150	Oral	4 h	141	-	46	**-**	**-**
Average ratios	N/A	N/A	N/A	N/A	N/A	N/A	**3.85**	**3.93**

*This study used tritium-labeled ivermectin and the biodisposition data are shown as total radioactivity expressed as either nanogram equivalents per gram lung tissue or ng/mL plasma.

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