S1 Data

Anatomical and Physiological Parameters use in Amiloride Oral PBPK Model

Organ Volumes

Organ	Volume (L)	Blood flow rate (L/min)	Endothelial Surface Area (cm2)	Fluid recirculation flow rate (L/min)	Lymph flow rate (L/min)
Venous Blood	0,96	N/A	N/A	N/A	N/A
Arterial Blood	0,42	N/A	N/A	N/A	N/A
Bone	11,89	0,33	384187,92	5,51E-06	1,18E-04
Brain	1,5	0,78	55910,26	5,75E-07	3,09E-05
Fat	21,19	0,46	362355,32	2,34E-05	1,90E-03
Gonads	0,04	0,003	2099,37	3,29E-06	1,96E-05
Heart	0,42	0,26	55416,44	1,16E-05	2,08E-04
Kidney	0,44	1,32	95581,5	3,41E-05	5,12E-04
Large intestine	0,41	0,26	7474,99	3,18E-05	2,04E-03
Liver	2,37	0,42	382274,29	7,26E-05	4,61E-03
Lung	1,21	6,25	666942,81	5,04E-08	1,21E-04
Muscle	33,37	1,14	792527,73	2,33E-05	1,25E-03
Pancreas	0,19	0,06	36091,14	9,01E-07	1,07E-03
Portal vein	1,04	1,2	N/A	N/A	5,67E-03
Saliva	0,006	N/A	N/A	N/A	N/A
Skin	3,8	0,33	166051,51	3,39E-05	6,31E-04
Small intestine	0,72	0,65	13223,45	1,18E-05	6,90E-04
Spleen	0,21	0,17	64693,85	2,83E-07	1,79E-03
Stomach	0,17	0,06	5111,25	5,73E-06	7,22E-05
Mucosa					
Duodenum	0,02	0,05	2019,2	2,66E-07	1,56E-05
Upper jejunum	0,05	0,11	5779,08	7,62E-07	4,47E-05
Lower jejunum	0,03	0,11	4270,48	5,63E-07	3,30E-05
Upper ileum	0,03	0,11	3759,88	4,96E-07	2,91E-05
Lower ileum	0,01	0,11	1810,31	2,39E-07	1,40E-05
Cecum	0,006	0,02	818,03	5,12E-07	3,28E-05
Colon ascendens	0,01	0,03	1499,72	9,39E-07	6,01E-05
Colon transversum	0,03	0,06	3152,9	2,14E-06	1,37E-04
Rectum	0,0067	0,01	516,1	5,25E-07	3,36E-05

Gastrointestinal Tract Dimensions

Compartment	pH	Length (cm)	Area (cm2)
Stomach	2	20	628,32
Duodenum	6	22,85	245,23
Upper jejunum	6,25	52,83	524,77
Lower jejunum	6,92	52,83	490,92
Upper ileum	7,21	78,53	654,25
Lower ileum	7,46	78,53	553,6
Cecum	5,7	5,95	129,68
Colon ascendens	5,6	16,63	362,66
Colon transversum	5,7	32,7	560,11
Rectum	6,6	10,93	110,59

GIT - Transit Time

Organ	Value

Stomach	15 min
Small intestinal transit time	2.1 h
Large intestinal transit time	44.2 h

S2 Table

Table S1: Table with details of clinical studies used for the verification of oral PBPK model

Study Name	Sample size	Mean (Age Range)	Dose (mg)	Formulation	Measurement Method	Ref
Jones et al.	10	28 (20-43)	10	Oral Solution	HPLC	19
Brooks et al.	12	31 (18-65)	10	Tablet	HPLC	29
Somogyi et al.	8	NA(25-38)	5	Tablet	HPLC	30
Sabanathan et al.	6	24(22-29)	2.5	capsule	HPLC	31
Flouvat et al.	12	26.2 (NA)	5	capsule	HPLC	32

S3 Data

Time (h)	Conc (ng/mL)
144,0705	3,6917982
144,515	4,9215794
144,9836	4,4649024
145,4911	7,0619254
145,9118	10,046533
147,0066	12,551283
147,977	12,868491
149,9658	10,084413
151,9909	9,510832
153,9224	7,2054386
155,9769	6,609014
168,043	4,3071632

Differential Equations to Model Amiloride Delivery to the Brain Following Intranasal Administration:

Equation for drug concentration in olfactory epithelium (C_{OE})*:*

$$\frac{dC_{\mathrm{OE}}}{dt} = -P_{\mathrm{eff}} \cdot \mathrm{SA}_{\mathrm{OE}} \cdot C_{\mathrm{OE}}, \qquad C_{\mathrm{OE}}(0) = \frac{0.254 \cdot \mathrm{D}}{V_N} \qquad (1)$$

where, C_{OE} is the concentration of amiloride in the olfactory epithelium; P_{eff} is the effective permeability of amiloride; $\mathrm{SA}_{\mathrm{OE}}$ is the surface area of olfactory epithelium in humans; D is the total dose of drug administered as nasal spray and V_N is the volume of nasal cavity.

Equation for drug concentration in non-olfactory epithelium (C_{NE})*:*

$$\frac{dC_{\mathrm{NE}}}{dt} = -P_{\mathrm{eff}} \cdot \mathrm{SA}_{\mathrm{NE}} \cdot C_{\mathrm{NE}}, \qquad C_{\mathrm{NE}}(0) = \frac{0.746 \cdot \mathrm{D} - A_{\mathrm{GI}} - A_{\mathrm{RES}}}{V_N} \qquad (2)$$

where, C_{NE} is the concentration of amiloride in the non-olfactory epithelium; $\mathrm{SA}_{\mathrm{NE}}$ is the surface area of non-olfactory epithelium in humans; A_{GI} is the amount of dose entering the GI tract from nasal cavity; A_{RES} is the amount of dose entering the lungs from nasal cavity.

Equation for drug concentration in systemic circulation (C_{SC}):

$$\frac{dC_{\mathrm{SC}}}{dt} = \overbrace{P_{\mathrm{eff}} \cdot \mathrm{SA}_{\mathrm{NE}} \cdot C_{\mathrm{NE}}}^{via\ non-olfactory\ epithelium} + \overbrace{P_{\mathrm{eff}} \cdot \mathrm{SA}_{\mathrm{GI}} \cdot \frac{A_{\mathrm{GI}}}{V_{\mathrm{GI}}} \cdot e^{-P_{\mathrm{eff}} \cdot \mathrm{SA}_{\mathrm{GI}} \cdot t}}^{via\ GI} + \overbrace{P_{\mathrm{eff}} \cdot \mathrm{SA}_{\mathrm{RES}} \cdot \frac{A_{\mathrm{RES}}}{V_{\mathrm{RES}}} \cdot e^{-P_{\mathrm{eff}} \cdot \mathrm{SA}_{\mathrm{RES}} \cdot t}}^{via\ lungs} + \overbrace{\sum_{i\ \mathrm{in}} \frac{Q'_i}{V_i} \cdot C_i}^{via\ blood\ flow\ from\ all\ organs} - \overbrace{\frac{C_{\mathrm{SC}}}{V_{\mathrm{SC}}} \cdot \left(\sum_{i\ \mathrm{in}} Q_i\right)}^{via\ blood\ flow\ to\ all\ organs} - \overbrace{\mathrm{Cl}_T \cdot C_{\mathrm{SC}}}^{Total\ clearance},$$

$$C_{\mathrm{SC}}(0) = 0 \qquad (3)$$

where, C_{SC} is the concentration of drug in systemic circulation, $\mathrm{SA}_{\mathrm{GI}}$ is the surface area of vasculature in gastrointestinal tract in humans, $\mathrm{SA}_{\mathrm{RES}}$ is the surface area of vasculature in the respiratory tract in humans; V_{GI} and V_{RES} are the volumes of gastrointestinal and respiratory tracts, respectively; Q_i is the blood flow rate to organ i in the set of organs $\{S\}$, Q'_i is the blood flow rate from organ i in the set of organs $\{S\}$, V_i

is the vascular volume of organ i in the set of organs $\{S\}$, V_{SC} is the volume of systemic circulation, Cl_T is the total clearance of amiloride from systemic circulation.

Equation for drug concentration in brain plasma (C_{BP}):

$$\frac{dC_{\mathrm{BP}}}{dt} = \frac{Q_{\mathrm{B}}}{V_{\mathrm{SC}}} \cdot C_{\mathrm{SC}} + P_{\mathrm{eff}} \cdot \mathrm{SA}_{\mathrm{OE}} \cdot C_{\mathrm{OE}} + P_{\mathrm{PCSF}} \cdot \mathrm{SA}_{\mathrm{PCSF}} \cdot (C_{\mathrm{CSF}} - C_{\mathrm{BP}}) - \frac{Q'_B}{V_P} \cdot C_{\mathrm{BP}}, \quad C_{\mathrm{BP}}(0) = 0 \qquad (4)$$

where, C_{BP} is the concentration of drug in brain plasma, Q_{B} is the blood flow rate to the brain, V_P is the volume of plasma in brain, Q'_B is the blood flow rate from brain to systemic circulation, P_{PCSF} is the permeability of drug between brain plasma and CSF, $\mathrm{SA}_{\mathrm{PCSF}}$ is the surface area of CSF interfacing with brain plasma, and C_{CSF} is the concentration of drug in CSF.

Equation for drug concentration in CSF (C_{CSF}):

$$\frac{dC_{\mathrm{CSF}}}{dt} = P_{\mathrm{PCSF}} \cdot \mathrm{SA}_{\mathrm{PCSF}} \cdot (C_{\mathrm{BP}} - C_{\mathrm{CSF}}) + P_{\mathrm{ICSF}} \cdot \mathrm{SA}_{\mathrm{ICSF}} \cdot (C_{\mathrm{IC}} - C_{\mathrm{CSF}}), \qquad C_{\mathrm{CSF}}(0) = 0 \qquad (5)$$

where, C_{CSF} is the concentration of drug in CSF, P_{ICSF} is the permeability of drug between brain intracellular region and CSF, $\mathrm{SA}_{\mathrm{ICSF}}$ is the surface area of CSF interfacing with intracellular region, C_{IC} is the concentration of drug in the brain intracellular region.

Equation for drug concentration in intracellular brain (C_{IC}):

$$\frac{dC_{\mathrm{IC}}}{dt} = P_{\mathrm{ICSF}} \cdot \mathrm{SA}_{\mathrm{ICSF}} \cdot (C_{\mathrm{CSF}} - C_{\mathrm{IC}}), \qquad C_{\mathrm{IC}}(0) = 0 \qquad (6)$$

For simplicity, the loss of drug from the nasal cavity was assumed to be negligible unless patients forcibly blow their noses after the spray. Therefore, we considered that the entire intranasal amiloride dose reached the nasal cavity. The absorption rate constants and effective permeability values are provided in Table 1.