Electronic Supplementary Information (ESI)

Transmembrane distribution of kanamycin and chloramphenicol: Insights into the cytotoxicity of antibacterial drugs

Text

1. The general Langmuir isothermal equation is expressed as

\[
\frac{1}{\gamma} = \frac{1}{N} + \frac{1}{KNc_L} \quad \text{(Eq. S1)}
\]

where \(\gamma\) is the mole ratio of solute adsorbed to SML, \(c_L\) equilibrium concentration of solute in mol·L\(^{-1}\), \(N\) saturated adsorption mole number of solute and \(K\) adsorption equilibrium constant in L·mol\(^{-1}\). From the regression line of plot \(\gamma^{-1}\) vs. \(c_L^{-1}\), a slope \((NK)^{-1}\) and an intercept \(N^{-1}\) were calculated.

2. The partition coefficient is calculated by the relation:

\[
P = \frac{[\text{solute}]_{\text{organic}}}{[\text{solute}]_{\text{aqueous}}} \quad \text{(Eq. S2)}
\]

where \(P\) is the partition constant of solute in L·kg\(^{-1}\), \([\text{solute}]_{\text{organic}}\) the mass concentration of solute in organic phase in mg·kg\(^{-1}\), \([\text{solute}]_{\text{aqueous}}\) the equilibrium concentration of solute in aqueous phase in mg·L\(^{-1}\). From the regression line of plot \([\text{solute}]_{\text{organic}}\) vs \([\text{solute}]_{\text{aqueous}}\), the slope \(P\) was calculated.
Fig.S1 A: Chromatogram and calibration curves (a, b) of standard PEA (0.2 g·L⁻¹) and PC (2 g·L⁻¹) mixing solution with the solvent of hexane - isopropanol mixture (hexane/isopropanol = 3:1, v/v, HPLC grade); B: Chromatogram of the commercial lecithin (4 g·L⁻¹).

**Determination:** The PEA, PC and TP contents in lecithin were quantitatively analyzed by HPLC-ELSD using a normal-phase column (Luna 5 μ Silica 100 Å, 5 μm, 250 × 4.60 mm, Phenomenex, USA). For gradient analyses, the binary gradient had a constant flow rate of 1.5 mL·min⁻¹, with solvent A - hexane/isopropanol as 13:16 (v/v), solvent B - hexane/isopropanol/water as 13:16:3. Gradient timetable: at 0 min, 30/70 (%A/%B), at 6 min, 0/100, at 10 min, 0/100; at 12 min, 30/70 and at 15 min, 30/70. The ELSD at post-column was kept at an evaporation temperature of 33 °C and at 3.5 bar pressure (2.5 L·min⁻¹) for nebulization gas i.e. compressed air. The injection volume of sample is 20 μL.
Fig. S2 A: Calibration curve and chromatogram for KAN determination eluted at 3.107 min (300 mg·L⁻¹ KAN) by HPLC-ELSD; B: that of CAP at 3.480 min (30 mg·L⁻¹ CAP) by HPLC-DAD.
Fig. S3 Effect of exposure time on the binding of antibiotics on SML (A) (1, 0.50 mM KAN and 2, 2.00 mM CAP) and embryos (B) (1, 0.70 mM KAN and 2, 4.80 mM CAP, embryos number: 5)

Fig. S4 Effects of ionic strength (A), pH (B), and temperature (C) on the binding amounts of KAN and CAP on embryos (number: 5): 1, 0.700 mM KAN; 2, 0.300 mM CAP.
Fig. S5 Cartoon illustration for the procedures of separating membrane and cytoplasm (1-7), and separating antibiosis from membrane (9-11):

1- 20 embryos were incubated (a) in KAN or CAP solutions for 8 h in 5.0 mL glass tubes;
2- The concentration of excess antibiotic in the supernatants ($c_{L1}$) was separated from embryos and determined;
3- The exposed embryos were left in the bottom of the tube;
4- Embryos were suspension (b) in 3 mL of deionized water;
5- Embryos were disrupted by ultrasonication (c) for 10×5 s at 120 w and interspersed by 5 s intervals of rest;
6- After centrifugation (d) for 5 min at 6000 rpm, the cytoplasm with adsorbed antibiotics was dispersed in the supernatant and the concentration ($c_{L2}$) of the antibiotics was determined;
7- Membranes with adsorbed antibiotics were left in the residue;
8- Membranes were suspension (b) in 1 mL dichloromethane;
9- Membranes were disrupted by ultrasonication (c) for 90×30 s at 240 w and interspersed by 15 s intervals of rest, then the adsorbed antibiotics were separated from membranes;
10- After centrifugation (d) for 10 min at 12,000 rpm, the adsorbed antibiotics were dissolved in the supernatant, then the supernatant was diluted to 5 mL with methanol and the concentration ($c_{L3}$) of antibiotics was determined;
11- Membrane without of the antibiotics was left in the residue.
Fig. S6 3D-morphology observation of the embryo membrane surfaces exposed to KAN and CAP. A: Control group, B: CAP (2 mM) exposure group, C: KAN (4 mM) exposure group.

Fig. S7 Toxicity characteristics of zebrafish larvae exposed to KAN and CAP. A-1, Control; A-2, KAN (0.36 mM) exposure group; A-3 to A-5, same as A-2 but 1.44 mM KAN; B-1, Control; B-2 & B-4, CAP (0.24 mM) exposure groups; B-3 & B-5, same as B-2 but 0.48 mM CAP. AM, Axial malformation; E, Edema; HE, Hemoglutination; PE, Pericardial edema; SB, Swim bladder; SBD, Swim bladder deficit; YSE, Yolk sac edema.
Fig. S8 Mortality of zebrafish larvae for evaluating the acute and chronic toxicity of antibiotics: A: KAN exposure groups; B: CAP exposure groups.