Size Does Matter But Swinging Both Ways (Amphiphilic) Boosts Systemic Uptake of Instilled Drugs

Abstract # 555

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Introduction

- Drugs are instilled in urinary bladder primarily for their localized action, but a fraction of instilled dose is bound to reach the systemic circulation (ref.1-6, Table 1)
- Here, we delved into wide variability in the absorbed dose fraction by testing whether physiochemical properties of drugs are deterministic in their systemic uptake ?
- Size- molecular weight (MW) range from 23-66500 Daltons and the solubility ratio in 1-octanol/water for drugs/probes: ^{24}Na+,^{14}C-urea, lidocaine and ^{125}I-Albumin, etc... can determine the entry of the absorbed drug fraction into extracellular and intracellular spaces (Volume of distribution-Vd) as well as concurrent renal or non-renal clearance
- It is plausible that "one size fits all" blood sampling time points underestimates the true systemic uptake of instilled small MW drugs: 1-4% formalin, 50% dimethyl sulphoxide (DMSO) and lidocaine.

Methods

- Here, we studied the physicochemical properties of 23 drugs and probes that have been instilled into human bladder or mammalian bladder
- A first-order multiple regression model was constructed for the dependent variable of reported systemic uptake and physicochemical properties as independent variables (determinants): MW in Daltons, hydrodynamic diameter= 2x of Stokes-Einstein radius in Ångstrom, partition coefficient (P), polar surface area in Ångstrom^2 and ionization constant pKa.
- Wide range and skewed distribution of properties required their log-transformation for computing a predictive equation for systemic uptake
- Significance of the linear-log model was assessed by the F test and the 95% confidence interval (CI) and whether least-squares line slope was different from 0 was determined by Student's t-test.

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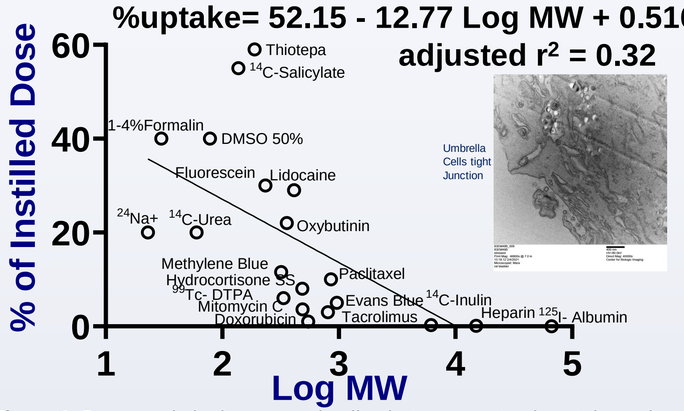


Figure 1. Downward sloping regression line between systemic uptake and Log MW of instilled drugs reflects an inverse relationship and the slope of least squares line is different from 0 ($p<0.05$). First-order linear-log model predicts that systemic uptake decreases by 12.77 ± 9.29 % (95% CI) for a unit rise in Log MW. A >3-fold higher systemic uptake of instilled oxybutynin over mitomycin C (Log P 4.2 vs -0.38; 357 vs 334.3 Daltons) highlights the benefits of amphiphilic nature in bladder absorbtion.

Probe	Molecular Mass (Daltons)	Hydrodynamic Diameter Ångstrom	Diffusion rate or Absorbed Dose Fraction	References
H^+	1	0.529	29.6 ± 18.6 mm/s	[4]Negrete et al 1996
$^3H_20$	18	2.75	4.35± 0.65 μm/s	[4]Negrete et al 1996
Ammonia	17	3.26	5 ± 0.48 μm/s	[4]Negrete et al 1996
^{14}C-Urea	60	2.3	0.0435± 0.0065 μm/s ; 25% dose in blood	[4]Negrete et al 1996 [1]Eldrup et al. 1983
^{24}Na+	24	2.6	20% in blood	[1]Eldrup et al. 1983
Fluorescein	412.3	7	29% in blood	Eichel et al 2001
^{99m}Tc- DTPA	487	11	3.6% in blood	Chelsky et al 1994
Gadobutrol	604.7	8	10% in bladder	[5,6]Singh et al 2020
^{125}I-Albumin	66500	35.5	0.01% in blood	[1]Eldrup et al. 1983

Results

- Downward sloping least squares line of systemic uptake and log MW of drugs (Fig.1) conforms to the Stokesian Diffusion model and diffusivity equation: $D= k_BT/6\pi*r*\mu$ for drug molecules assumed as spheres of Stokes-Einstein radius (r)= 0.5 x of hydrodynamic diameter
- Only variable in diffusivity equation is 'r' in the denominator and other three terms are constants: Boltzmann constant (k_B), temperature (T) and T dependent viscosity (μ) at 37°C for *in vivo*.
- Increase in MW increases size (hydrodynamic diameter) to slow down paracellular diffusion
- Amphiphilicity of drugs is indexed by Log P or solubility ratio between water and 1-octanol mixture, with hydrophilic, ionized fraction partitioning into water layer and hydrophobic, unionized fraction partitioning into 1-octanol layer, the drug fraction that crosses cell membrane.
- Our parsimonious regression model passed the Kolmogorov-Smirnov log normality test and global F test for significance ($p<0.05$) after we excluded correlated variables- problem of multicollinearity- highlighted by the correlation coefficient, r= 0.37 between log P and polar surface area.
- While simple regression of systemic uptake and log MW is defined by coefficient of determination (r^2) = 0.39 , adjusted r^2 = 0.32 for multiple regression implies that log P attenuates the negative impact of log MW on the systemic uptake of instilled drugs.

1-Octanol = Log P
Water

Figure 2. Unlike Log MW, Log P exerts a positive impact and amphiphilic nature boosts systemic uptake.

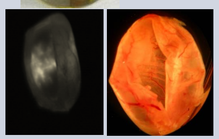
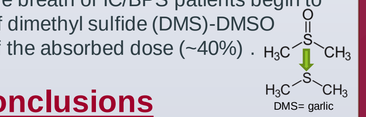


Figure 3. Photographic evidence for the rat bladder uptake of fluorescein (gel), 24h after instillation.

Table 1. The MW or hydrodynamic diameter of drugs/probes exerts an inverse relationship on published rate of diffusion and instilled dose fraction reaching bladder or blood (systemic circulation).

Discussion

- Size matters- is manifested by the inverse relationship of log MW and systemic uptake. Larger MW raises hydrodynamic diameter, a determinant for the passive paracellular diffusion across tight junctions.
- Our predictive equation conforms to the dilation of tight junctions by inflammatory cytokines preceding the higher uptake of instilled lidocaine and radiolabeled probes in IC/BPS patients and >50% systemic uptake for salicylate (137 Daltons) and thiotepa (189.23 Daltons) (ref. 1,2,3,5).
- Fluorescein uptake highlights passive paracellular diffusion of most xenobiotics(Table 1; ref.2) as energy-dependent transcellular absorption in Umbrella cells is reserved for endogenous substances (^{24}Na+,^{14}C-urea).
- Amphiphilicity (Log P) contributes heteroscedasticity (unequal variances) of linear-log model, leads to >3 fold higher uptake of oxybutynin over mitomycin C
- With rapid distribution half-life of <2min and rapid hepatic clearance, true systemic uptake is grossly underestimated by first blood sample drawn 15min post-instillation of small MW drugs: formalin, DMSO and lidocaine…..
- Within 2 min of instillation, lidocaine affects blood pressure of SCI patients, and the breath of IC/BPS patients begin to emanate garlic odor of dimethyl sulfide (DMS)-DMSO metabolite- only 3% of the absorbed dose (~40%) .



DMS= garlic

Conclusions

- While size (Log MW) reduces but higher Log P boosts the systemic uptake of instilled drugs
- Delayed blood sampling can underestimate the systemic uptake- kinetic process dependent on paracellular diffusion from urothelium, rapid distribution in large Vd and rapid clearance- for DMSO, formalin, lidocaine and other small MW drugs.

References

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