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

Abstract # 555

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Introduction

- To seek localized action, drugs are routinely instilled in urinary bladder, but a fraction of instilled dose (F) is bound to reach the systemic circulation (ref.1-7, Table 1)
- Here, we delved into wide the variability of F by testing whether physiochemical properties of drugs are deterministic in their systemic uptake ?
- Size- molecular weight (MW) ranging from 23-66500 Daltons and the solubility ratio in 1-octanol/water for drugs/probes (Log P): ^{24}Na+,^{14}C-urea, lidocaine and ^{125}I-Albumin, etc... can determine the entry of F 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 F 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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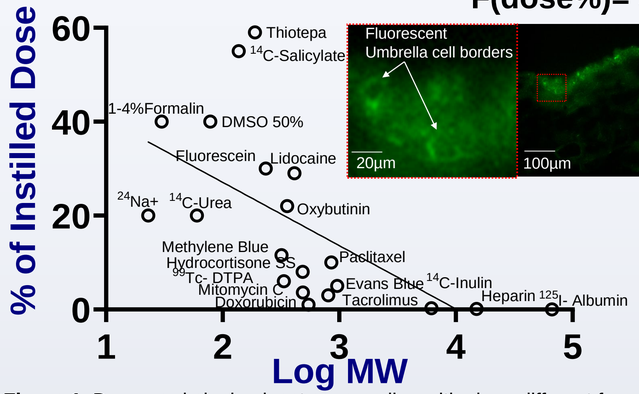
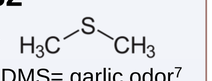
F(dose%)= 52.15 - 12.77 Log MW + 0.516 Log P

adjusted r^2 = 0.32





DMS= garlic odor[7] sensed immediately

Figure 1. Downward sloping least squares line with slope different from 0 ($p<0.05$) displays the inverse relationship of log MW and F. First-order linear-log model predicts that a unit rise of Log MW decreases F by 12.77 ± 9.29 % (95% CI), resisted by the amphiphilic nature of oxybutynin to raise F >3 fold over mitomycin C (Log P 4.2 vs -0.38; 357 vs 334.3 Daltons). Exclusive paracellular diffusion of instilled fluorescein is evinced by green hexagonal borders and dark apical surface of mammalian umbrella cells[1,2].

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	[2]Sonn et al 2009, 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]Singh et al 2020
^{125}I-Albumin	66500	35.5	0.01% in blood	[1]Eldrup et al. 1983
Colloidal Gold	197.6	100	Transmission EM probe	[6]Rajaganapathy et al 2015.

Results

- Downward sloping least squares line of systemic uptake and log MW (Fig.1) conforms to the Stokesian Diffusion model and diffusivity equation: $D= k_BT/6\pi*r*\mu$ with drug molecules assumed as spheres of Stokes-Einstein radius (r)= 0.5 x of hydrodynamic diameter
- Only variable in the 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*.
- Dark grains of colloidal gold (10nm) in TEM illustrates how larger size slows down paracellular diffusion of large MW drugs
- 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 drug fraction partitioning into 1-octanol layer, enters cell.
- 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.

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[5,6] or blood (systemic circulation).

Discussion

- Size matters- is manifested by the inverse relationship of log MW and F (Fig.1). Larger MW raises the hydrodynamic diameter, a determinant for the passive paracellular diffusion across tight junctions as evinced by instilled fluorescein in rat (Fig.1) and in human bladder (ref.2)
- Most instilled xenobiotics (Table 1) are absorbed paracellularly as energy-dependent transcellular absorption is typically reserved for electrolytes ^{24}Na+ and ^{14}C-urea[1].
- Our predictive equation conforms to the dilation of tight junctions by inflammatory cytokines preceding the higher F of instilled lidocaine and radiolabeled probes by IC/BPS patients and >50% F of salicylate (137 Daltons) and thiotepa (189.23 Daltons) by patients (ref. 1,2,3,5).
- Amphiphilicity (Log P) lends heteroscedasticity (unequal variances) to linear-log model by bestowing >3 fold higher F of oxybutynin over mitomycin C
- First blood sample drawn >15min post-instillation of small MW drugs: formalin, DMSO[7] and lidocaine grossly undercounts the true F owing to rapid distribution half-life of <2min and hepatic clearance of instilled drugs
- Instilled lidocaine affords local anesthesia and affects the blood pressure of SCI patients, and IC/BPS patients begin to emanate garlic odor of dimethyl sulfide (DMS), a DMSO metabolite representing 3% of the absorbed dose (~40% F)[7].

Conclusions

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

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