Abstract
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Leveraging Log-Linear Relationship of Molecular Weight (Daltons) and Size (Angstrom) to Probe Tight Junctions In Urothelium

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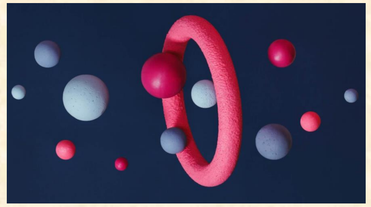

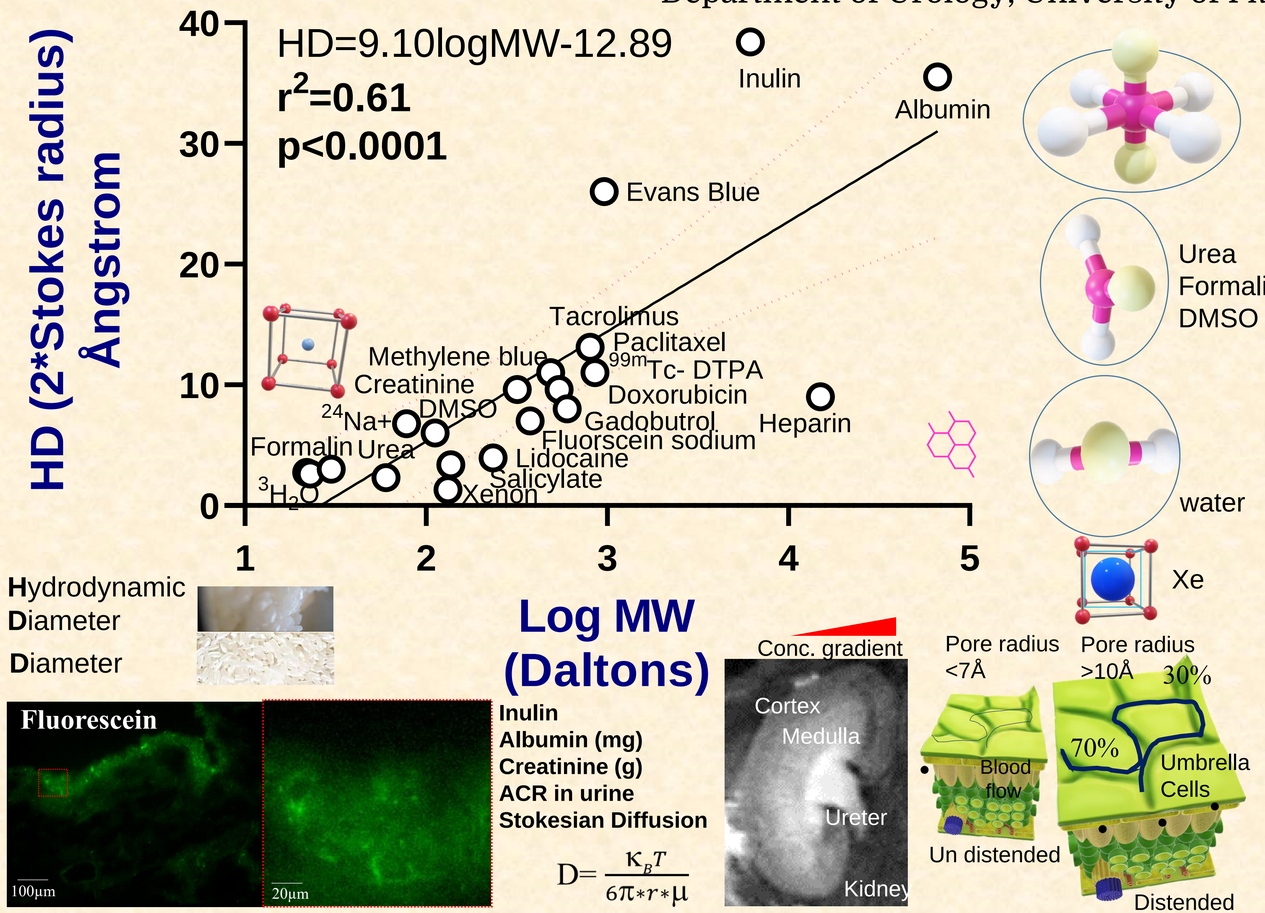

Inulin
Albumin (mg)
Creatinine (g)
ACR in urine
Stokesian Diffusion

$$D=\frac{\kappa_B T}{6\pi * r * \mu}$$

Figure 1- Size matters- Positive log-linear relationship between log-transformed molecular weight (Log MW) and hydrodynamic diameter (HD) of instilled drugs is displayed by least squares line (solid black), with slope is different from 0 (p<0.0001) and the bands for 95%CI are indicated by dotted pink lines. The paracellular diffusion pathway traced by green fluorescence of instilled Fluorescein in rodents[1,2] and in humans conforms to Stokesian diffusion and prioritizes spherical radius (size) above weight (MW) as evinced by the faster systemic uptake of heavier xenon than H_20 and Na+. Likewise, heavier albumin is also smaller than swollen inulin (polyfructose). CKD mediated widening of tight junctions in glomerulus (cortex) elevates ACR[9] in urine to >300 mg/g from <30mg/g in normals.

Background

- Does molecular weight (MW) predict the size of drugs ? We sought to answer this question by examining the relationship between two covariates.
- The relationship is critical for understanding the pathology of interstitial cystitis/bladder pain syndrome (IC/BPS) as ultrastructural studies[1] of past used lanthanum tracer to evince the inflammation mediated dilatation of urothelial tight junctions and other structural deficiencies in bladder biopsies
- However, the impact of IC/BPS must be benchmarked to the pore size of tight junctions in healthy individuals who may not be willing to cede their bladder tissue and tissue processing artifacts may generate aberrant dimensions of native pore size in porous tight junctions as seen on-
- Confocal laser endomicroscopy of live humans[2] visualized fluorescent umbrella cell borders (Figure 1) to confirm poor transcellular permeability and only paracellular diffusion is feasible for instilled Fluorescein and other polar dyes: Methylene blue and Trypan blue/Evans blue dye
- Since drugs and dyes are assumed to diffuse as spheres in Stokesian diffusion and the radius (r) of "spherical" drugs in aqueous medium can be determined by electron microscopy or dynamic light scattering, we hypothesized that the pore size of native tight junctions can be non-invasively discerned from the systemic uptake of drugs of known diameter analogous to authenticating a sieve/filter by particle size that pass through

Methods

- We collated the hydrodynamic diameter (HD) = 2* of Stokes-Einstein r in Angstrom (Å) of 20 drugs and dyes that have been instilled in human bladder
- Owing to the skewed distribution, log-transformation of molecular weight (MW) was necessary for linear regression with HD
- Whether the slope of least-squares line is significantly different from 0 was determined by Student's t-test with its two-sided 95% confidence interval(CI)

Results

- We discovered a hitherto unreported log-linear relationship between MW and size (HD) of drugs with a positive β of 9.10± 3.63 (95%CI)
- A unit rise in log MW raises HD by 9.10 Å with the coefficient of determination, r^2 of 0.61
- Size dependent decline in the intravesical absorption is determined by "spherical radius r" of drugs in the denominator of Stokesian diffusion equation as other three terms are constants: Boltzmann constant (k_B), temperature (T) and T dependent viscosity (μ) at 37°C for *in vivo*.
- Paired electrons in outer orbitals shrink size as cubic face centered Xe is 5 times heavier than H_20 or cubic body centered Na+
- Polysaccharide nature of inulin allows it to swell to higher HD than its physical diameter akin to rice whereas albumin and lipophilic drugs (Paclitaxel) are less likely to be engulfed by water molecules

Interpretation

- Size is the overriding factor in diffusion- affirmed by the comparable uptake of hydrophilic inulin[13] and of ten times heavier albumin (less hydrophilic) [2]
- One size does not fit all- Evans blue/Trypan blue (r=13Å)[4] do not reliably predict the systemic uptake of smaller drug molecules[1-13]
- Analogous to the invisible and visible sweating on skin at low and high humidity, respectively, the luminal surface accumulation of Fluorescein and Tc-DTPA (r≤5.5Å)[5] and Evans blue/Trypan blue (r=13Å) is also visible at 25-40% and >90% of anaesthetized bladder filling capacity[6], respectively.
- Since 2-unit rise of log MW raises HD of albumin by 3-fold over creatinine, a log-linear decline in the uptake with r indexes that tight junctions have effective pore radius of ~5-7Å unless widened by distension or inflammation[2-3]
- Dilation of glomerulus tight junctions secondary to CKD is tracked by a rise of albumin to creatinine ratio (ACR)[9] in urine from <30mg/g to >300 mg/g
- Likewise, bladder inflammation is manifested by a 4-fold rise in instilled creatinine uptake[11] and delayed elimination of lidocaine[8]
- Widened tight junctions of small intestine are estimated by exogenous LMR- 2h urinary ratio of lactulose (HD~10Å) and mannitol (HD 8Å)[10]
- Though pore size may differ between jejunum and colon, uniformity is expected in the indirect estimation of tight junction's pore diameter across bladder luminal surface[1-3] , indirect method previously used for intestinal and buccal epithelium[12]
- Disconnect between *ex vivo* lidocaine absorption rate and rapid (~2min) ascent of lidocaine serum levels of 0.16mg/L in human adults after instillation highlights the disconnect between *ex vivo* and *in vivo* research

CONCLUSIONS

- Given that clinical use of ACR and LMR is predicated on Stokesian diffusion in diseased kidney and intestine, respectively, urology must also rely on Stokesian diffusion to resolve the confusion over bladder permeability in IC/BPS and the dilatation of tight junctions relative to healthy volunteers
- Traditional ultrastructural studies are only feasible in IC/BPS patients and are vulnerable to tissue processing artifacts

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