Comparative Aspects of Topical Delivery

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Overview

- Review structure and function of skin
- Interspecies differences
- Stress that selection of experimental model system may impact prediction across species
- Particles versus molecules
Biological Functions of Skin include:

- Physical and metabolic barrier to the environment: stratum corneum
- Thermoregulation: hair, apocrine, eccrine, sebaceous sweat glands, blood flow shunts
- Mechanical support: collagen, water
- Endocrine (e.g. Vitamin D)
- Neurosensory reception
- Immunological response: Keratinocytes, Langerhans Cells
- Metabolism, Biotransformation

Species differences in all of these functions could occur. No reason why they all should change in the same direction!
Anatomical Considerations

- Primary barrier to drug absorption is the **stratum corneum**
- Composed of dead keratinocytes embedded in a lipid matrix, through which most drugs are absorbed.
- Lipid matrix excreted by cells in lower layer
- Basal layer consists of viable keratinocytes which migrate to surface and are ultimately shed. Other cell types present: melanocytes, Merkel cells, Langerhans cells, etc.)
- Dermis, vasculature and appendages
**Skin**: PORTAL of entry **and** TARGET for toxicity

- **IL-8, TNFα, Others**
- **SYSTEMIC EFFECT**

Diagram showing skin structure with labeled parts and pathways for the systemic effect.
ABSORPTION versus PENETRATION

Absorption data in both *in vivo* and *in vitro* systems must be interpreted in terms of penetration into the skin versus absorption through the skin.

- **PENETRATION** relates to the amount of chemical which gets to targets within the skin and should be correlated to **LOCAL** cutaneous activity or modulation of a systemic **IMMUNOLOGIC** response.

- **ABSORPTION** relates to the amount of chemical which penetrates the skin but is then absorbed into the bloodstream which correlates to **SYSTEMIC** activity.

Data from all absorption models may be used to study both if the correct target is understood.
Experimental Model Systems

In Vitro vs In Vivo

Human vs Animal
Animal Models

- **Practicality**
- **Absorption**
  - Toxicology - Increased absorption compared to man to assess worse case scenario
    - Rats, Mice, Rabbits
  - Pharmacology - Similar absorption to man
    - Pigs, Primates, Hairless Rodents

*No one has this worked out for veterinary species*

- **Toxicity**
  - Immunological considerations
  - Rabbits, Guinea Pigs, Rodents
## Comparative Epidermal Thickness and Number of Cell Layers From the Back of Nine Species

<table>
<thead>
<tr>
<th>Species</th>
<th>Epidermis (µm)</th>
<th>Stratum Corneum (µm)</th>
<th>Number of Cell Layers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat</td>
<td>12.97 ± 0.93</td>
<td>5.84 ± 1.02</td>
<td>1.28 ± 0.13</td>
</tr>
<tr>
<td>Cow</td>
<td>36.76 ± 2.95</td>
<td>8.65 ± 1.17</td>
<td>2.22 ± 0.11</td>
</tr>
<tr>
<td>Dog</td>
<td>21.16 ± 2.55</td>
<td>5.56 ± 0.85</td>
<td>1.89 ± 0.16</td>
</tr>
<tr>
<td>Horse</td>
<td>33.59 ± 2.16</td>
<td>7.26 ± 1.04</td>
<td>2.50 ± 0.25</td>
</tr>
<tr>
<td>Monkey</td>
<td>26.87 ± 3.14</td>
<td>2.05 ± 2.30</td>
<td>2.67 ± 0.24</td>
</tr>
<tr>
<td>Mouse</td>
<td>13.32 ± 1.19</td>
<td>2.90 ± 0.12</td>
<td>1.75 ± 0.08</td>
</tr>
<tr>
<td>Pig</td>
<td>51.89 ± 1.49</td>
<td>12.28 ± 0.72</td>
<td>3.94 ± 0.13</td>
</tr>
<tr>
<td>Rabbit</td>
<td>10.85 ± 1.00</td>
<td>6.56 ± 0.37</td>
<td>1.22 ± 0.11</td>
</tr>
<tr>
<td>Rat</td>
<td>21.66 ± 2.23</td>
<td>5.00 ± 0.85</td>
<td>1.83 ± 0.17</td>
</tr>
</tbody>
</table>

**Blood Flow Measurements of Nine Species at Five Cutaneous Sites**

<table>
<thead>
<tr>
<th>Species</th>
<th>BUT</th>
<th>EAR</th>
<th>HSJ</th>
<th>TLJ</th>
<th>VAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat</td>
<td>1.82 ± 0.59</td>
<td>6.46 ± 2.30</td>
<td>1.86 ± 0.70</td>
<td>2.39 ± 0.35</td>
<td>6.19 ± 0.94</td>
</tr>
<tr>
<td>Cow</td>
<td>6.03 ± 1.84</td>
<td>6.98 ± 2.19</td>
<td>5.51 ± 2.32</td>
<td>5.49 ± 1.49</td>
<td>10.49 ± 2.13</td>
</tr>
<tr>
<td>Dog</td>
<td>2.21 ± 0.67</td>
<td>5.21 ± 1.53</td>
<td>5.52 ± 1.31</td>
<td>1.94 ± 0.27</td>
<td>8.78 ± 1.40</td>
</tr>
<tr>
<td>Horse</td>
<td>3.16 ± 1.22</td>
<td>-----</td>
<td>6.76 ± 1.49</td>
<td>2.99 ± 0.86</td>
<td>8.90 ± 1.46</td>
</tr>
<tr>
<td>Monkey</td>
<td>3.12 ± 0.58</td>
<td>20.93 ± 5.37</td>
<td>8.49 ± 3.28</td>
<td>2.40 ± 0.82</td>
<td>3.58 ± 0.41</td>
</tr>
<tr>
<td>Mouse</td>
<td>3.88 ± 0.92</td>
<td>1.41 ± 0.48</td>
<td>10.10 ± 3.51</td>
<td>20.56 ± 4.69</td>
<td>36.85 ± 8.14</td>
</tr>
<tr>
<td>Pig</td>
<td>3.08 ± 0.48</td>
<td>11.70 ± 3.02</td>
<td>6.75 ± 2.09</td>
<td>2.97 ± 0.56</td>
<td>10.68 ± 2.14</td>
</tr>
<tr>
<td>Rabbit</td>
<td>3.55 ± 0.93</td>
<td>8.38 ± 1.53</td>
<td>5.38 ± 1.06</td>
<td>5.46 ± 0.94</td>
<td>17.34 ± 6.31</td>
</tr>
<tr>
<td>Rat</td>
<td>4.20 ± 1.05</td>
<td>9.13 ± 4.97</td>
<td>6.22 ± 1.47</td>
<td>9.56 ± 2.17</td>
<td>11.35 ± 5.53</td>
</tr>
</tbody>
</table>

Units = ml/min/100g (mean ± SE)

But = buttocks; Ear = pinnae; HSJ = humeroscapular joint; TLJ = thoracolumbar junction; VAB = ventral abdomen.

## Hair Follicle Density

<table>
<thead>
<tr>
<th>Species</th>
<th>Area of Skin</th>
<th>Number of Hair Follicles/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Abdomen</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Pig</td>
<td>Back</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Rat</td>
<td>Back</td>
<td>289 ± 21</td>
</tr>
<tr>
<td>Mouse</td>
<td>Back</td>
<td>658 ± 38</td>
</tr>
<tr>
<td>Hairless Mouse</td>
<td>Back</td>
<td>75 ± 6</td>
</tr>
</tbody>
</table>

*From Bronaugh*
Effective Tortuosity

\[ \tau = 1 + \frac{2g}{h}\ln\left(\frac{d}{2s}\right) + N\frac{k}{sh} + \frac{d}{(1+w)}\left(\frac{d}{w/hg}\right)^2(N-1) \]

minpath = \left( (k + g + O)^*(N-1) \right) + k

\( \tau \) = effective tortuosity (ratio of diffusivity in stratum corneum with impermeable corneocytes to diffusivity in stratum corneum without impediments)

minpath = minimum pathway length

d = corneocyte diameter
k = corneocyte thickness
N = the number of corneocyte layers
h = the stratum corneum thickness
g = the vertical gap between corneocytes
s = the lateral gap between corneocytes
w = the ratio between the long overlap and short overlap of successive corneocytes

Are any of these parameters different across species?
Human Skin

Porcine Skin

Monkey Skin
Pig Skin is Similar to Human Skin

- Large surface area makes it amenable to test transdermal patches for human use
- Similar surface characteristics
- Body masses
- Skin to body surface area ratio
- Sparse hair coat

Churchill

I like pigs. Dogs look up to us.
Cats look down on us.
Pigs treat us as equals.
Pig Skin vs. Human Skin

- Thick epidermis
- Hair follicle density
- Epidermal turnover kinetics
- Lipid composition
- Biophysical properties of lipid
- Carbohydrate biochemistry
- Arrangement of collagen and elastic fibers

*But don’t use skin from abattoir if hide is scalded!*
NC STATE UNIVERSITY

Skin Forum June, 2008

Rat Skin

Mouse Skin
Cow Skin
Dog Skin

Foot pad
Cat Skin

Abdomen

Lumbar
Ear Skin is Different than other Areas!

- Thickness is thinner compared to other body sites and is thicker on the outside (convex) surface than the inner (concave) surface
- Hair follicle hair density is greater on the convex surface
- Glandular density is different between surfaces
- Cartilage is present
- Blood vessels traverse cartilage and is different than other body regions
Body Site Differences

- The rate of penetration and absorption differs across various body sites
- Scrotum > Forehead > Axilla > Scalp > Back = Abdomen > Palm and Plantar surface
- Seen in humans and animals
- The major reasons are due to:
  - Differences in anatomy: skin thickness
  - Differences in physiology: blood flow and distribution of blood vessels
  - Stratum corneocyte cell size?
Topical Parathion in Pigs: Site Differences

Cutaneous Biotransformation

- Although P450 activity is less in skin, it can have a profound effect on bioavailability
- Phase I and II occur in basal layer
- Applications
  - Prodrugs: conversion of lipid ester to free drug
  - detoxification of pesticides (parathion)
  - bioactivation of toxicants (benzo(a)pyrene)
- No Work published on species differences in absorption!
Experimental Models

■ *In Vivo*
  - Blood AUC
  - Total excretion analysis
  - Non-invasive tape-stripping

■ “Target” of all other studies

■ Absorption reflects the influence of all anatomical, biochemical, physiological and immunological “barriers”

■ Some animal models (monkey, pig and some hairless rodents) may serve as surrogates for humans
In Vitro Absorption Models

- **Diffusion Cells**
  - Static
  - Flow Through
  - Stratum Corneum / epidermal membranes
  - Split (dermatomed) or full thickness skin
  - Species (rodent vs. pig vs. human)

- **OECD Guidelines 427 and 428**
  - Adopted April 13th, 2004
Topical Site Differences in Piroxicam Absorption in Pigs: *In Vitro vs. In Vivo*

Table V. In Vitro Absorption of Piroxicam Using Diffusion Cells in Cranial and Caudal Sites (Mean ± SE)

<table>
<thead>
<tr>
<th>Sites</th>
<th>Flux (0-12hr) (µg/cm²/hr)</th>
<th>Cumulative Amount Transported (µg/24hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cranial</td>
<td>0.0062 ± 0.0009</td>
<td>0.4100 ± 0.0500</td>
</tr>
<tr>
<td>Caudal</td>
<td>0.0068 ± 0.0003</td>
<td>0.4400 ± 0.0800</td>
</tr>
</tbody>
</table>

n = 7 replicates

Table I. In Vivo Absorption of Piroxicam in Cranial and Caudal Sites (Mean ± SE)

<table>
<thead>
<tr>
<th>Sites</th>
<th>Cumulative Mass (mg)</th>
<th>Mean Penetration Depth (μ)</th>
<th>% Applied Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cranial</td>
<td>8.36 ± 2.00</td>
<td>1742.70 ± 353.51</td>
<td>0.00430 ± 0.00140</td>
</tr>
<tr>
<td>Caudal</td>
<td>2.69 ± 0.51</td>
<td>1214.66 ± 103.98</td>
<td>0.00018 ± 0.000062</td>
</tr>
</tbody>
</table>

1Estimated from AUC of penetration profile at the depth of 9480μ
2Discrete compound values estimated at 9480μ

Table IV. Depth Correlation with Cutaneous Structure

<table>
<thead>
<tr>
<th>Approximate Depth (μ)</th>
<th>Microanatomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 15</td>
<td>'stratum corneum</td>
</tr>
<tr>
<td>16 - 45</td>
<td>epidermis</td>
</tr>
<tr>
<td>46 - 1749</td>
<td>dermis</td>
</tr>
<tr>
<td>1750 - 4850</td>
<td>subcutaneous layer</td>
</tr>
<tr>
<td>4851 - 10000</td>
<td>muscle layers</td>
</tr>
</tbody>
</table>

'Stratum corneum may be slightly thinner due to tape stripping
**Isolated Perfused Porcine Skin Flap (IPPSF)**

- Isolated system with control over physiological parameters and perfusate composition
- Intact functional microcirculation
- Viable epidermis and dermis
- Relatively large dosing area
- Predictable extrapolation to *in vivo*
- Allows for simultaneous assessment of absorption, skin disposition, pharmacokinetics and irritation
- Humane alternative animal model
- Cost-effective compared to *in vivo* studies

![Graph showing IPPSF vs In Vivo Human with R² = 0.91](image)
# In Vivo Human vs IPPSF Absorption

<table>
<thead>
<tr>
<th>Compound</th>
<th>Man (7 days)</th>
<th>IPPSF (8 hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicylic Acid</td>
<td>6.5 ± 5.0</td>
<td>7.5 ± 2.6</td>
</tr>
<tr>
<td>Theophylline</td>
<td>16.9 ± 11.3</td>
<td>11.8 ± 3.8</td>
</tr>
<tr>
<td>2,4-Dimethylamine</td>
<td>1.1 ± 0.3</td>
<td>3.8 ± 0.6</td>
</tr>
<tr>
<td>DHP</td>
<td>1.8 ± 0.5</td>
<td>3.9 ± 2.4</td>
</tr>
<tr>
<td>PABA*</td>
<td>15.3 ± 8.4</td>
<td>5.9 ± 3.7</td>
</tr>
</tbody>
</table>

* PH difference in pigs vs human skin, PABA pKa is in between

IPPSF Surgery and Perfusion Systems
BARIUM ANGIOGRAPHY
IPPSF Absorption versus Time Profiles (%D/ml)

Skin Depth of Penetration Profiles

\[\text{\textsuperscript{14}C-Naphthalene} \bigcirc \text{\textsuperscript{3}H-Dodecane}\]
Transdermal Drug Delivery in Veterinary Species

- Antiparasite compounds (flea and heartworm prevention)
  - Fipronil
  - Imidacloprid
  - Ivermectin
  - Selamectin
- Analgesics (fentanyl)
- Nitroglycerin
Limitations for Transdermal Patches in Animals

- Fur !!
  - Adhesion
  - Altered absorption
- Small surface area (cats)
- Selection of site of application that animal cannot get to
- Dosing across wide weight ranges
  - Can’t cut reservoir patches to reduce area !
- No approved veterinary patches
- Occlusive nature of patch often increases irritation
Drug absorption across species is dependent on formulation. Beware of extrapolations!!

Abamectin Partitioning in Different Formulations

Partition Coefficient - Porcine Skin

Formulation

- Ivomec
- Eprinex
- 100% Isopropanol
- 70/30 Isopropanol:Water
- 50%:50% Isopropanol:Water + SLS
Selamectin in Dogs and Cats

<table>
<thead>
<tr>
<th></th>
<th>Dogs</th>
<th>Cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous T ½</td>
<td>14 hr</td>
<td>69 hr</td>
</tr>
<tr>
<td>% Absorbed Oral</td>
<td>62%</td>
<td>109%</td>
</tr>
<tr>
<td>% Absorbed Topical</td>
<td>4.4%</td>
<td>74%</td>
</tr>
</tbody>
</table>
Fentanyl (Duragesic®) Transdermal Delivery in Dogs

- **Duragesic®** fentanyl patches
  - 25, 50, 75, 100 µg/hr
- Lag-time to reach steady-state 12-24 hr
- Steady-state concentrations maintained for 72 hr after application
- One 50 µg/hr patch delivers equivalent of 23-37 mg morphine / day
Transdermal Fentanyl in Dogs
(Kyles, Papich & Hardie AJVR 57:715, 1996)

Duragesic-50 patch (50 mcg/hr)

- Lag time: 24 hours.
- Rate of delivery: 35.7 mcg/hr
  (mean 71%, range 27-100%)
- % absorbed: 64% (range 25-90%)
- Steady-state concentrations:
  approximately 1.6 ng/ml
Transdermal Fentanyl in Dogs (50 mcg/hr)

Time (hours)

Fentanyl (ng/ml)

Patch removed

IV
Transdermal Fentanyl in Cats
(Lee, Papich, Hardie: AJVR 61: 914-919, 2000)

Duragesic-25 patch (25 mcg/hr)

- Lag time: 12 hours (variable)
- Rate of delivery: 8.5 mcg/hr (34%; range 19-59%)
- % absorbed: 36% (range 18-63%)
- Steady-state concentrations: approximately 2.0 ng/ml
Fentanyl Study in Cats (25 µg/hr)

- IV (25 µg/cat)
- Patch removed

Concentration (ng/ml) vs. Time (hours)
Fentanyl in Cats versus Dogs

- Increase lag time in dogs
- Depot in cats after patch removal
- Reduced absorption and steady state flux in cats
Dermal Absorption / Penetration of Topically Applied Nanomaterials: Pig versus Humans
Skin Penetration of Quantum Dots

- (3) Core sizes
  - QD 565 (~4.6 nm) **GREEN**
  - QD 655 (~12 nm) **RED**
  - QD 621 (~ 5 x 8 nm) **RED**

- (3) Surface coatings
  - Polyethylene glycol (PEG) (neutral charge)
  - Carboxylic acids (negative charge)
  - Amines (PEG-amine) (positive)


Ryman-Rasmussen, Riviere, Monteiro-Riviere: Penetration of intact skin by quantum dots with diverse physiochemical properties. *Toxicological Sciences* 91:159-165, 2006
QD 565: All Penetrate by 8 h – Pig Skin
Flow-Through Diffusion Cells  QD-PEG-621 24h – Pig Skin

Control  2μM  10μM  10μm

Scale Bar: 100 μm
Flow-Through Diffusion Cell-10μM QD-PEG621 for 24h

Human Skin - QD565-NH$_2$ 8 h
Target for immune localization and potential lymphatic transport are Langerhans Cells located just beneath stratum corneum.
**Penetration Summary**

- In pigs, QD 565/655 showed penetration but QD 621 did not, suggesting that these QDs are at the cutoff for QD penetration.
- In human skin, no penetration for three types of QD

**SPECIES DIFFERENCES?**
- Hair follicle density
- Size of the HF opening
- Lipid structure
  - Pig has 6-hydroxy sphingosine & human does not
  - Pig has shorter chain length ceramides
  - Pig has hexagonal packing of lipids
  - Human orthorhombic lipid packing

**VEHICLE, DOSE EFFECTS?**
Dangerous to extrapolate to other nanomaterials as some penetration was observed for smaller fullerenes (3.5 nm)

All studies could be a function of particle size, size distribution, shape, surface coatings, pH and vehicle
Overall Conclusions

- All species are not created equal
- Vehicles (hence formulations) are important
- Technique is important
- Modeling may be useful
- Absorption ≠ Penetration
- Chemicals ≠ Particles
The End