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Studies in Drug Transport vs. Current in Iontophoretic Onychomycosis Treatment

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Abstract— **An iontophoretic treatment system for onychomycosis, using drug applicators targeting either toe nail only or nail and surrounding tissue, is analyzed. Phase 1 clinical data shows levels of drug delivery that differ unexpectedly from relative dosing level to multiple tissue types. Current monitoring and analysis techniques, coupled with assays of drug delivery into excised nail and cadaver toe, were used to evaluate drug delivery vs. current flow. The results indicate good correlation with piecewise linear models of current flow and extracted drug in the nail-only application. For the nail and surrounding tissue application, assayed drug levels indicate that on average, drug load per unit dose (mA-min) is more efficient into nail than into surrounding tissue (2.38:1 ug/mA-min nail vs. surrounding tissue, n=6, p=0.009).**

I. INTRODUCTION

TRADITIONALLY, iontophoretic drug delivery has been applied to a single, living tissue type, e.g., stratum corneum and dermis. Iontophoretic treatment of onychomycosis employs this technology to deliver drug actives into saline soaked nail, nail bed and other soft tissues. These tissue types are significantly different. Models reported in the literature [4] have predominantly focused on single tissue type delivery. Those models take into account the complex electro-chemical and physiologic factors that influence drug flow in that "relatively" homogenous environment. It is perhaps not unexpected that drug delivery into soft tissue alone at one efficiency level might not be the same as drug delivery into nail & nail bed.

A drug applicator is under development a.) That is used with a mask to limit contact and delivery to a portion of the nail and the nail bed beneath (Masked Drape); or b.) That is used without a mask to deliver drug to nail, nail bed and surrounding soft tissue (Full Drape).

Both applicator designs were used in a recently completed Phase 1 clinical study [7].

Table I summarizes the data compiled from phase 1 clinical nail samples at various iontophoretic dose levels taken 24 hours post treatment. For an average dose of 8 mA-min, Masked Drape clipping assays averaged 423 ug/g and Full Drape clippings averaged 842 ug/g. The clipping sizes and volumes varied. (n=80, mean=0.017 mL, sd=0.01) The amount of drug found in nail clippings for the Masked Drape treatments appeared to be lower than expected given the higher current concentration in nail vs. levels found for the Full Drape treatments at a lower level of nail current.

TABLE I
PHASE 1 CLINICAL DATA

Table I: Phase 1 Clinical Data				Mean Nail Levels (ug/g) Applicator Design	
Group #	Dose	Current	Duration	Nail Only (N)	Nail/Skin (N)
1	3 mA-min	0.3 mA	10 min	201 (8)	631 (8)
2	6 mA-min	0.5 mA	12 min	600 (7)	736 (8)
3	6 mA-min	0.3 mA	20 min	638 (5)	988 (8)
4	10 mA-min	0.5 mA	20 min	492 (7)	970 (7)
5	15 mA-min	0.5 mA	30 min	185 (4)	886 (4)

Our hypothesis is that 1.) The lower levels in the Masked Drape case are the result of sampling in the extremities of the delivery gradient, and 2.) That the amount of terbinafine found in the Full Drape nail samples was higher than might be expected because drug transfer efficiency into nail is higher than into soft tissue.

The results of drug loading experiments in cadaver toes reported by Nair [1] were compared with current measurements made using a current test fixture and models for the two tissue groups; first, to determine if a drug delivery gradient existed in the masked drape case and how that correlated with drug assays in cadaver toe. Second, to determine the proportion of current flowing in soft tissue vs. into nail in cadaver toe delivery and to compare that with drug loading found by Nair in the two tissue types. It is believed that the transfer efficiency is higher in nail than into soft tissue.

II. METHODS AND MATERIALS

Fig. 1 illustrates the current test fixture. The device is designed to support cadaver nail over agarose (skin analogue) and measure current distribution in two dimensions during iontophoresis using the masked drape and full drape applicator designs. It measures and records current flow in real time using a 45 node sensor with 3 mm spacing and associated data acquisition system. The overall fixture covers a 12 – 20 mm diameter circle.

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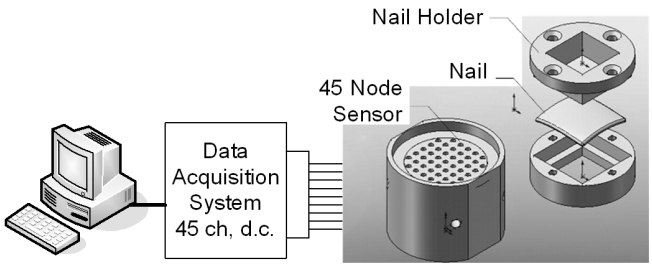

Figure 1: Current Test Fixture

Cadaver nails were obtained for current distribution testing using the test fixture. Each nail was soaked in physiologic saline for 1 hour prior to each treatment. The nails were placed on top of approximately 5 mm thick agarose gel[1] and exposed to iontophoretic treatments of terbinafine at 0.5 mA for 5 min for the masked drape tests. For full drape testing, the nail was placed on top of agarose with agarose added on one end to simulate the cuticle area. Current sensed through each of 45 nodes were recorded.

The methods used and experimental details for terbinafine delivery into cadaver toes are well documented in Nair's paper [1]. In summary, 6 cadaver toes were soaked in physiologic saline prior to treatment. Each terbinafine loaded applicator type was placed on the toe and iontophoretic treatment of 0.5 mA for 20 min was applied. Post treatment, the nails, and sections of nail bed and surrounding tissue were excised and analyzed. The amount of loaded terbinafine was compiled for each section. The iontophoretic power source and general configuration for applicator on cadaver toe are shown below in Fig. 2a and Fig. 2b.

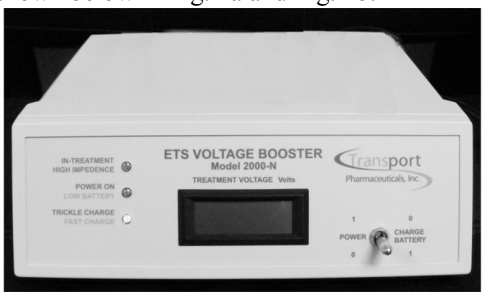

Figure 2a: Iontophoretic Power Source for Onychomycosis Treatment

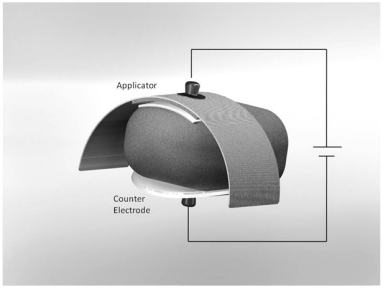

Figure 2b: Full Drape Applicator Illustration
(Mask between applicator and nail for Mask Drape)

[1] Agarose gel: 1% by weight Type II agarose and 20 mM saline.

III. RESULTS

A. Masked Drape

A gradient of current and drug is evident for the Masked Drape case in figures 3a and 3b, resulting from iontophoretic delivery through the mask opening into the larger conductive medium of nail and underlying tissue. Fig. 3a illustrates drug assay results from cadaver nails following iontophoresis using the masked drape applicator[1]. Extracted levels of terbinafine are plotted over distance. Fig 3b shows current measurements taken over the surface of the nail at the same current levels through cadaver nail into agarose. The terbinafine sample data was compiled allocating samples to the same relative spacing as current sensing nodes in the current test fixture.

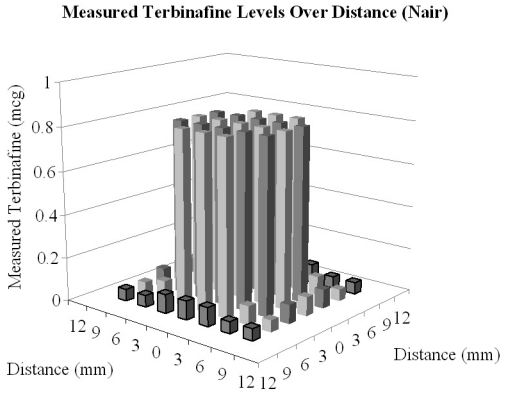

Figure 3a: Masked Drape Drug Delivery. Extracted levels of terbinafine [1] following iontophoresis (10 mA-min) plotted over surface of nail.

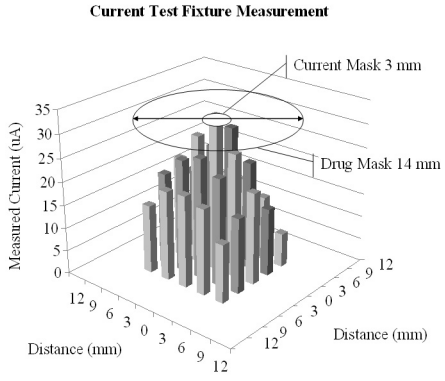

Figure 3b: Masked Drape Current Measurement. Current measured during iontophoretic delivery of terbinafine under nail surface at 0.5 mA. Note: Kresteva and Papazov [6] provide results of finite element analysis for essentially masked electrodes over tissue, illustrating the resulting gradients with higher resolution.

A piecewise linear analysis was performed in lieu of a finite element analysis and compared with terbinafine extractions performed by Nair in cadaver toes. Volume

current flow was estimated by approximating parallel resistances within each drug sample volume. The areas of each drug sample were estimated based upon the relative weight of the samples to the overall weight and dimensions of the nail. Fig. 4 shows the relative sample areas and a simple illustration of the model used. A series of resistances (> 150 per nail) were modeled based upon the thickness and surface areas of samples taken from cadaver toes. The current flowing through each resistance was summed per area to create an estimate of current distribution.

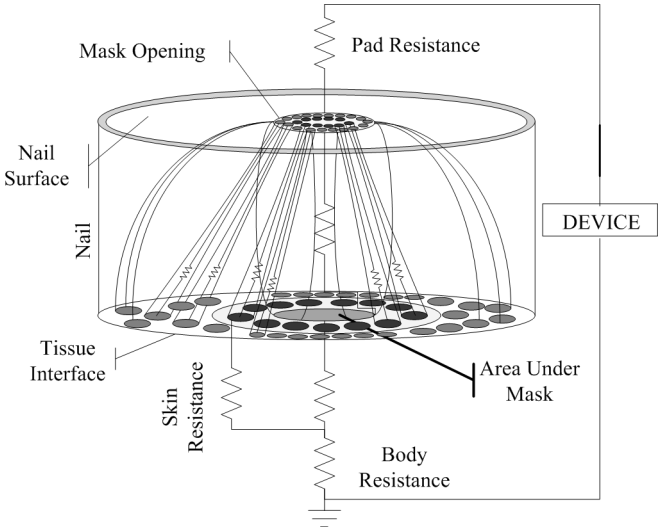

Figure 4: Current Distribution Parallel Resistance Model for Masked Drape. Current flow through concentric volumes of nail and "tissue" were estimated based upon piece-wise linear parallel resistance calculations.

The results of predicted current flow and extracted terbinafine are shown in Fig. 5. The model correlates well with experimental data.

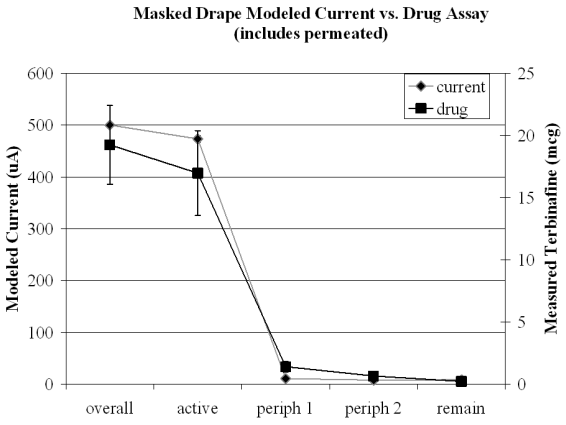

Figure 5: Drug Assay vs. Resistance Model Masked Drape. Drug assays from sectioned nail is plotted per section vs. the value predicted by the current model within each section. (n=3, r=0.998, p< 0.001)

B. Full Drape

For the Full Drape applicator, the conductivity of soft tissue is in parallel with the conductivity of nail. Parallel conducting tissues have been modeled as parallel resistances [5]. The amount of applied current that flows in each may be estimated as the relative percentage of one of those resistances to the sum of both as:

$$i_{skin} = i_{applied} r_{nail} / (r_{nail} + r_{skin}), \quad (1)$$

where i is current and r is resistance.

The current sensing test fixture was used to measure current flow as terbinafine was delivered from a Full Drape applicator to a cadaver nail. The nail was placed on top of agarose which extended to the side of one end of the nail (simulating the cuticle area and full drape coverage of nail and skin). Fig. 6 shows the resulting current flow measurement. Approximately 0.17 mA was measured in nail vs. 0.33 mA in skin. (34% nail, 66% skin)

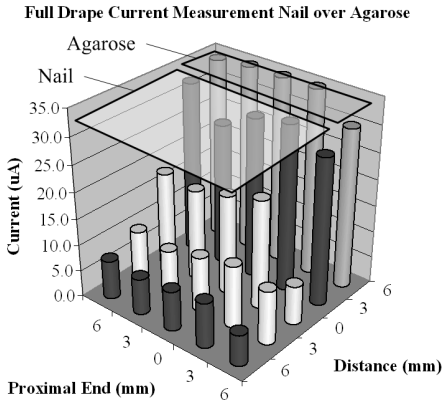

Figure 6: Current Measurement Full Drape. Current flow measured at each of 25 nodes under the surface of the nail and agarose combination when the Full Drape applicator is applied, delivering 0.5 mA of current.

Voltages measured during masked drape cadaver experiments were used to establish intrinsic nail impedance using the formula [5]

$$\rho = rA / h, \quad (2)$$

where r=resistance in k-ohms, A=nail area in cm^2 and h=nail thickness in cm. An intrinsic impedance for nail (n=3) was found to be 2753 k-ohm-cm, sd=217 from the cadaver toe data. The intrinsic impedance was paired with nail size and thickness measurements for the full drape applicators, solving for r, yielding an average R_{skin} = 64 k-ohms and an average R_{nail}=152 k-ohms. The resistance ratio results in a ratio of current in nail and skin of 30% nail, 70% skin.

Fig. 7 shows voltage measurements taken and the resulting calculated resistances for skin and nail for Full Drape and Masked Drape treatments during the clinical study (Fig. 8). Average skin resistance was 67 k-ohms and nail resistance varied during treatment between 202 k-ohms and 584 k-ohms. At time 0 (resistance fluctuates over time), the ratio *in-vivo* is 25% nail, 75% skin. (n=26)

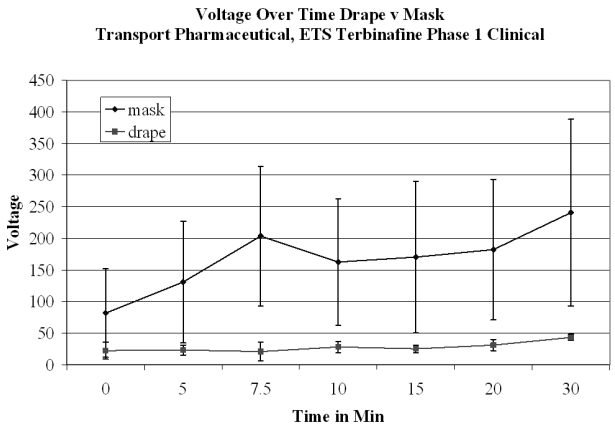

Figure 7: Clinical voltages measured over time for Mask and Full Drape

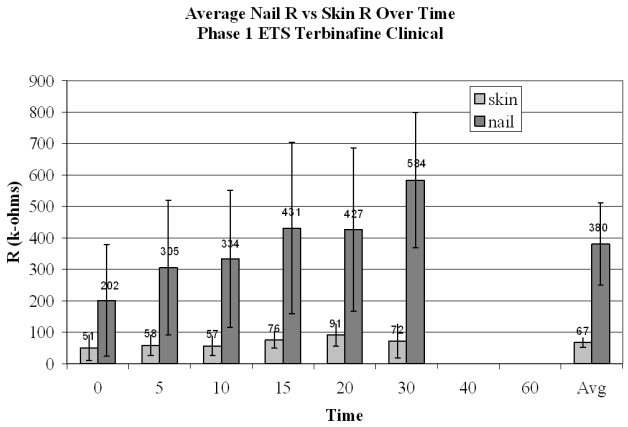

Figure 8: Average nail and skin "resistance" calculated from measured voltages during clinical study over time

Table II summarizes the relative resistances and current delivery ratios for nail with agarose, cadaver nail and skin and in-vivo nail and skin. Treatment area and thickness for skin, nail or agarose as well as hydration levels vary the impedances. The current ratios favor soft tissue as expected.

TABLE II
CURRENT DELIVERY RATIOS

Table II: Current Delivery Ratio's

	Agarose/Nail (k-ohms)	Cadaver (k-ohms)	*In-vivo* (k-ohms)
Skin or Agarose	10.7	64	67
Nail	20.7	152	202 - 584
Current Ratio Skin/Nail	1.9:1 (66%, 34%)	2.33:1 (70%, 30%)	3 – 9 (75%, 25%) – (90%, 10%)

IV. DISCUSSION

A. Masked Drape

The hypothesis that lower than expected drug levels found during the clinical study from nail clippings in Masked Drape cases was the result of samples at the extremities of the nail and a drug delivery gradient is supported by the location of clinical nail samples, the results of current distribution modeling and cadaver toe experiments.

The current measurement and drug loading maps for cadaver nail and toe shown in Fig. 3 clearly indicate a significant gradient. Drug loading at the periphery of the nail was 26 to 77 times less than loading in the center of the nail. Nail clippings for the Masked Drape group in the phase 1 clinical study were taken 24 hours post treatment from the distal nail. Samples varied in size and volume but averaged 1.8 mm long. This size and location corresponds with nail sampling performed in the cadaver toe experiments in the category of "peripheral 2" or "remaining" and shown in Fig. 3a in the 9 to 12 mm range. The current levels predicted by the model in the same areas correlate well with drug distribution samples, $r=0.998$, $p<0.001$.

B. Full Drape

From Full Drape cadaver toe experiments, the amount of current expected to flow in the cadaver skin based upon calculated values of R_{skin} and R_{nail} are 70% skin, 30% nail (See Table II). Current ratios for Phase 1 clinical measurements are similar at treatment time. If terbinafine delivery into dissimilar tissues were strictly related to current ratio (the same efficiency of delivery into both tissue types), then the relative ratio of delivered terbinafine in skin and nail would be 2.33 to 1 (70% skin / 30% nail); however, extracted terbinafine from cadaver toes showed statistical equivalence ($p=0.78$, $n=6$) of delivery into skin and nail & nail bed (shown in Fig. 9), suggesting that there was a difference in drug loading efficiency between the two tissue types.

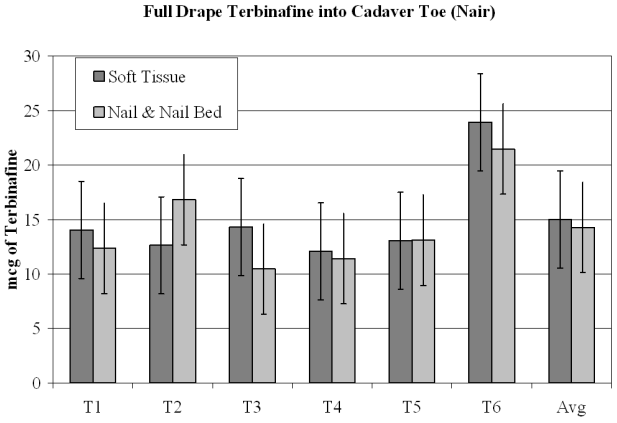

Figure 9: Extracted terbinafine levels in skin vs. nail & nail bed following iontophoresis with Full Drape of 10 mA-min in cadaver toes [1].

Other studies report similar differences in the effect of tissues and electro-chemical influences on drug uptake during iontophoresis.

L. Wearley [3] studied the effect of binding on the iontophoretic transport of amino acids in skin. In the study, he demonstrated that the stratum corneum exhibits binding behavior which retards iontophoretic delivery of amino acids. It is believed that differences in keratin content, lipid content combined with pre-soaked nail acting as a porous hydrogel, may enhance hydrophilic drug formulation transfer characteristics; however this has not been studied specifically.

D. de Berker [2] in his article on nail biology, published in the International Journal of Cosmetic Science, indicates that healthy nail is 1000 times more permeable to water than

skin. This property of nail may influence the electroosmotic element of iontophoretic drug delivery in nail and further differentiate the two tissue types from a drug delivery perspective.

Kalia [4], in his analysis of the process of electro-migration and electroosmosis of molecules into tissue, shows that molecular transfer does not follow a simple 1:1 ratio with current. Rather, the efficiency of transfer is dependent upon many factors including charge mobility and drug concentration (not to mention time varying effects of pH or local charge concentrations). In addition, Kalia notes apparent saturation effects at higher current levels related to physiochemical properties.

For purposes of this discussion, suggesting that overall, different drug transfer efficiencies should be considered when delivering into dissimilar tissues simultaneously with the Full Drape applicator, we use Kalia's analysis as an example. Drug flux is:

$$J_x^T = J_x^{EM} + J_x^{EO} = (1/z_x AF)t_x i + vc_x \qquad (3)$$

where t_x is the transport number, F is Faraday's constant, A is area of application, i is current, z_x is charge and vc_x is an electroosmotic term.. For a particular "membrane" or "tissue type", the transport number (t_x) may be estimated as the ability to move charged drug molecules relative to the other ions present. This is calculated as product of the ion's concentration, mobility, and charge divided by the summation of the same product for all of the ions in the system.

Rather than determine individual elements of Kalia's formula, an aggregate transport number for skin (t_{skin}) and nail & nail bed (t_{nail}) was calculated, essentially aggregating the factors and segmenting them into soft tissue plus nail/nail bed.

$$J_x^T = J_x^{Tskin} + J_x^{Tnail} = (1/z_{skin} AF)t_{skin} i_{skin} + vc_{skin} + (1/z_{nail} AF)t_{nail} i_{nail} + vc_{nail} = l_{skin} + l_{nail} \qquad (4)$$

Where *l* is the amount of drug loaded for skin or nail. For a given dose in uA-min, the amount of terbinafine loaded into cadaver nail & nail bed vs. cadaver skin vs. delivered dose and calculated transfer number is

$$l_{total} = l_{skin} + l_{nail},\ l_{skin} = d_{skin} \times t_{skin} \text{ and } l_{nail} = d_{nail} \times t_{nail} \text{ in ug/mA-min,} \qquad (5)$$

where d_{skin}, d_{nail} is the product of current through skin (or nail) and time (dose), t_{skin}, t_{nail} is an aggregate of all factors related to delivery into skin (or nail) and l_{skin}, l_{nail} is the amount of terbinafine loaded into skin (or nail).

Table III lists the averages of areas of delivery, current and the result of transfer number calculations for the 6 cadaver toe trials using equation 5.

TABLE III
DRUG VS. CURRENT PER UNIT AREA

Drug per unit area vs. current per unit area (from Cadaver Toe)		
n=6	**avg**	**sd**
skin area (cm^2)	3.288	0.322
nail area (cm^2)	2.753	0.565
skin current density (mA/cm^2)	0.106	0.013
nail current density (mA/cm^2)	0.054	0.010
dose per unit area: skin ($mA\text{-}min/cm^2$)	2.119	0.258
dose per unit area: nail ($mA\text{-}min/cm^2$)	1.089	0.209
transfer number: skin=$(ug/cm^2)/(dose/cm^2)$	2.181	0.650
transfer number: nail=$(ug/cm^2)/(dose/cm^2)$	5.180	2.114

Fig. 10 illustrates the relative values of the average transport numbers including values calculated for current (if delivery were dictated by current alone), Masked Drape and Full Drape from Table III.

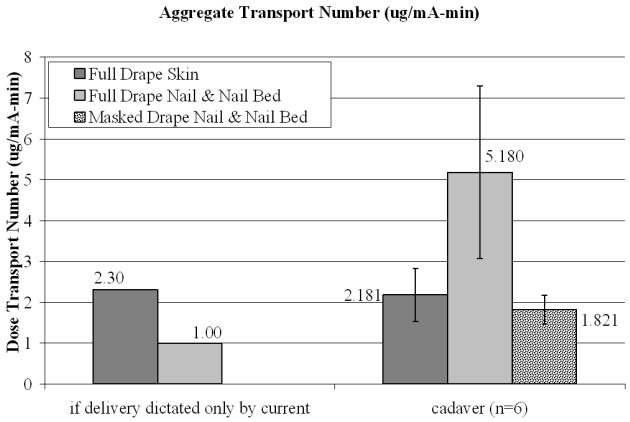

Figure 10: Aggregate Transport Number Mask vs. Full Drape. The product of applied dose and transport number yields delivered drug in ug.

For cadaver toe with the Full Drape design, the ratio of skin to nail aggregate transport number is 1 to 2.38. Transfer to nail is 2.38 times more per mA-min than into skin (n=6, p=0.009). This contrasts with a current "delivery" ratio of 2.3 to 1 (2.3 times more current in skin than in nail). Interestingly, the aggregate transport number for the nail-only application is less than the factor for nail in the Full Drape design. (n=4, p=0.032) It is possible that the decreased nail treatment area of the masked drape reduces terbinafine loading vs. the applicator with an increased coverage area; however this has not been evaluated.

Applying the cadaver transfer number for nail vs. skin to the *in-vivo* results yields an estimate for nail and drug delivered for both tissues. (Based upon resistance ratios calculated from measured voltages during the clinical study.)

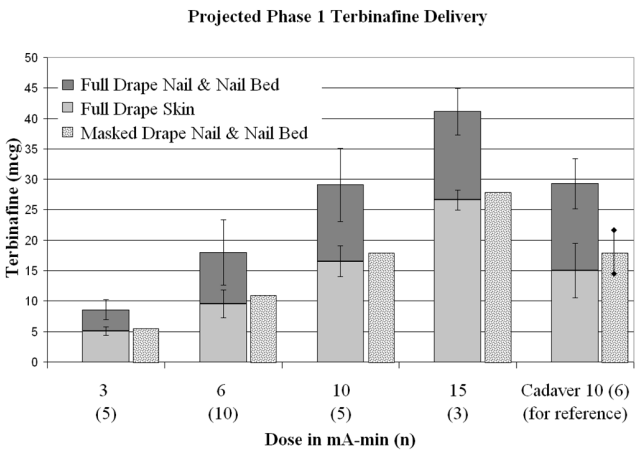

Figure 11: Projected Delivery of Terbinafine into Skin and Nail for Full Drape and Masked Drape using Cadaver Transfer Number Calculation

The mean level of terbinafine estimated to have been delivered with Full Drape treatments to patients in the phase 1 clinical using this method at 10 mA-min is 16.5 ug skin, 12.6 ug nail & nail bed, compared with a mean of 15 ug skin and 14 ug nail & nail bed for cadaver toes.

V. CONCLUSION

Terbinafine concentrations in peripheral areas of nail following iontophoretic treatment, where the drug is introduced into the nail using a mask, were shown to be less than drug concentrations directly under the mask due to current and drug delivery gradients.

Terbinafine concentrations resulting from simultaneous iontophoretic delivery to dissimilar tissues were found to be impacted by different transfer efficiencies, resulting in higher percentage delivery in nail than soft tissue. Notably for terbinafine delivery into nail & nail bed versus surrounding soft tissue, transfer efficiencies were found to be more than 2 to 1 in favor of nail per mA-min in cadaver toe (p=0.009).

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