phenytoin. Teratological studies in this strain are ongoing. Phenytoin concentrations in fetal brain determined by HPLC were about 60% higher than those in fetal liver, which were similar to those in both maternal liver and brain. These results suggest than the acatalasemic mouse strain may be relatively resistant to phenytoin-enhanced ROS formation and teratogenicity, requiring further optimization of the treatment regimen to evaluate the embryoprotective role of endogenous catalase. The lower constitutive and phenytoin-enhanced DNA oxidation in catalase overexpressing embryos suggests a protective role for endogenous catalase, although the teratological relevance remains to be determined. The increased concentration of phenytoin in fetal brain compared to fetal liver and maternal tissues may explain the high frequency of neurodevelopmental deficits compared to other anomalies caused by this drug, and raises interesting questions as to the mechanisms underlying this selectivity.

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50. Hepatic Expression of ABC Drug Transporters in Rodent Models of Inflammation During Pregnancy

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Purpose: While various rodent models of inflammation and infection have been established, few have focused on using pregnant rats. Pregnancy induces a number of physiological changes which may affect the response of these rats to an infectant. Our objective was to compare three different inflammation models using pregnant rats in order to determine the best one for use in future pharmacokinetic studies. We examined the models in terms of their effect on the expression of several key hepatic drug transporters and the metabolizing enzyme Cyp3a, as these genes are known to be affected by the inflammatory response and also have many clinically relevant substrates.

Methods: Pregnant SD rats (G17-18, n=3-6/group) were administered single i.p. doses of the following infectants: bacterial endotoxin (LPS, 0.1 - 1.0 mg/kg), polyinosinic:polycytidylic acid (poly I:C, 0.75 - 5.0 mg/kg), or interleukin-6 (IL-6, 1□g/rat). Control pregnant rats received saline. Animals were sacrificed 6-24 hrs later and hepatic mRNA levels of P-glycoprotein (Mdr1a and Mdr1b), Mrp2, Bcrp, Oatp2, and Cyp3A were measured via real-time PCR.

Results: At the given doses, LPS and poly I:C models were the only to have a significant effect on hepatic drug transporter and Cyp3a expression levels as compared to controls. No significant differences were observed with the IL-6 model at the given dose. Expression profiles of mRNA levels were similar between the LPS and poly I:C models, with significant downregulation ($p<0.05$) of all examined genes except for Mdr1b, which was significantly induced. However, LPS was more toxic for pregnant rats than poly I:C was.

Conclusion: Both, LPS and poly I:C-induced inflammation in pregnant rats are effective for studying the impact of inflammation on hepatic drug transporters and drug disposition of their substrates. From our observations, poly I:C was safer for use in pregnant rats as it resulted in less mortalities. IL-6 dosing would have to be optimized in order to provide an effective model, but this may be costly as cytokines are an expensive medium.

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51. Quantitation of Stratum Corneum Depth in the Skin Surface Biopsy by Tape Stripping

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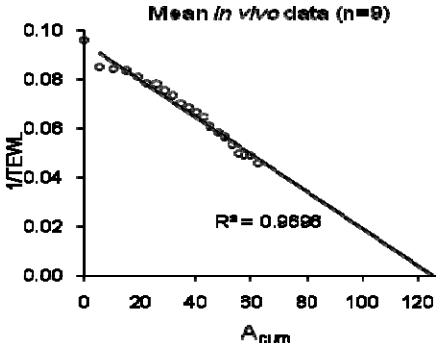
Purpose: The skin surface biopsy by tape stripping allows successive sampling of the stratum corneum (SC) at increasing depths. However, SC thickness varies between subjects and sites and the amount of SC material removed varies between strips. This work shows a method by which the relative SC depth and amount removed with each strip can be determined.



Methods: A site on the inner volar forearms of nine normal human volunteers was stripped 20 times with 14 mm CuDerm discs. After each strip, transepidermal water loss (TEWL) was measured at the site with a Tewameter (Courage-Khazaka). The absorbance of each strip was read at 850 nm (SquameScan 850). These procedures were repeated using excised human skin from a single female donor (11 replicates). Recognizing that Fick's 1st Law describes water diffusion through the SC membrane, a linear relationship exists between the inverse of TEWL and the thickness of the membrane after each strip. The thickness of the unstripped SC is given by the x-axis intercept of such a plot. As absorbance (A) is proportional to the thickness of the absorbing material (Beer-Lambert Law), the cumulative absorbance (A_{cum}) of the material on the strips can be substituted for thickness to obtain a similar relationship. This allows us to obtain a depth at each tape strip relative to the total depth. It also allows comparison between individual subjects.

Results: The predicted theoretical relationship between $(TEWL)^{-1}$ and A_{cum} was verified experimentally for the human subjects (see figure). The mean percentage of SC removed after 20 strips was 49.2% (%CV=21.6). There was significant variability in the SC thickness removed by the 20 strips (represented by A_{cum} values; mean=62.5, %CV=26.2). The same linear relationship was also verified for excised skin (R^2=0.9916). The percentage of SC removed by 20 strips (mean=82.6, %CV 3.6%) was greater than seen for *in vivo* skin. As well, a greater absolute amount of SC was removed from the excised skin (mean A_{cum} = 96.72, compared to 62.5 for in vivo skin).

Conclusions: We demonstrated a simple method for determining relative depth in SC after tape stripping. While comparable methods were previously applied to *in vivo* skin, we showed for the first time that theoretical predictions also hold true *in vitro*. Inter-individual differences may explain the differences between *in vivo* skin and the single sample of excised skin, or they may reflect different rates of diffusion in non-living tissue.

Reference: Herkenne, C. et al (2008) In vivo methods for the assessment of topical drug bioavailability Pharm Res 25(1):87-103.

52. A Cree Anti-Diabetic Botanical Alters Gene Transcript Changes in Caco-2 Cells

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Purpose: To evaluate a Cree botanical, AD09, used by the Cree of Eeyou Istchee to treat symptoms of type II diabetes (T2D), for its role in altering gene transcript changes in human Caco-2 intestinal cells. Also, to undertake a quantitative comparison of the phytochemical profiles of ethanol (EtOH) and traditional water (DW) extracts to determine the relevance of using an EtOH extract in laboratory studies.

Methods: Human Caco-2 intestinal cells were exposed to AD09 at a concentration of 100 □g/mL for a period of 4 and 24 hrs, the RNA was extracted, and microarray experiments were performed using human 19K cDNA arrays to establish gene changes with the extract versus 0.1 % DMSO control. Furthermore, cells were exposed to compound M, a major component of AD09 for 4 hours, at a concentration of 8.8 µg/mL, and microarray experiments performed. Both extracts were analyzed by HPLC coupled with diode array detector and evaporative light scattering detector.

Results: Microarray experiments for AD09 and compound M for 4 hrs revealed no statistically significant gene transcript changes. However, the 24-hr exposure to AD09 yielded 304 downregulated mRNAs ($p<0.05$) with a fold-change greater than 1.5 with significant downregulation of key transcription factors and members of different signaling pathways. A phytochemical evaluation yielded many similarities between the extracts in the different markers examined. Only slight differences were observed in the polar and non-polar regions of the chromatograms.

Conclusions: AD09 did not cause transcript changes of the cytochrome P450s which are usually altered in the intestinal model of Caco-2 cells upon exposure to a xenobiotic, signifying that the botanical may be quite safe to use especially with