

# ABSTRACTS: GROUP 4: CREIGHTON UNIVERSITY SUMMER RESEARCH INSTITUTE 2004

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## DISSOLUTION PROFILES OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAID) WITH DIFFERENT PARTITION COEFFICIENTS

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*Background.* Any drug to be absorbed from oral route must first be dissolved in the gastrointestinal fluids. The process by which a drug particle dissolves is termed dissolution. *In vitro* dissolution testing of pharmaceuticals is not a guarantee of therapeutic efficacy, but can reveal the physiological availability (bioavailability) of a drug. The oil/water partition coefficient, on the other hand, is a measure of a molecule's lipophilic character, ie, its preference for hydrophilic or lipophilic phase. To produce a pharmacological response, a drug molecule must cross the biological membrane, which consists of protein and lipid material, acting as a lipophilic barrier for many drug molecules. The resistance of this barrier to drug transport is related to the lipophilic nature of the molecule being transported.

*Objective.* The objective of this work was to select three NSAIDs based on the differences in their octanol/water partition coefficient and determine their *in vitro* dissolution profiles. The dissolution profiles of generic vs brand products for all three NSAIDs will also be evaluated.

*Experimental.* Aspirin, acetaminophen and ibuprofen with octanol/water partition coefficients 0.45, 1.2 and 4.5, respectively, were used as model drugs for this investigation. Dissolution studies were carried out in USP Dissolution Apparatus-2. Simulated intestinal fluid without enzyme was used as the dissolution medium. NSAID were analyzed by spectrophotometric method.

*Results.* Among the three NSAIDs studied, ibuprofen had the highest octanol/water partition coefficient. Therefore, one should expect the solubility of this drug in aqueous-simulated intestinal fluid to be lower than the other two drugs. This low aqueous solubility affects the dissolution of this drug in the intestinal fluid. The dissolution profiles of all three NSAIDs and brand vs generic products are presented.

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## PERCEPTUAL MOTOR SKILLS AND DENTISTRY

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Chalk carving of a geometric figure is a widely accepted test for perceptual motor skills, which are highly important for dental students. Analyzing the effect of specific dimensions of dental student's chalk carving on grades and the improvement of perceptual motor skills from freshman to senior year via chalk carving exercises will help lead to preventing subjectivity and determining hand dexterity among dental students. Sixty-seven incoming freshmen dental students performed a chalk carving exercise. Sixty-two of those students repeated the same exercise as seniors. The following dimensions of the carvings were measured and observed: notch length, notch depth, tip size, notch center, and overall length. A team of three faculty members graded the carvings. The students' grades were based on their carving's measurements, surface flatness, angles, and symmetry.

A Pearson correlation coefficient was performed to relate the aggregate measurement differences to grade for each carving. Individual dimensions were also tested for correlation to grades. Freshman and senior grades were analyzed with a *t* test to determine significance of improvement.

In the dimensions measured, the aggregate difference for the freshmen showed  $r = -.21$  and  $P = .081$ . The aggregate difference for the seniors showed  $r = -.29$  and  $P = .023$ . With these results, a strong correlation was found between chalk-carving dimensions and grades in freshman and senior groups. The improvement in grades from freshman to senior year was significant. Stronger correlation in the senior data may be explained by the subjectivity of other criteria. Senior carvings were more notably executed. Measurement deviations became secondary to subjective criteria of surface smoothness, angles, and symmetry in assigning grades. Development of grading methods to quantify these subjective criteria may prove useful in the future.

**THE ER- $\alpha$  GENE IS ASSOCIATED WITH AGE AT MENARCHE IN CAUCASIAN WOMEN**

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Menarche is a significant milestone in a female's life. The estrogen receptor  $\alpha$  (ER- $\alpha$ ) is important in mediating estrogen signaling. In the present study, we evaluated the effects of the ER- $\alpha$  gene on the age at menarche in 397 unrelated Caucasian women by genotyping seven single nucleotide markers (SNPs). DNA was extracted from whole blood using a commercial isolation kit (Gentra Systems, Minneapolis, MN, USA) following the procedure detailed in the kit. The genotyping procedure for all SNPs was similar, involving polymerase chain reaction (PCR) and invader assay reaction (Third Wave Technology, Madison, WI, USA). The ages of subjects were averaged at 60.5 years with the range of 19.7 to 85.8 yrs. The mean age at menarche was 12.9 years with the range of 9 to 17 years. The minor allele frequencies for the seven SNPs, rs2077647, rs2234693, rs1514347, rs1801132, rs932477, rs3778082 and rs2228480, were 49.8%, 44.8%, 23.9%, 20.3%, 9.8%, 13.1%, and 19.5%, respectively. Among the seven SNPs, rs3778082 and rs2228480 were significantly associated with the onset of menarche. For the marker rs3778082, menarche occurred, on average, about four months later in women with allele *A* than in those without it, 13.20 years vs 12.85 years ( $P < 0.05$ ). For the marker rs2228480, women carrying allele *A* had an earlier menarche of about four months than found among non-carriers, 12.74 years vs 13.05 years ( $P < 0.05$ ).

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**DEVELOPMENT OF A FOAM-FILM FORMULATION FOR THE TREATMENT OF BURNS**

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The American Burn Association estimated that more than one million burn injuries occurred during 2000. Burns can be classified into two major categories: partial thickness burns and full thickness burns. Partial thickness burns affect the epidermis and the dermis, while full thickness burns include damage to the subcutaneous layers. A burn can also be classified into three classes according to the degree of its severity. First- and second-degree burns are partial thickness burns, affecting the epidermis and both the epidermis and the dermis, respectively. Third-degree burns are full thickness burns that penetrate into the deep tissues of the body.

Burn injuries are treated with debridement, biologic wound coverings, early excision and grafting, temporary wound closure with biological or synthetic dressings, and antimicrobials. Antimicrobials, such as sulfadiazine and mafenide acetate, are used in topical creams for the treatment of burns. All of these treatments require mechanical application that causes the burn patient immense pain and discomfort. The objective of this study was to develop a foam-film formulation for the topical delivery of an anti-infective. We hypothesize that this foam-film formulation will enhance patient compliance and effectiveness in the treatment of burns.

The foam was formed using acetic acid, cetrimide, chitosan, gelatin and distilled water. Mafenide acetate was used as the model anti-infective. A spectrophotometer was used to quantitate the mafenide acetate. Different amounts of mafenide acetate were dissolved into a phosphate buffer. The phosphate buffer had a pH of 7.4 and consisted of sodium phosphate dibasic, potassium phosphate monobasic, and distilled water. The wavelength was set at 226nm. The foam was formed using a foam former containing a mechanical pump. The stability as well as the permeability of the drug through films formed by these foam-film systems was evaluated. The *in vitro* release of mafenide acetate from the foam was then investigated using a Precision<sup>®</sup> reciprocal shaking bath.

A stable foam-film system was developed using chitosan and a cationic surfactant, cetrimide. Chitosan and cetrimide concentration has minimal effect on the stability of the foam. The *in vitro* release study revealed that release of mafenide from this foam delivery system is very quick.

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## **EXPRESSION OF SUPPRESSOR OF CYTOKINE SIGNALING (SOCS)-2 PROTEIN IN HUMAN COLON CANCER CELLS**

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The proliferation and survival of human colon cancer Caco-2 cells are stimulated by insulin-like growth factor-I (IGF-I) receptor. Our laboratory has cloned suppressor of cytokine signaling (SOCS)-2 protein as an intracellular binding partner of the IGF-I receptor. The primary goal of this research was to examine the expression and localization of SOCS-2 protein in Caco-2 colon cancer cells. Caco-2 cells are responsive to IGF-I and the IGF-I receptor is expressed and phosphorylated in these cells. We hypothesized that endogenous SOCS-2 protein is expressed in colon cancer Caco-2 cells. To test this theory, we formulated the following experimental approach: cell culture, immunoblotting with SOCS-2 antibody, immunoblotting with MAPK and phosphoMAPK antibodies, immunoblotting with IGF-I receptor and phosphotyrosine antibodies, and immunolocalization of SOCS-2 protein in intact cell. SOCS-2 protein expression led to inhibition of MAPK phosphorylation, suggesting that SOCS-2 may play a negative regulatory role in IGF-1 receptor signaling.

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## **EXPRESSION OF SOCS-1 AND 3 IN TNF-ALPHA AND IGF-1 TREATED VASCULAR SMOOTH MUSCLE CELLS**

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This research centered around the concept of calculating and analyzing the expression of SOCS proteins in the presence of cells treated with pro-inflammatory cytokines, as well as exploring the clinical applications of our findings. The cytokine used in our experiment was a pro-inflammatory cytokine called TNF-alpha or tumor necrosis factor- alpha. The proteins in our experiments are known as SOCS or suppressors of cytokine signaling proteins. There are three different types of SOCS: SOCS-1, 2, and 3. Recent research has shown that SOCS proteins, and SOCS-1 in particular, have been found to inhibit the signaling in the Jak/STAT (Janus Kinase) pathway.

The purpose of this research was to attain, calculate, and analyze the expressions of SOCS1 and SOCS3 in pig aortic smooth muscle cell cultures treated with TNF- $\alpha$ , as well as to look at the effect of IGF-1 in relation to TNF- $\alpha$  and the effect it may have on protein expressions of SOCS1 and SOCS3. In addition, we hoped to identify any possible clinical applications concerning the treatment of patients undergoing arterial inflammation.

To complete the experiments, we used culture pig aortic smooth muscle cells (PASMC) treated with TNF- $\alpha$  (100 ng/mL) at time periods of 12, 24, 48, and 72 hours. Treated PASMCs were then harvested and protein was extracted. A protein assay was conducted to calculate concentrations for each individual sample in order to load equal amounts in each well. Samples were collected and stored at a temperature of  $-80^{\circ}$  C. SOCS1 and SOCS3 were detected using antibodies against these proteins. Quantitative analysis of the protein bands was performed by a densitometer. These same techniques were completed for the expressions of both SOCS1 and SOCS3.

Preliminary results revealed a dose-dependent increase by TNF- $\alpha$  in SOCS1 and SOCS3 expression by more than two-fold compared to the control group. More experiments are currently underway in order to attain a better comprehension of the nature of SOCS1 and SOCS3 expression in response to treatment with TNF- $\alpha$  and the effect of IGF-1 on TNF- $\alpha$ .

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## OSTEOCLAST ACTIVATION WITH SMOKING

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Past experiments have shown the bone mass of smokers to be lower than in nonsmokers. The purpose of this experiment was to determine if smoking increases osteoclast activation, resulting in bone remodeling. Bone remodeling is the process in which bone cells remove and rebuild bone. There are three steps to bone remodeling: activation, resorption, and formation. In activation, the osteoclasts (bone removing cells) attach to the bone. During resorption, the osteoclasts release acid phosphatase, an acidic chemical that removes the bone and creates resorption pits. In the final step, formation, osteoblasts (bone-building cells) rebuild the bone that the osteoclast has removed. If smoking increases osteoclast activation and the osteoblasts cannot match the osteoclast work output, then the bone mass would gradually decrease.

To test our hypothesis, rats were exposed to low-dose smoke, high-dose smoke, or sham exposed (N=12/group). The rats were exposed to the smoke for 11 sessions/week for a duration of one hour each session. The high-dose mice were exposed to  $164 \pm 34$  TSP (total suspended particles, mg/mL<sup>3</sup>), low-dose mice to  $91 \pm 17$  TSP, and the control group mice were sham exposed. The proximal tibia was processed for histomorphometric analysis of the number of osteoclasts and the percentage of bone surface that these cells covered.

Differences among groups were tested by analysis of variance (ANOVA) with Tukey Post Hoc tests. Significance was set at  $P < 0.05$  and data was reported as mean and standard deviation. The high-dose group was found to have greater osteoclast number ( $2.8 \pm 1.9$ ) and percentage osteoclast surface ( $0.4 \pm 0.3\%$ ) than the low-dose and control groups. The low-dose and control groups did not differ from each other. Their osteoclast number averaged  $0.4 \pm 0.8$  and osteoclast surface averaged  $0.03 \pm 0.06\%$ . From these results, the conclusion was made that high doses of smoke exposure led to greater osteoclast activation. Greater osteoclast activation will lead to greater bone loss if the osteoblasts are not able to keep up with the osteoclasts.

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## QUALITATIVE RESEARCH IN COLORECTAL CANCER SURVEILLANCE

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*Purpose.* Colorectal cancer (CRC) is one of the leading causes of death worldwide, with an incidence of a million new cases per year, 10% of which are estimated to be hereditary. Early detection by colonoscopy or genetic testing is the key to preventing CRC. However, many at high-risk are reluctant to participate in genetic testing. Therefore, it is crucial to identify the reasons for their reluctance, and to provide a solution in order to increase the participation rate and save lives. The purpose of our study was to identify critical issues in cancer surveillance participation and to assess the application of qualitative research in cancer surveillance and prevention.

*Methods.* Literature was searched online for relevant articles. We compared the ability of qualitative vs quantitative research to identify critical issues in genetic screening for hereditary cancers, specifically hereditary non-polyposis colorectal cancer (HNPCC).

*Results.* Participation rates were related to participants' perceived (rather than actual) risk for CRC, socioeconomic status, culture, race, sex, and familial and social relations. Patients of minority backgrounds tended to not trust the medical system to keep their results confidential, and believed genetic testing results would be used by their insurance companies to deny benefits or that their companies would raise rates. Although quantitative research may provide statistical information, such as different rates of participation in different populations, socio-psychological bases for these findings are often left unexplored. In contrast, qualitative research provides researchers with the ability to ask questions which go beyond numbers, and thus may provide solutions for raising participation rates. Researchers have begun qualitative research in cancer prevention as well as surveillance of breast and other cancers; however, qualitative research for HNPCC prevention and surveillance has not yet been reported.

*Conclusions.* Qualitative research appears to be a powerful tool for improving participation in genetic testing for cancer. Qualitative research coupled with cancer education is a promising approach to improve cancer prevention.

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## A COMPARATIVE STUDY OF DIAGNOSTIC INTERPRETATION OF CONVENTIONAL BITEWING RADIOGRAPHS VS INDIRECT DIGITIZED RADIOGRAPHS

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New methods for reading dental x-rays are constantly being developed and evaluated. Diagnosis of dental caries by independent examiners is subjective and can be unreliable.

*Objective.* To assess and compare the diagnostic yield regarding the subjective recognition depth of dental caries observed in conventional bitewing radiographs and scanned digitized images.

*Materials and Methods.* Four conventional bitewing radiographs were generated using DenOptix Gendex Imaging unit on fifty dental students. Conventional films were scanned with TigerView Scanning system version 5.00.01 on Epson Expression 1600 flatbed scanner at 150 and 300 dpi. The scanned images were read on a Gateway EV700 cathode ray tube monitor at 800×600 pixels, a relatively low resolution. Gray level was set at 32 bits. Three observers detected the presence and depth of proximal caries recorded on a 3-point depth scale (1 = less than half of the enamel, 2 = over half of the enamel and up to the dentin-enamel junction, and 3 = into dentin and less than half of the pulp). Observers read conventional, 150 dpi, and 300 dpi images on two separate occasions. Inter-observer, intra-observer and inter-viewing condition results were analyzed for agreement using a Kappa and Weighted Kappa test. 1642 proximal surfaces were evaluated.

*Results.* There was moderate agreement (0.4–0.6) for inter-observer and intra-observer comparisons for all three viewing conditions.

*Conclusions.* There was no improvement of diagnostic interpretive yield using scanned digital x-rays over conventional x-rays.

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## ISOPROSTANE-INDUCED INHIBITION OF [<sup>3</sup>H]D-ASPARTATE RELEASE FROM ISOLATED RETINAE: ROLE OF ANTIOXIDANTS

Student Researcher: Arthur Stallworth

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*Purpose.* There is evidence that isoprostanes, a series of prostaglandin-like compounds, can regulate excitatory neurotransmission in bovine retinae. Isoprostanes are formed *in situ* by a non-enzymatic, free-radical catalyzed peroxidation of arachidonic acid in retinal tissues and, because of this, we considered the possibility that these novel compounds may mediate their effect via oxidant stress-mediated mechanisms. The aim of the present study is therefore to evaluate the effect of antioxidants on isoprostane-mediated inhibition of K<sup>+</sup>-induced [<sup>3</sup>H]D-aspartate release from bovine retinae.

*Method.* Isolated neural retinae were incubated in oxygenated Krebs solution containing 200 nM of [<sup>3</sup>H]D-aspartate for 60 minutes. The retinae were prepared for studies of neurotransmitter release using the superfusion method. Release of [<sup>3</sup>H]D-aspartate was evoked by iso-osmotic concentration of K<sup>+</sup> (50 mM) stimuli applied at 90 minutes (S<sub>1</sub>) and at 108 minutes (S<sub>2</sub>) after the onset of superfusion. When used, the antioxidants were present throughout the experiment.

*Results.* Preliminary studies indicate that in the presence of the antioxidant, ascorbic acid (90 μM), the isoprostane, 8-iso prostaglandin F<sub>2α</sub> (8-isoPGF<sub>2α</sub>; 0.1–30 μM) caused a concentration-dependent inhibition of K<sup>+</sup>-evoked [<sup>3</sup>H]D-aspartate release without affecting basal tritium overflow. Similarly, in the presence of the antioxidant, trolox (10 μM), 8-isoPGF<sub>2α</sub> caused an inhibition of the neurotransmitter release. In the presence of trolox (10 μM) and ascorbic acid (90 μM), 8-isoPGF<sub>2α</sub> (0.01 μM) caused 55% and 15% inhibition of K<sup>+</sup>-evoked [<sup>3</sup>H]D-aspartate release respectively. It was interesting to note that pretreatment of retinal tissues with both ascorbic acid and trolox reversed 8-isoPGF<sub>2α</sub>-mediated inhibition of K<sup>+</sup>-evoked [<sup>3</sup>H]D-aspartate release.

*Conclusion.* Preliminary studies show indication that oxidant stress regulates the 8-isoPGF<sub>2α</sub>-mediated inhibition of K<sup>+</sup>-evoked [<sup>3</sup>H]D-aspartate release in bovine isolated retinae.

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