Analysis of Gene Expression of Enzymes Contributing to Pain
via Formation of Oxidized Lipids in Burn Patients

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INTRODUCTION: The transient receptor potential vanilloid subtype-1 (TRPV1) receptor is a ligand-gated cation channel that is expressed on peripheral neurons where it is activated by painful stimuli such as heat, and mediates inflammatory thermal and mechanical pain. Although medications to relieve acute pain exist, they have a narrow spectrum of action and come with a multitude of adverse effects. In response to heat injury and inflammation linoleic acid is metabolized to form oxidized linoleic acid metabolites (OLAMs), which are direct agonists for TRPV1. The mechanism by which linoleic acid could be oxidized to these metabolites include enzymatic reactions (lipoxygenases (LOX) or cytochrome p450 enzymes (CYP)) or non-enzymatic via oxygen free radicals. The list of possible enzyme candidates involved in metabolizing linoleic acid is large and before additional studies can begin to target those enzymes involved in OLAM formation, a narrower focus must be established. In this study, we analyzed a large database of gene transcripts to evaluate any alterations in expression levels for a specific set of enzymes that may be involved in OLAM synthesis in burn pain.

METHODS: The Glue Grant Trauma-Related Database (TRDB) was used to identify changes in expression patterns of transcripts from skin biopsies and blood circulating leukocytes after both burn and traumatic injury. The Affymetrix HG-U133_Plus_2 human microarray was used to analyze a total of 102 genes, including 86 genes known to oxidize poly unsaturated fatty acids (the general class of lipids that includes linoleic acid). A total of 87 burn patients had associated 238 microarrays from skin biopsies and 253 burn patients had 602 blood circulating leukocyte microarrays conducted up to 1 year after injury. This was compared to a control dataset of 41 skin samples and 95 blood circulating leukocyte samples. Within the trauma population, a total of 187 patients had an associated 785 microarrays from blood circulating leukocytes that was compared against 95 control samples. Data was analyzed by log2 expression differences from control with adjustment of alpha levels for multiple comparisons.

RESULTS: As compared to control samples, burn injury in humans triggered significant upregulation of specific (CYP and LOX) gene transcripts in blood circulating leukocytes and skin biopsies. The genes that were upregulated with burn injury were not conserved across tissue type as a separate set of genes was upregulated in the two different tissue types. Within both the skin and blood circulating leukocyte datasets there was significant gene upregulation up to one year from injury. When the burn group blood circulating leukocyte expression pattern was compared with the trauma group blood circulating leukocytes the genes that were upregulated were not conserved over the two different injury types. The genes that encode for certain Cytochrome P450 enzymes are among the top upregulated genes in each tissue and injury group.

CONCLUSION(S): Thermal injury triggers a massive, selective and sustained alteration in the expression pattern of enzymes capable of forming OLAMs in both skin and blood. The CYP family appears to play a critical role in OLAM formation, thus identifying and targeting specific CYP enzymes responsible for OLAM synthesis after burn injury could provide potential strategies for development of novel analgesics.

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