217

Investigative Studies on the Mechanism of Toxicity of a TNF-Alpha Converting Enzyme (TACE) Inhibitor

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SCH 900567 is a specific inhibitor of TNF-a converting enzyme (TACE) and was developed for the treatment of Rheumatoid Arthritis. In 6-month toxicology studies, SCH 900567 was found to cause toxicity consisting of multifocal inflammatory lesions in both rats and dogs. The nature of the lesions observed with SCH 900567 suggested that this toxicity could be due to the pharmacological effects of TACE inhibition. TACE is a membrane-integrated metalloproteinase involved in cleaving membrane-bound TNF-a, releasing the soluble and active form of this cytokine into circulation. TACE is also involved in cleaving and shedding the ectodomain of the TNF receptors 1 and 2 into the circulation. It was hypothesized that blocking the shedding of these receptors by SCH 900567 contributes to its toxicity through local amplification of TNF receptor-mediated signaling pathways. To investigate this possibility, the effects of SCH 900567 on the expression of these receptors was examined using RAW264.7 cells as well as Kupffer cells isolated from mouse liver and mouse peritoneal macrophages. Results from these studies show that SCH 900567 caused an accumulation of TNF-R2 at the surface of these cells as determined by flow cytometry. Furthermore, RT-PCR and ELISA showed that expression of a number of inflammatory genes including Il-1a, Il-6 and Ptgs2 was enhanced in cells exposed to SCH 900567. These findings are consistent with and proposed to contribute to the development of inflammatory lesions observed in the preclinical safety studies.

218

Acute Central Neurotoxicity of Inhaled Alpha-Diketone Butter Flavoring Compounds in the Rat Brain

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Workers inhaling butter flavoring vapors have increased risk of fixed airways obstruction. Lung disease risk increases with increasing exposure to the alpha-diketone, 2,3-butanedione (diacetyl), a major component of most butter flavoring, and a chemical that also imparts the aroma and flavor of butter to many natural compounds. A related alpha-diketone, 2,3-pentanedione, is a potential substitute for 2,3-butanedione in flavorings. However, a structurally related beta-diketone, 2,4-pentanedione, causes neurotoxicity after subchronic inhalation. To investigate 2,3-pentanedione neurotoxicity, rats inhaled 2,3-pentanedione (270 ppm, 6 hr 41 min) and were sacrificed the following day. No histopathologic alterations were seen in sagittal brain sections stained with H&E or dual immunofluorescence for activated caspase-3, an indicator of apoptosis, and glucose transporter-1, a vascular marker. Apoptotic bodies and caspase-3 expressing cells occurred at similar low baseline levels in control and 2,3-pentanedione-exposed rats. In 2,3-pentanedione-exposed rats, interleukin-6 (IL6) transcripts increased in olfactory bulb, striatum, and hippocampus. Transcripts of claudin-1, a component of blood-brain barrier, increased in olfactory bulb and striatum. Nitric oxide synthase-2 (NOS2) increased in olfactory bulb. To determine if another alpha-diketone caused similar changes, rats were exposed to air or 25, 249 or 346 ppm 2,3-butanedione (6 hr). 2,3-butanedione increased IL6 in olfactory bulb, striatum, and hippocampus at 249 or 346 ppm concentrations. NOS2 was increased in olfactory bulb, striatum, and hippocampus after 346 ppm 2,3-butanedione. Claudin-1 increased in hippocampus at 349 ppm and in olfactory bulb at 249 and 346 ppm. These findings indicate that acute 2,3-pentanedione and 2,3-butanedione exposures alter claudin-1, IL6 and NOS2 expression in brain and suggest the need for detailed neuropathologic studies after longer alpha-diketone exposures.

219

Acquired Multiple Acyl-CoA Dehydrogenation Deficiency (Ethylmalonic-Adipic Aciduria) in Dogs Associated With Striated Muscle Toxicity

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Acquired multiple acyl-CoA dehydrogenation deficiency (ethylmalonic-adipic aciduria) has been reported in horses, but not dogs. Following treatment with a cannabinoid (CB-1) receptor antagonist, dogs (but not rats or monkeys) developed degeneration and lipid accumulation in the skeletal muscle and heart. Muscle degeneration was monitored clinically with muscle enzyme activities: creatine kinase (CK), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Lipid accumulation, confirmed with Sudan Black histochemistry and electron microscopy, was associated with alterations in biomarkers of lipid and carbohydrate metabolism in serum and plasma. These changes included dysregulation of fatty acid oxidation evident by increased serum nonesterified fatty acids (NEFA), cholesterol, triglycerides, phospholipids and plasma acylcarnitines (C2 –C22) and decreased glutamine. There were also changes that indicated increased glycolysis (decreased glucose) resulting in metabolic acidosis (decreased bicarbonate and increased lactate). Increases in urinary metabolites included ethylmalonate and methylsuccinate (indicative of short chain acyl-CoA dehydrogenase [SCAD] inhibition), adipic acid and suberic acid (indicative of medium or very long chain acyl-CoA dehydrogenase [MCAD or VLCAD] inhibition), dimethylglycine and sarcosine (indicative of inhibition of dimethylglycine and sarcosine dehydrogenases), and lactate (indicative of increased anaerobic metabolism) using a combination of NMR and MS/MS analytical methods. Acyl-CoA, sarcosine, and dimethylglycine dehydrogenases are flavin-containing enzymes which converge at the level of electron transfer flavoprotein (ETF)/ETF oxidoreductase. ETF acts as an electron acceptor for mitochondrial flavin-containing enzymes. The combined serum, plasma and urinary findings in these dogs indicate inhibition of fatty acid oxidation at the level of ETF or ETF oxidoreductase. In conclusion, this is the first report of this condition in dogs.

220

Cytokine and Chemokine Changes in the Serum, Blood and Liver of Rats Exposed to Lipopolysaccharide

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Pretreatment with low, non-hepatotoxic doses of lipopolysaccharide (LPS) can predispose rats to develop hepatotoxicity after exposure to drugs that are otherwise not toxic. This phenomenon suggests that a modest underlying inflammatory state in the liver may represent a mechanism for the development of idiosyncratic hepatotoxicity in humans. In this study, our objective was to identify serum and blood biomarkers of exposure to LPS in rats as a first step to identify subjects that may be more sensitive to develop hepatotoxicity with certain drugs. Male Sprague-Dawley rats (n = 3/time point/group) were administered saline or LPS (5 mg/kg) once by tail vein injection and were sacrificed 2, 6 and 24 hours post-administration. Traditional hematology and serum chemistry parameters were evaluated. Serum and liver were profiled for a panel of cytokines and chemokines with the Luminex xMAP platform. RNA samples from blood and liver were profiled with Affymetrix RAE230 microarrays. There was time-dependent moderate neutrophilia associated with moderate lymphopenia. Serum chemistry changes were limited to mild increases in activity of alanine aminotransferase, aspartate aminotransferase, and glutamate dehydrogenase at the 24-hour time point only. Serum TNFa, interleukin (IL)-1, IL-5, IL-6, IL-12 and MCP-1 were markedly increased as early as 2 hours following LPS administration, while in the liver changes were limited to mostly IL-1 and MIP-1. These changes correlated to changes in mRNA levels in the blood and liver. These results indicated that several serum key markers (especially IL-1, IL-6 and MCP-1) would be sufficient to monitor for the presence of an inflammatory hepatic state.