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MEETING REPORT

SOCIETY OF TOXICOLOGY (SOT) 51ST ANNUAL MEETING AND TOXEXPO

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SUMMARY

The Society of Toxicology's (SOT) 51st Annual Meeting and ToxExpo was held in San Francisco, California, on March 11-15, 2012, attracting over 7,200 professionals to discuss new findings in toxicology, toxicity biomarkers and pharmaceuticals. This report reviews presentations focused on therapies for radiation sickness, cancer and fungal infections.

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TOWARD PROTECTION AGAINST IONIZING RADIATION

A study aimed at evaluating the small-molecule compound ISMTR-283 on human myeloid, erythroid and mesenchymal lineages for its potential to protect marrow progenitor cells from radiation damage was discussed by Emer Clarke from ReachBio. Bone marrow (BM), comprising both stem and progenitor cells, as well as marrow stromal/mesenchymal cells, is highly susceptible to radiation damage. Patients treated with medical radiation for leukemia are usually "rescued" using BM from matched donors. However, this is not an option in the event of an accidental radiation exposure. In collaboration with ISM Therapeutics, in vitro studies were conducted in which mesenchymal progenitor cells were treated with the drug (0.1-0.4 μM) for 2 hours prior to radiation exposure of 2 or 4 Gy. Following culture for 14 days, cells were scored microscopically. Mean scores for cells exposed to no radiation, 2 Gy radiation, 4 Gy radiation, 2 Gy radiation plus ISMTR-283 (0.5 μM) and 4 Gy radiation plus ISMTR-283 (0.5 μM) were 170, 109, 49, 138 and 75, respectively. It was found that a concentration of 0.4 μM of drug protected erythroid and myeloid progenitor cell proliferation when administered prior to exposure to 2 but not 4 Gy radiation.

Further studies found that treatment of BM cells with ISMTR-283, 2 and 24 hours following radiation exposure, resulted in a protective effect. Mean scores were 16 and 27, respectively, for cells exposed to 4 Gy radiation and those treated with the drug (0.1 μM) 24 hours after radiation exposure. There were differences in the protective effect with time; the greatest effect was observed with prior treatment of the drug. These data suggest that the agent can protect BM cells from radiation exposure when administered prior to or following exposure to ionizing radiation. They also demonstrated a protec-

tive effect of ISMTR-283 on BM stem and progenitor cells when administered both prior to or following ionizing radiation. Thus, ISMTR-283 may provide a prophylactic or treatment option for accidental radiation exposure.

NANOPARTICLES FOR THE TARGETED TREATMENT OF CANCER

Sandra Casinghino (CytImmune Sciences) discussed clinical studies of CYT-6091, a nanoparticular formulation of recombinant human TNF-α bound to the surface of PEGyated colloidal gold particles, for the treatment of cancer. A phase I study found that CYT-6091 effectively targeted the tumor, which was confirmed by the presence of gold nanoparticles in patient biopsies. The drug had no dose-limiting toxicities at the highest dose of 0.6 mg/m^2, equivalent to > 1 mg TNF-α per dose, and did not induce an anti-TNF response. The drug caused fever, which was eliminated by pretreatment with acetaminophen/indomethacin, and acute lymphopenia that resolved within 24 hours. A phase II study will assess the efficacy of CYT-6091 in combination with docetaxel administered systemically.

PEDIATRIC STUDIES OF ANIDULAFUNGIN AND VORICONAZOLE

The ergosterol synthesis inhibitor voriconazole (Vfend®) and the 1,3-β-glucan synthesis inhibitor anidulafungin (Eraxis™, Ecalta®) are indicated for the treatment of fungal infections, including *Aspergillus* and *Candida* infections. The combination of voriconazole and anidulafungin is currently in phase III trials for the treatment of *Aspergillus* infection. Studies are ongoing to investigate the pediatric administration of voriconazole alone or in combination with anidulafungin. Christopher J. Bowman (Pfizer) discussed in vivo toxicology studies, which investigated the combination administration of anidulafungin and voriconazole as part of the voriconazole pediatric investigation plan. Juvenile Fischer rats were administered anidulafungin or voriconazole either alone or in combination (once daily) from postnatal day (PND) 21 to 56, with a recovery period to PND 84. The doses for both agents were those of the adult rat no observed adverse event level (NOAEL) or above. Systemic exposure at juvenile rat NOAEL was comparable to that previously found for adult rats. Systemic exposure was also comparable to anidulafungin exposure alone when evaluated in a previous study in juvenile rats. Adverse events associated with anidulafungin included transient, reversible reductions in body weight, hematology, serum chemistry, liver weight and minimal liver changes. A voriconazole-associated increase in γ-glutamyltransferase was observed in female rats only. It was found that the juvenile rats were not more sensitive to each drug alone compared with adult rats administered the agents alone. Furthermore, there were no new, additive or synergistic toxicities observed with the combination dose. This study supported pediatric clinical studies for the coadministration of anidulafungin and voriconazole.

TWO NOVEL RADIATION COUNTERMEASURE THERAPIES: YEL-001 AND YEL-002

The recent Fukushima accident has highlighted the need to develop novel therapies against radiation exposure. Robert Schiestl (University of California Los Angeles) presented data on the development of two novel radiation countermeasure therapies: Yel-001 and Yel-002. These compounds are small, biologically active, drug-like molecules that were shown to reduce radiation-induced cyto- and genotoxicity in yeast. In vitro studies found that adding either Yel-001 or Yel-002 to irradiated cultures reduced cell death and genomic instability. Also, these compounds were found to be effective at reducing lethality to an average of 25% in vivo following an LD100/30 dose of ionizing radiation, following administration at 24 hours postexposure and subsequent injections at 48, 72, 96 and 120 hours. It was mentioned that treatment with Yel-001 and Yel-002 reduced radiation-induced leukemia from 90% to 50% and 40%, respectively. In the case of Yel-002, the treatment after radiation exposure improved the recovery of the hematopoietic cells after sublethal exposures. Furthermore, the drugs reduced the rate of spontaneous leukemia formation from 10% to 0%. The in vitro and in vivo studies performed have shown an acceptable toxicity profile. These results support Yel-001 and Yel-002 as potential novel radiation countermeasure therapies.

OCTN2 IN DRUG-INDUCED TOXICITY

Peter Calcraft (AstraZeneca) reported the results of a study on drug-induced toxicity. It is well known that cardiotoxicity is one of the main reasons leading to discontinuation of a candidate drug's development. Several mechanisms may be involved, including the interaction of drugs with transporter proteins, such as members of the solute carrier family. In the study presented, the organic cation/carnitine transporter 2 (OCTN2), an Na^+-dependent, high-affinity carnitine transporter, was evaluated. Carnitine is essential for the beta-oxidation of fatty acids, which are the main source of energy in the heart, and homozygous mutations in the human *OCTN2* gene lead to primary systemic carnitine deficiency and hypertrophic cardiomyopathy. Drug interactions with OCTN2 may modify drug disposition and carnitine homeostasis. Using HEK-293 cells expressing either OCTN2 (HEK-OCTN2) or an empty vector (HEK-control), 46 known cardiotoxins, 19 non-cardiotoxins and 2 nontoxic control compounds were evaluated for OCTN2 interaction. The compounds were tested either at a concentration of 100 μM or a concentration range of 0.1-100 μM to determine the IC_{50}/EC_{50} values. Cytotoxicity was determined at 40 minutes and 24 hours using the Promega Cell Titre GloTM ATP viability assay. A total of 14 of the 65 compounds tested inhibited OCTN2-mediated carnitine uptake, with a higher prevalence of inhibitors observed among the known cardiotoxins (28%) than non-cardiotoxins (5%), with 7 of the cardiotoxins exhibiting an IC_{50} value < 100 μM. After 24-hour incubation, 30 of the 74 compounds tested exhibited cytotoxicity in the HEK-OCTN2 cells. The HEK-OCTN2 and HEK-control cells were equally sensitive to the cytotoxic effects of all compounds tested, indicating that these compounds are unlikely to be substrates of OCTN2. In conclusion, the lack of OCTN2-dependent drug-induced cytotoxicity in HEK-OCTN2 cells suggests that OCTN2 is unlikely to act as a polyspecific drug transporter. It was suggested that in vitro evaluation of OCTN2–drug interactions may be useful for cardiotoxic hazard identification.

AN IN VITRO APPROACH TO IDENTIFY DRUGS THAT CAUSE SEVERE DILI

Severe drug-induced liver injury (DILI) is another reason that drug development is discontinued in the early phases. Jie Zhang

(National Center for Toxicological Research/FDA) presented an in vitro approach in primary human hepatocytes to identify drugs causing DILI. In addition, individual mechanistic endpoints were evaluated to associate mechanisms underlying severe DILI. Primary human hepatocytes represent the closest in vitro model to human liver due to the presence of a full complement of xenobiotic metabolizing enzymes. The cells were treated with various drugs at seven concentrations, and luminescence and fluorescence assays were performed at 24 hours to measure ATP content, glutathione depletion, caspase-3 activity and generation of reactive oxygen species (ROS). The drugs tested were divided into three groups based on the intensity of severe DILI drug response in the ROS assay; each group was then associated with specific DILI types. This in vitro screening method was proposed as a mechanism to first distinguish severe DILI drugs and then to allow prioritization of drug candidates based on DILI type.

ZEBRAFISH AS A MODEL TO EVALUATE TYROSINE KINASE INHIBITOR CARDIOTOXICITY

The many advantages that the zebrafish model offers include small size, ease of manipulation and embryo transparency, allowing evaluation of different internal organs with no need for invasive methodologies, as well as the quick development and low cost of maintenance. Olaia Holgado (Biobide) presented a study that used an automatic device to evaluate the cardiotoxicity of a series of tyrosine kinase inhibitors in a zebrafish model. The early detection of cardiotoxicity, along with the ease and reduced cost of this model, may considerably optimize the drug development process. In order to validate the quality of the analysis system and the zebrafish model, a panel of previously described, blind-coded tyrosine kinase inhibitors was used. The results showed that all compounds that were reported to be cardiotoxic in the clinic, with the exception of pazopanib, were also positive for cardiotoxicity in the zebrafish assay. Thus, this automated method may offer key data to identify potential cardiotoxic drugs at a very early stage of development, making the selection of new non-cardiotoxic candidates easier.

NO PRODUCTION AS A BIOMARKER FOR PDE4 INHIBITOR-INDUCED VASCULAR INJURY

Phosphodiesterase PDE4 inhibitors are potential antiinflammatory and antiasthmatic drugs, but often the finding of drug-induced vascular injury (DIVI) in preclinical models has led to discontinuation of development. Therefore, the identification of a reliable biomarker would provide an important reference for the future development of PDE4 inhibitors. Grainne McMahon Tobin (Center for Drug Evaluation and Research/FDA) presented a study on the role of nitric oxide (NO) as such a biomarker. Previously, the effect of the PDE4 inhibitor CI-1044 on DIVI was investigated in a rat model, and it was found that CI-1044 treatment led to elevation of serum nitrite, resulting in increased vascular injury in the mesentery tissue. In contrast, the addition of an NO synthase (NOS) inhibitor prevented DIVI, suggesting a correlation between serum NO levels and the extent of vascular lesions. In the present study, the level of endothelial cell-specific NOS (eNOS) phosphorylation was observed to elucidate the mechanism by which PDE4 inhibition leads to the elevation of NO and DIVI. Rats were treated with CI-1044, and serum and mesentery tissue collected over a 24-hour time period after the last dose. The results suggested that treatment with CI-1044 led to modulation of the phosphorylation of eNOS, thus increasing NO, as measured by serum nitrate levels. In conclusion, this study supports the usefulness of NO as a biomarker for PDE4 inhibitor-induced DIVI.

DISTRIBUTION, METABOLISM AND EXCRETION RESULTS FOR A NOVEL ANTI-HCV THERAPY

Hepatitis C virus (HCV) infection targets the liver and chronic infection causes liver damage, which can eventually lead to hepatocellular carcinoma. Current therapy regimens are focused on inhibition of viral replication through different mechanisms; however, the preclinical candidate ITX-7650 (iTherX Pharmaceuticals) acts to inhibit the cellular entry of HCV via binding of cells expressing the scavenger receptor class B member 1 (SRB1). Carol E. Green (SRI International) discussed the progression of this novel compound through excretion, distribution and metabolic studies in Sprague-Dawley rats. Both male and female rats were administered a single dose of [^{14}C]-ITX-7650 (10 mg/kg i.v. or 30 mg/kg p.o.). Tissues (brain, kidney, liver, lung and spleen), urine and feces were collected up to 24 (i.v.) or 48 (p.o.) hours post-dose for analysis of total radioactivity. Results showed that excretion via both the urine and feces was involved in the elimination of radioactivity for both dose routes. The percentage of the total dose of radioactivity recovered was comparable for urine and feces for the i.v. group (7.3-15.5%), while significantly more radioactivity was recovered in the feces (36-45.2%) than the urine (13-14.5%) for the p.o. group.

The greatest concentration of radioactivity was found in the liver, with approximately 7-10% of the total radioactive dose found. Tissue concentrations peaked at 1 and 2 hours, respectively, for i.v. and p.o. doses, and declined with time. Male lung and spleen concentrations showed peak concentrations at later time points for both routes of administration: 16.7 and 11.9 µg-equivalent (eq)/g at 4 hours, respectively, for i.v., and 33.6 and 24.1 µg-eq/g at 6 hours, respectively, for p.o. Radioactivity was threefold higher in the tissues compared with that in plasma, and plasma concentrations were higher than in the brain. Plasma and blood levels of [^{14}C]-ITX 7650 radioequivalents were higher for the p.o. group than for the i.v. group. Mean ratios of 0.55-0.82 for blood:plasma radioequivalent concentrations were determined and AUC values were approximately 220 and 50 h.µg-eq/g, respectively, for the p.o. and i.v. routes. Half-life values were approximately 10 and 16.5 hours, respectively for the i.v. and p.o. routes. It was found that female rats had greater plasma exposure to the radioequivalents than male rats. A number of rat in vivo metabolites were formed, with one metabolite representing ≥ 24% of the total peak area, as determined by HPLC studies. One metabolite in the urine was found at higher concentrations than [^{14}C]-ITX-7650. In addition, in vitro studies with liver microsomes from different species showed that [^{14}C]-ITX-7650 was extensively metabolized in dogs and cynomolgus monkeys.

UCB-1017471 – A NEW ANTIEPILEPTIC CANDIDATE

Olympe Depelchin of UCB Pharma presented preclinical data on the company's novel antiepileptic drug candidate UCB-1017471. It was previously found that the drug induced hepatic porphyria, brown pigment deposits suggested to consist of hepatic porphyrins, in dogs but not rats. This study aimed to understand why this toxic event did

not occur in rats and predict the potential incidence of hepatic porphyria in humans. Thus, the effort was focused on elucidating the mechanisms involved and probing the assumption of a dog-specific reactive metabolite that would disrupt the heme biosynthetic pathway. Beagle dogs were administered the drug (200 mg/kg/day p.o.) for 4 weeks and liver samples were analyzed for cytochrome P450 (CYP) activities, porphyrin levels, ferrochelatase activity and porphyrin adducts. Hepatocytes from rats, dogs and humans were incubated with [^{14}C]-UCB-1017471 and the metabolites were analyzed by radio-HPLC. It was found that total porphyrin levels were increased in the liver of treated dogs. However, this was not observed in the liver of rats treated with the highest dose. A 75% decrease in the activity of the enzyme ferrochelatase was also observed in dog liver. In vitro and in vivo studies found that the main metabolite in dogs resulted from beta-oxidation of the butyramide side-chain of the drug via CYP2B11, and this metabolite was not found in rat or human hepatocytes. It was suggested that this metabolite was likely to cause the dog-specific protoporphyria due to binding to the heme moiety of the CYP, which then inhibits ferrochelatase, allowing accumulation of protoporphyrin in the liver. Because this bioactivation was limited to dogs, it was suggested to be unlikely to cause clinical concern.

CELGENE PRESENTS THE TOXICOLOGICAL PROFILE OF CC-485118

Inhibition of tyrosine-protein kinase SYK, which has a critical role in downstream signaling from immunoglobulin receptors involved in the inflammatory process, is one of the mechanisms for the potential treatment of autoimmune diseases. Dinah Misner (Celgene) discussed an in vivo toxicology study for the company's small-molecule SYK inhibitor CC-485118 (CC-118). The compound had a potent effect against SYK, with an IC_{50} value of 7 nM, and some potency toward tyrosine-protein kinase JAK2 and colony-stimulating factor 1 receptor (CSF-1 receptor) targets, with IC_{50} values of 30 and 28 nM, respectively. A 7-day, repeat-dose toxicology study was conducted in both male and female cynomolgus monkeys. Male and female cynomolgus monkeys were administered the drug (0, 100, 500 and 1000 mg/kg/day) for 7 days. Doses of 500 and 1000 mg/kg/day were not tolerated, with deaths and/or early sacrifice by days 3 to 7. The 100 mg/kg/day dose was well tolerated, with higher AUC and C_{max} levels upon repeat dosing. Hematological parameters were also measured, showing a decrease in red blood cell count, hemoglobin, hematocrit, lymphocytes and eosinophils, and an increase in neutrophils and platelets at ≥ 100 mg/kg/day. Alanine and aspartate transaminase serum levels were increased, while alkaline phosphatase, calcium and phosphorus levels were decreased at the same doses. Adverse events of discolored skin and hunched posture were noted in both male and female monkeys. The red discoloration was observed in a number of organs in all dose groups and myelosuppression was observed at doses of 500 mg/kg/day. Multiorgan acute hemorrhage, gastric erosion and lymphoid depletion were also observed at the 100 mg/kg/day dose.

DISCLOSURES

The author states no conflicts of interest.

The website for this meeting can be found at http://www.toxicology.org/ai/meet/am2012/am.asp.