Spring 2013

IN FOCUS
ITR Newsletter

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Society of Toxicology Annual Meeting and ToxExpo 2013

Booth 1120

ITR Canada will be present at the 52nd Society of Toxicology Annual Meeting and ToxExpo held at the Henry B. Gonzalez Convention Center in San Antonio, Texas on March 10-14, 2013.

Drop by booth 1120 to meet one of our marketing or toxicology specialists and learn more about the services we have to offer.

Not going to SOT?
Visit our website at:
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INFORMATION ON UPCOMING SOT POSTERS

Growth Hormone and IGF-1 Measurements in Beagle Dogs by ELISA: Assay Implementation and Variations in Baseline Levels

Measurements of GH and IGF-1 in Beagle dogs are complicated by several technical and biological issues. In this poster, ITR presents data from ELISA methods that were specially adapted and qualified at our facility for the quantification of GH & IGF-1 in canine serum. During assay implementation and routine use, several observations have been made with these hormone baseline levels and some biological & technical parameters.

Beagle dog serum was obtained from males and females, originating from two different breeders; Marshall and Covance. Samples were tested from dogs housed at three different preclinical testing facilities; ITR and two other undisclosed facilities ("Lab B" & "Lab C"). A commercial GH ELISA kit designed for rat/mouse GH and another commercial human IGF-1 kit were adapted for use with dog serum samples. Typical basal GH levels in Beagle dogs vary from < 6.25 to 40 ng/mL. No significant differences were observed in the GH basal level between different genders, breeding source or test facility. However, GH levels generally increased with higher body weight and age. GH varied between individuals of the same study. Typical basal IGF-1 levels in Beagle dogs varied greatly, from < 42 to 150 ng/mL (ex. from Mashall-bred dogs housed at ITR), while the normal range increase to 150-500 ng/mL with Covance-bred dogs housed at "Lab C". Age and body weight only had minor impact on the IGF-1 basal levels, while a gender difference was only seen within the Covance-bred dogs housed at "Lab C".

The GH/IGF-1 data gathered internally from several preclinical studies with Beagle dogs of various origins have shown that basal IGF-1 levels can vary significantly depending on the dog breeding source and housing facility, in addition to age and body weight. It was also found that due to the natural cyclic activity of GH, multiple pre-dose samplings are useful, with the last pre-dose sample being ideally taken as close as possible to dosing with the test item.

Use of an In Vitro Flow Cytometric Method as a Replacement for Live Animal Experimentation

Animal experimentation is indispensable to drug development however a challenging goal for the preclinical research industry is to apply the 3Rs of animal experimentation.

ITR's aim was to develop a method to evaluate the potency of two test items to trigger intracellular events in lymphocyte subsets expressing or not the IL-2 high affinity receptor subunit (CD25). As one of the major alternatives to *in vivo* animal testing is *in vitro* cell-based assays, we have developed an *in vitro* flow cytometry method using cynomolgus peripheral blood mononuclear cells (PBMC) to replace the use of living monkeys in our experimentations.

Cynomolgus PBMC were stimulated with increasing doses of the test items under evaluation. The PBMCs were then stained using conjugated monoclonal antibodies specific to different lineage markers such as CD3, CD4, CD8 and CD25 to allow distinction between the different T lymphocyte subsets. In addition, in order to evaluate the potency of the test items to differentially stimulate CD25+ and CD25- lymphocytes, we have evaluated the activation of a downstream mediator of the IL-2 receptor signaling by measuring the phosphorylation of the Signal Transducer and Activator of Transcription (Stat5). Intracellular staining using a Phospho-Stat5 (pStat5) specific antibody was thus performed and data was acquired by flow cytometry. The pStat5 signal fold-increase was plotted against the test items concentration and the half maximal effective dose (EC50) was determined. The results obtained showed that the calculated EC50 for the $CD4^+CD25^+$ was of approximately 100 to 1000 times lower compared to $CD4^+CD25^-$ cells and $CD8^+$ cells. In addition, when comparing the potency to the two test articles to stimulate $CD4^+CD25^+$ cells, a 2-log difference was observed between calculated EC50.

By developing an innovative approach, ITR was able to apply the 3R principles and replace *in vivo* testing by an *in vitro* cell-based assay. The method allowed comparing the potency of two test items to signal through the IL-2 receptor in different lymphocyte subsets.

Research on peptide analysis using dry blood spot technology combined with mass spectrometry for toxicology studies: a practical approach for minimizing number of animals in line with 3R concepts

ITR Canada will be presenting data obtained using a recently developed assay for the quantitation of the calcitonin-salmon peptide using dry blood spots (DBS) at the upcoming SOT 2013 conference in San Antonio, Texas. Actual in-vivo data was obtained on the peptide by subcutaneous injection to a CD-1 mouse, and 10 mcL of blood was collected by tail clipping for a total of 18 timepoints. The blood was immediately transferred to Whatman 903 filter paper and allowed to dry before placing with desiccant. The dry blood samples were then punched out, and calcitonin-salmon was extracted from the spots and analyzed using electrospray ionization on a triple quadrupole API 4000 mass spectrometer monitoring the parent and fragment ions of both calcitonin-salmon and its analog internal standard.

Results clearly show that this approach provides a reliable and complete toxicokinetic profile based on the 18 timepoints collected from a single mouse. The TK profile and information on the assay will be presented at the SOT conference. This would lead to a significant reduction of mice required for a study and is well aligned with the 3R concept.

If you are interested in further information on any of these poster or would like to obtain a copy of a posters, please contact us at marketing@itrlab.com.

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