**INTRODUCTION**

Intrathecal administration and sampling continues to be critical in non-clinical development of many pharmaceuticals to allow for their administration and evaluation. A surgically implanted catheter offers numerous advantages for administration of pharmaceuticals required to be delivered across the blood-brain barrier or to allow sampling of cerebral spinal fluid (CSF) to investigate their pharmacokinetics. Historically, administration of compounds in the intrathecal space or sampling of CSF has been invasive and the use of anesthetics was required causing possible interference with results and requiring a larger number of animals for sampling. In alignment with the 3Rs (reduction, replacement, refinement) we have developed a vascular access port based method to allow long term intrathecal administration of test compounds and/or of CSF. In addition, a modified functional observation battery (FOB) often conducted as part of the ICH 37A safety pharmacology core battery was developed for the evaluation of neurological and/or behavioral changes in non-human primates.

**MATERIALS AND METHODS**

A total of 3 female Cynomolgus monkeys originating from China were assigned to the study, ranging between 5 and 6 years of age with body weights from 2.8 to 3.4 kg at the onset of the study. Under deep anesthesia, a hemi-laminectomy was performed in the vertebral lamina (L3) and a cerebrospinal fluid catheter was inserted into the intrathecal space and advanced cranially approximately 10 cm. The catheter was subcutaneously tunneled to the interscapular site where the catheter was connected to a vascular access port. The animals were first used to study the pharmacokinetics of a candidate drug by CSF sampling (0.2 mL) at 10 timepoints over a period of 36 hours. The catheter, accessed via the port, was locked with phosphate buffered saline (PBS) which was removed prior to sampling and replaced following sampling. Following the end of the first (pharmacokinetics) phase and a washout period the animals were fitted to a vascular access port/catheter. The animals were first used to study the pharmacokinetics of a candidate drug by CSF sampling (0.2 mL) at 10 timepoints over a period of 36 hours. The catheter, accessed via the port, was locked with phosphate buffered saline (PBS) which was removed prior to sampling and replaced following sampling. Following the washout period, the 28 day intrathecal infusion was administered. At the end of the 28 day infusion period, the catheter was disconnected across the blood-brain barrier or to allow sampling of cerebral spinal fluid (CSF) to investigate their pharmacokinetics. Histologically, administration of compounds in the intrathecal space or sampling of CSF has been invasive and the use of anesthetics was required causing possible interference with results and requiring a larger number of animals for sampling. In alignment with the 3Rs (reduction, replacement, refinement) we have developed a vascular access port based method to allow long term intrathecal administration of test compounds and/or of CSF. In addition, a modified functional observation battery (FOB) often conducted as part of the ICH 37A safety pharmacology core battery was developed for the evaluation of neurological and/or behavioral changes in non-human primates.

**RESULTS**

In the preliminary pharmacokinetic study, all animals were patent for the duration of the sampling period and all CSF samples were collected up to 36 hours following administration. Following a washout period, the 28 day intrathecal infusion administration was performed. At the end of the 28-day infusion period, no changes in body weight or food consumption were observed. There were no pronounced effects on general behavior or the various neurologic parameters evaluated during the Function Observation Battery. Macroscopically, procedure-related changes included catheter-associated dark area in the skin at the entry of the catheter in one female or secondary regenerative and degenerative changes including enlarged iliac lymph node and small thymus, respectively in a second female. In all 3 animals, minimum to mild catheter-associated chronic granulomatous inflammation was associated with the catheter in the subarachnoid space at the injection site, next to the spinal cord and spinal nerve roots. The granulomatous inflammation was characterized by thin bundles of fibrovascular connective tissue admixed with numerous foreign body multinucleated syncitial giant cells. Similar moderate catheter-associated chronic granulomatous inflammation was also noted along the catheter track beside the vertebral body and dorsal spinal process below the lumbar muscles. In addition, one monkey had minimal infarction of the cervical spinal cord meninges and mild infarction of the brain meninges (forebrain, midbrain, cerebellum, and medulla). Similar mild infarction of the meninges was noted around the spinal cord at the injection site associated with the intrathecal catheter. The infarction was characterized by a perivascular mixed inflammatory infiltrate of neutrophils, lymphoplasmacytic, and histiocytic cells, distending the meninges, predominantly the pia. Changes occasionally noted in bone marrow and lymphoid tissue of these animals were considered to be degenerative or regenerative changes secondary to the procedure, including bone marrow myeloid hypercellularity; lymphoid hypercellularity in the thymus, correlating to macroscopic findings (small and meseptic and mandibular lymph nodes; lymphoid hypercellularity with concurrent histiocytosis in the iliac lymph node, correlating to enlagement noted macroscopically and chronic inflammation with hamohagence and foreign body granuloma (suture material) in the skin and subcuts around the catheter. The inflammatory or regenerative histopathology findings were generally considered to be an adaptive response to the presence of a foreign body and allow for the identification and distinction from test item related changes. Despite compression of the spinal cord, there was no indication of degenerative changes and no corresponding neurological clinical findings. The minimal to mild degree of the findings confirms the potential utility of this approach in toxicology studies or other investigations requiring 28 days administration, or potentially even longer periods of administration.

**CONCLUSION**

In alignment with the 3Rs (reduction, replacement, refinement), our laboratory has demonstrated that CSF sampling and continuous infusion for 28 days to Cynomolgus monkeys is feasible using a vascular access port based approach. Minimal to mild catheter-associated chronic granulomatous inflammation was associated with the catheter in the subarachnoid space at the injection site, next to the spinal cord and spinal nerve roots. Similar moderate catheter associated chronic granulomatous inflammation was also noted along the catheter track beside the vertebral body and dorsal spinal process below the lumbar muscles and was not considered to be an adaptive response to the presence of a foreign body (catheter). Our results indicate that the improved method may be appropriate for long term toxicological, neurologic and pharmacokinetic evaluations in the Cynomolgus monkey allowing for the use of a reduced number of animals.

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