Attachment

Column E Explanation Form

This form is intended as an aid to completing the Column E explanation.
Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. IACUC approved protocol number and date: 15-04, 2/2/15
2. Number of animals used under Column E conditions in this study. 83
3. Species (common name) of animals used in this study. Guinea pig
4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (Cut/paste from the approved docket and or amendments).

Guinea pigs are the major animal infection model established for the human herpes virus-2 (HSV-2) to evaluate protective efficacy of potential HSV-2 vaccines and therapeutic test compounds. The Hartley guinea pig model was chosen to observe clinical signs, body weight and mortality post HSV-2 infection and treatment with potential therapeutic test substances. Post infection, animals were monitored daily for clinical signs of disease. The viral infection of guinea pigs with HSV-2 can have varying clinical readouts from no clinical signs to advanced morbidity. To evaluate the viral disease, we followed the established published literature for viral clinical scoring listed in the table below. Animals that reached a moribund state or exhibit a score of (4) or a loss of 30% body weight were immediately euthanized.

Observation	Score
No clinical signs of disease	0
Vaginal erythema	1
Single to a few modest herpetic lesions	2
Large or fused vesicles	3
Severe vaginal ulceration and paralysis	4
Found dead	5

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Analgesics may interfere with immune responses and physiological processes to the infection and the display of disease symptoms and interfere or obscure immune protection results. The measurement of clinical scores is vital to the determination of the efficacy of the vaccine. Therefore, we did not to use analgesics to avoid interference with test results. For example, morphine is known to suppress the innate immune system and reduce severity of HSV-1 infection; the opioid derivative buprenorphine may have similar effects; the cyclooxegenase-2 (Cox-2) pathway is required for efficient herpes virus replication: NSAIDS down-regulate this pathway and Cox-2 inhibitors limit the replication of herpes viruses; Lidocaine destabilizes the HSV virion. Therefore, animals exhibiting pain or unrelieved distress (reached a moribund state or exhibit a score of (4) or a loss of 30% body weight were humanely euthanized.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency___None_____CFR_____

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1. IACUC approved protocol number and date: 14-13, approved on 10/03/14
2. Number of animals used under Column E conditions in this study: 28
3. Species (common name) of animals used in this study: Rabbit
4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (Cut/paste from the approved docket and or amendments). The objective of this study was to see if test articles are effective in reducing the signs and symptoms associated with ocular allergic conjunctivitis in rabbits caused by microsphere administered to both eyes. Rabbit eye gives good parallel to the human eye model and there is historical reference data accumulated over decades for such experiments.
The microsphere injections cause mast cells to release histamine which causes inflammation- the main component of concern in allergies. Inflammation causes some discomfort and pain. This animal model is then used to study the potential treatments for reducing histamine release.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. The pain and distress associated with ocular allergic reactions was not remedied with analgesics because these have anti-inflammatory effects and which could have altered the disease course and thus confounded the results of test articles under investigation.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency___None_____CFR_____

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1. IACUC approved protocol number and date: 15-16, 11/12/15
2. Number of animals used under Column E conditions in this study. 19
3. Species (common name) of animals used in this study. Common Marmoset
4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (Cut/paste from the approved docket and or amendments).

Multiple sclerosis (MS) is an autoimmune disease affecting approximately 250,000 - 350,000 people in the US where the immune system targets a number of central nervous system (CNS) antigens, such as myelin oligodendrocyte glycoprotein (MOG). An initial loss of tolerance to these self-antigens results in inflammation and neurodegeneration. To study the disease and potential treatments, experimental autoimmune encephalomyelitis (EAE) which mimics the disease, is caused by injecting 100µg rhMog/ CFA in non-human primate models. Our model of EAE was selected based on methods and results from the published literature, with consideration for the number of immunizations needed, reliability of incidence of disease, and established humane endpoints. Animals were assessed for EAE onset twice daily. Humane end points were exercised to end pain/distress and suffering caused by the disease.

To evaluate the disease progression, we followed the established published literature for clinical scoring listed in the table below:

Clinical Scoring
0 = No clinical signs
0.5 = apathy, loss of appetite, vomiting, altered walking without ataxia
1 = lethargy, anorexia, tail paralysis, tremor
2 = ataxia, optic disease
2.5 = incomplete paralysis of one (monoparesis) or both sides (paraparesis), sensory loss, brainstem syndrome
3 = complete paralysis of one (hemiplegia) or two sides (paraplegia)
4 = complete paralysis (quadriplegia)
5 = moribund or spontaneous death attributable to EAE

Note: A score ≥ 2 reflects an overt neurological deficit. Humane endpoint is defined as a clinical score ≥ 3.

5. Provide scientific justification why pain and/or distress could not be relieved.
State methods or means used to determine that pain and/or distress relief would interfere with test results.

In experimental studies of autoimmune conditions inflammation plays important causative role. This ongoing immune response may be compromised by the use of anti-inflammatory agents (e.g., NSAIDs). Therefore, such care was withheld during the course of the study. As part of the autoimmune disease, some potential pain and distress may be observed. Humane endpoints, were, therefore, pre-established and observed throughout the study. Humane endpoints were assessed as clinical score of ≥ 3, progressive body weight loss (≥ 20%), and clinical presentation. Where it was possible without confounding the study, supportive care was provided per veterinary advice in consultation with the PI. If such measures were not compatible with study objectives, the animal was humanely euthanized with the approval of the PI and veterinarian.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency___None_____CFR_____