University of California, San Francisco
USDA Registration Number: 93-R-0440

Column E Explanation

The University of California, San Francisco is committed to using laboratory animals in such a way as to minimize pain and discomfort. Attached are explanations of the procedures with the potential for producing pain or distress in animals covered by Subchapter A – Animal Welfare and reported in Column E during the period of 10/1/2021 through 9/30/2022 and the reason anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretations of the research.

1. Number of animals used in this study: 4

1a. Species (Common name) of animals used in the study: Syrian Hamster

1b. Explain the procedure producing pain or distress:

To test a new antiviral therapy for SARS-CoV-2, Syrian hamsters are used as the *in vivo* model of SARS-CoV-2 infection. Human SARS-CoV-2 isolates replicate efficiently in the lungs of hamsters, causing pathological lung lesions following intranasal infection, and these lesions share characteristics with SARS-CoV-2–infected human lungs. SARS-CoV-2–infected hamsters mount neutralizing antibody responses, are protected against subsequent rechallenge with SARS-CoV-2, and respond to convalescent serum treatment. Collectively, these findings demonstrate that the Syrian hamster model is a useful model to understand SARS-CoV-2 pathogenesis and test vaccines and antiviral drugs.

Antiviral therapy will be administered before, during or after virus infection, and the infection will be tracked in animals for up to 2 weeks to assess signs of infection, histology and molecular biology. Animals inoculated with antiviral therapy are expected to develop a milder disease and have lower viral loads than control animals. Syrian hamsters typically recover from SARS-CoV-2 infection, but there is potential for distress with respiratory pathogen infection. Signs of illness may include malaise, fatigue, and possibly respiratory distress.

1c. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and distress would interfere with test results. (For federally mandated testing, see item 1d.)

Pain-relieving drugs or other methods for relieving pain may interfere with the antivirals being tested. However, animals will be monitored twice daily and euthanized if moribund.

In this study we are unable to alleviate the disease manifestations potentially associated with SARS-CoV-2 infection as treatment would interfere with the outcome of the study, the goal of which is to better understand the pathogenesis of this virus and to establish a model for preclinical evaluation of vaccines and antivirals, and may render the data scientifically useless. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis and stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise which would likely not be alleviated by opioids.

Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production (1, 2). Moreover, opioids can suppress Natural Killer cell activity (3). Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release (4, 5) and respiratory depression (6). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (7), inhibit interferon-alpha release from dendritic cells (8), and increase the synthesis and release of IL-10 from human macrophages (9). The analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease (10).

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1d. What, if any, Federal Regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number and the specific section number.

N/A

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1. Number of animals used in this study: 102

1a. Species (Common name) of animals used in the study: New Zealand White Rabbit

1b. Explain the procedure producing pain or distress:

Staphylococcus aureus, *Pseudomonas aeruginosa,* and *Klebsiella pneumoniae, and Acinetobacter baumannii* cause pneumonia. Rabbit models reproduce many of the important salient features of these microorganisms as they occur in humans*. S. aureus, P. aeruginosa,* and *K. pneumonia* are also natural pathogens of rabbits as they are known to cause disease outbreaks in rabbit farms. The long-term goal is to dissect mechanisms by which these agents cause different biofilm-related infection types using rabbit models of pneumonia. To establish pneumonia, endotracheal tube placement and agent administration in the trachea are performed on an anesthetized animal. Pneumonia and ventilator-associated pneumonia caused by these bacteria are particularly severe infections, both in rabbits and in humans.

1c. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and distress would interfere with test results. (For federally mandated testing, see item 1d.)

Pneumonia and ventilator-associated pneumonia caused by these bacteria are particularly severe infections, both in rabbits and in humans. Interventions short of euthanasia (e.g., administration of antibiotics) would either invalidate the model by altering the natural progression of the disease which is under study, alter the pathophysiology or host response. Deaths are not unexpected in these infection models, and they cannot be accurately or reliably predicted in the individual animals. Animals may manifest >15% weight loss, become moribund or otherwise unable to access food and water. Animals are monitored at least twice daily and any animal with these findings will be humanely euthanized.

1d. What, if any, Federal Regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number and the specific section number.

N/A