

UTAH STATE UNIVERSITY
REGISTRATION NUMBER: 87-R-0002
EXPLANATION FOR THE USE OF ANIMALS LISTED IN COLUMN E

The 2022 Annual Report of Research Facility for Utah State University indicates that 130 guinea pigs have been used in studies involving pain and distress without analgesic or other palliative care. All such work conducted at this institution involves experiments examining virus infections in animal models, and associated work to evaluate potential antiviral therapeutic agents. This involves three related categories of experiments, virus titration studies in animals, dose range finding studies for novel therapeutic agents or treatments, and antiviral experiments in which virus infected animals are treated with potential antiviral agents. In the case of all experiments involving viral infections animals are monitored several times daily during virus infection. Based upon previous experience with virus infection models specific criteria for each viral disease indicating likely death have been identified. Animals meeting these criteria are euthanized to minimize pain and distress.

The purpose of preliminary virus titration studies is to identify the minimum viral dose required to produce mortality in approximately 90% of the animals inoculated. Titration experiments are only necessary when evaluating new virus stocks or new virus strains, or new animal models such as different animal strains. As such titration experiments are performed infrequently. Titration experiments are vital to properly establish the animal model, and the information gained from the titration studies is used to determine the dose of virus used in subsequent antiviral experiments. The viruses being studied are often emerging infectious agents, or surrogates for such agents. As such, little is known regarding treatment, means to alleviate pain and distress, and any possible interaction between the virus and potential palliative care. Attempts to alleviate pain or distress in animals involved in virus titration experiments have the potential to alter the outcome of the infection, and thereby provide inaccurate data for the planning of future experiments.

Antiviral experiments conducted at this institution often involve the use of experimental therapeutic agents. Due to the novel and experimental nature of the compounds involved little if any information is known regarding their toxicity profile. Oftentimes, there is no toxic effect associated with any dose of agent tested in dose range finding studies, and when available, previous toxicity information or previously published data regarding compound use is used to determine appropriate drug dose and method of administration. Dose range-finding experiments using small numbers of animals are conducted to identify the maximum tolerable dose and appropriate route of administration. This ensures that animals treated with experimental compounds in subsequent antiviral experiments are not treated with an overtly toxic dose. The experimental status of the agents being tested also means that little or no information is available regarding possible drug-drug interactions. Co-administration of pain relieving compounds could alter their antiviral activity or could enhance drug toxicity. Therefore, the use of pain relieving substances is avoided in these experiments.

A literature search on PubMed identified several published reports where commonly used pain medications such as opioids¹⁻⁷ and non-steroidal anti-inflammatory agents⁸⁻¹² altered virus infections, including potential interaction with COVID-19¹³. Furthermore, published reports identified potential interactions between analgesics and known antiviral drugs¹⁴⁻¹⁷. Even vitamins or antibiotics can alter disease outcomes in animal models testing antiviral drugs^{18,19}. Due to such possible drug-virus and drug-drug interactions pain relieving or other palliative substances are not routinely administered to animals in either virus titration or antiviral experiments. Power analysis and other statistical analyses are

performed prior to conducting antiviral experiments to determine the correct number of animals that must be used. This minimizes the number of animals that need to be subjected to the pain and distress associated with a virus infection.

1. Chuang RY, Suzuki S, Chuang TK, et al. Opioids and the progression of simian AIDS. *Front Biosci* 2005;10:1666-1677.
2. Davies PW, Vallejo MC, Shannon KT, et al. Oral herpes simplex reactivation after intrathecal morphine: a prospective randomized trial in an obstetric population. *Anesth Analg* 2005;100:1472-1476, table of contents.
3. Mahajan SD, Aalinkeel R, Reynolds JL, et al. Morphine exacerbates HIV-1 viral protein gp120 induced modulation of chemokine gene expression in U373 astrocytoma cells. *Curr HIV Res* 2005;3:277-288.
4. Chen YH, Wu KL, Tsai MT, et al. Methadone enhances human influenza A virus replication. *Addict Biol* 2015.
5. Coussons-Read ME, Daniels M, Gilmour MI. Morphine alters the immune response to influenza virus infection in Lewis rats. *Adv Exp Med Biol* 1998;437:73-82.
6. Chen YH, Wu KL, Tsai MT, et al. Methadone enhances human influenza A virus replication. *Addict Biol* 2017;22:257-271.
7. Ogbuagu O, Friedland G, Bruce RD. Drug interactions between buprenorphine, methadone and hepatitis C therapeutics. *Expert Opin Drug Metab Toxicol* 2016;12:721-731.
8. Gaylis N. Infliximab in the treatment of an HIV positive patient with Reiter's syndrome. *J Rheumatol* 2003;30:407-411.
9. Chen N, Warner JL, Reiss CS. NSAID treatment suppresses VSV propagation in mouse CNS. *Virology* 2000;276:44-51.
10. Rajic Z, Butula I, Zorc B, et al. Cytostatic and antiviral activity evaluations of hydroxamic derivatives of some non-steroidal anti-inflammatory drugs. *Chem Biol Drug Des* 2009;73:328-338.
11. Lauder SN, Taylor PR, Clark SR, et al. Paracetamol reduces influenza-induced immunopathology in a mouse model of infection without compromising virus clearance or the generation of protective immunity. *Thorax* 2011;66:368-374.
12. Zheng W, Fan W, Zhang S, et al. Naproxen Exhibits Broad Anti-influenza Virus Activity in Mice by Impeding Viral Nucleoprotein Nuclear Export. *Cell Rep* 2019;27:1875-1885 e1875.
13. Terrier O, Dilly S, Pizzorno A, et al. Antiviral Properties of the NSAID Drug Naproxen Targeting the Nucleoprotein of SARS-CoV-2 Coronavirus. *Molecules* 2021;26.
14. Bergasa NV, Boyella VD. Liver derived endogenous opioids may interfere with the therapeutic effect of interferon in chronic hepatitis C. *Med Hypotheses* 2008;70:556-559.
15. Crain SM, Shen KF. Neuraminidase inhibitor, oseltamivir blocks GM1 ganglioside-regulated excitatory opioid receptor-mediated hyperalgesia, enhances opioid analgesia and attenuates tolerance in mice. *Brain Res* 2004;995:260-266.
16. Amici C, Di Coro A, Ciucci A, et al. Indomethacin has a potent antiviral activity against SARS coronavirus. *Antivir Ther* 2006;11:1021-1030.
17. Romanowski EG, Gordon YJ. Effects of diclofenac or ketorolac on the inhibitory activity of cidofovir in the Ad5/NZW rabbit model. *Invest Ophthalmol Vis Sci* 2001;42:158-162.
18. Bitetto D, Fabris C, Fornasiere E, et al. Vitamin D supplementation improves response to antiviral treatment for recurrent hepatitis C. *Transpl Int* 2011;24:43-50.

19. Zhou Y, Yang Y, Wang P, et al. Adefovir accumulation and nephrotoxicity in renal interstitium: Role of organic anion transporters of kidney. *Life Sci* 2019;224:41-50.

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The 2022 Annual Report of Research Facility for Utah State University indicates that 1,697 hamsters have been used in studies involving pain and distress without analgesic or other palliative care. All such work conducted at this institution involves experiments examining virus infections in animal models, and associated work to evaluate potential antiviral therapeutic agents. This involves three related categories of experiments, virus titration studies in animals, dose range finding studies for novel therapeutic agents or treatments, and antiviral experiments in which virus infected animals are treated with potential antiviral agents. In the case of all experiments involving viral infections animals are monitored several times daily during virus infection. Based upon previous experience with virus infection models specific criteria for each viral disease indicating likely death have been identified. Animals meeting these criteria are euthanized to minimize pain and distress.

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