



Citizens for Alternatives to Animals Research & Experimentation

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September 23, 2016

Robert D. Hall, PhD, JD
Associate Vice Chancellor for Research and Director of Compliance
205 Jesse Hall
University of Missouri
Columbia, Missouri 65211
Via email: HallR@missouri.edu

Dear Vice Chancellor Hall:

I am writing on behalf of Citizens for Alternatives to Animal Research (CAARE), to express our concerns over the corneal wounding experiments on dogs at the University of Missouri, Columbia. This letter expresses the opinion of our board, staff, advisory board, consultants and thousands of supporters.

That the dogs were unnecessarily killed as part of these experiments, as widely reported by the media, is only one of the reasons why this research exemplifies an unethical study that should never have been approved by your institution.

The study published in the April 7, 2016 edition of *Veterinary Ophthalmology* asserted that its aim was “to determine whether a commercially available ophthalmic solution containing 0.2% hyaluronic acid would accelerate canine corneal wound healing in vivo compared to a control treatment with similar viscosity.”¹

While one could question why this was necessary, given that the compound (Optimend™) is already commercially available and does not appear to be causing adverse reactions, the fact is that a study of this sort could have – and should have – been carried out as a clinical study in affected dogs, and not as an invasive, painful, permanently disabling, and ultimately lethal experiment on young, healthy dogs.

Clinical studies in ophthalmology to closely examine eye pathophysiology for the advancement of both diagnosis and treatment are prevalent in human and veterinary medicine, including corneal studies.^{2 3}

A current review article in the *British Journal of Ophthalmology* describes successful clinical study of microbial keratitis, a serious, sight-threatening corneal pathology in humans with rapid progression. It examined dozens of clinical studies in people, which investigated a range of therapies, including the use of topical compounds, similar to the lethal beagle study carried out at UMC.⁴

Non-lethal histological methods

Moreover, the dogs' lives could have been spared after the experiments. Killing dogs to study microscopic and detailed cellular pathways of the cornea is simply unnecessary. The use of in vivo confocal microscopy (IVCM), in use for over twenty years, enables clinicians to non-invasively examine corneal injury and disease at the cellular level.⁵

In this 2003 review article on confocal microscopy the authors summarize its powerful ability to examine the human cornea, stating, "Confocal microscopy allows ophthalmic clinicians and researchers to visualise living tissues at greatly increased resolutions." They also note its ability "to research the wound healing characteristics of the human cornea following penetrating keratoplasties and refractive surgery procedures."⁶

Another source states:

Corneal confocal microscopy is a novel clinical technique for the study of corneal cellular structure. It provides images which are comparable to in-vitro histochemical techniques delineating corneal epithelium, Bowman's layer, stroma, Descemet's membrane and the corneal endothelium. Because corneal confocal microscopy is a non invasive technique for in vivo imaging of the living cornea it has huge clinical potential to investigate numerous corneal diseases.⁷

Yet another overview of IVCM describes

IVCM is helpful in evaluating the morphological characteristics of corneal dystrophies at the histological level and may be helpful in diagnosis, determination of progression, and understanding the pathophysiology of disease.⁸

Another study demonstrated the detailed non-invasive examination of the cornea in living beagles using time- and Fourier-domain optical coherence tomography and ultrasound pachymetry.⁹

Tissue Cultures and 3D organotypic corneal cultures

Another option besides wounding and killing young, healthy dogs would be to use ex vivo human corneal organ cultures. These can be purchased from the National Disease Research Interchange (NDRI), a non-profit, federally funded repository of human tissue ethically obtained from consenting human donors.¹⁰

A 2012 study used human corneal fibroblasts in a 3D model obtained from donor tissue that demonstrated intricate cellular pathways and successfully modeled a human corneal disease, keratoconus disease.¹¹

The use of sophisticated 3D cultures to grow human skin and corneas has been well documented with increasing frequency. These techniques are being effectively used by clinicians to study and treat debilitating medical conditions.

Recent advances in three-dimensional (3D) culture techniques, coupled with the ability to independently manipulate genetic and microenvironmental factors, have enabled the real-time study of mammalian tissues. These systems have been used to visualize the cellular basis of epithelial morphogenesis, to test the roles of specific genes in regulating cell behaviours within epithelial tissues and to elucidate the contribution of microenvironmental factors to normal and disease processes. Collectively, these novel models can be used to answer fundamental biological questions and generate replacement human tissues, and they enable testing of novel therapeutic approaches, often using patient-derived cells.¹²

According to the Organisation for Economic Co-operation and Development (OECD), which establishes scientific standards for non-animal tests in fulfillment of its mission to promote global well-being:

Organotypic models exist which “make use of reconstructed human cornea-like epithelium (RhCE) which closely mimics the histological, morphological, biochemical and physiological properties of the human corneal epithelium.”¹³

Moreover, pre-fabricated models, utilizing reconstructed human cornea-like epithelium (RhCE), are commercially available in the U.S. through the Institute for In Vitro Sciences (IIVS). They are made from “human-derived cells, cultured on specially designed cell culture inserts. The cells differentiate to form a multi-layered structure which closely models the corneal epithelium.”¹⁴ IIVS’ website states they offer specialized protocols on request.

Limitations of the UMC dog study

The use of intentional injury to model disease progression in animals has many scientific shortcomings. The research team readily acknowledges these quite clearly in their publication.

They point out that one-year-old dogs are considerably younger than the typical canine patient with ulcerative keratitis, and that changes in corneal diameter, decrease in keratocytes, and development of a hyaline membrane occur with age. They also discuss limitations in their choice of control substance that could affect the outcome.

Perhaps most disturbing is their admission that the small number of animals used adversely impacts the clinical significance of the data. Statistical analysis prior to the study indicated that

24 dogs should have been used, but because that was “deemed impractical given concerns of animal resources” they chose half that number, knowing their results would be compromised.

Alkali corneal burn experiments on dogs at UMC

CAARE was deeply disturbed to discover another published study carried out by researchers at UMC to study chemical ulceration of the cornea. In this experiment, alkali burns were inflicted on the eyes of seven young beagles who were also killed as part of the study. Just as with the corneal abrasion study, healing and treatment for alkali burns can be studied without inflicting serious and painful injuries on animals’ eyes, or killing them for histological exam.

Of concern is the indication that these studies may be ongoing or that there are plans to continue this line of research, since the authors conclude in their abstract that “Additional in vivo SAHA dosing studies with larger sample size are warranted.” There is also reference to this as a “pilot study” in the paper’s conclusion.¹⁵

Concluding remarks

CAARE maintains that harming and killing beagles to study corneal injury and disease is unjustifiable.

These studies violate the trust and expectation that is placed on research institutions to carry out ethical research. It is no small matter to inflict painful corneal wounds on animals in non-essential, non-lifesaving research, when better, humane methods are available.

Corneal ulcers arising from lacerations or chemical burns are known for being extremely painful. Indeed, and ironically, the motivation to develop an agent with hyaluronic acid to promote corneal ulcer healing in animals came from a veterinarian after his personal experience with a corneal laceration and experiencing just how painful it was.¹⁶

The Animal Welfare Act requires that alternatives to the use of animals be considered when there is any pain or distress involved.

9 C.F.R. § 2.31(d)(ii) stipulates that:

- (ii) The principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and has provided a written narrative description of the methods and sources, e.g. the Animal Welfare Information Center, used to determine that alternatives were not available;

CAARE appreciates UMC’s cooperation in sharing this written narrative supplied to USDA Animal Care in fulfillment of this requirement pertaining to the experiments addressed in this letter.

We trust you will treat this matter seriously. The taking of animal life and inflicting pain should not be taken lightly or dismissed with hollow verbiage about the university's commitment to humane animal care. Actions speak louder than words, and the details of these eye experiments on beagles indicate serious omissions in animal research at UMC.

I look forward to your reply addressing our concerns, in particular, the question of whether these experiments have continued or are ongoing.

Sincerely,



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President, CAARE

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¹ Gronkiewicz KM, Giuliano EA, Sharma A, Mohan RR. Effects of topical hyaluronic acid on corneal wound healing in dogs: a pilot study. *Veterinary Ophthalmology* 2016 Apr 7. doi: 10.1111/vop.12379.

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