Sepsis Experiments on Animals: Wasted Time, Money, and Lives

People for the Ethical Treatment of Animals

What Is Sepsis?

Sepsis, defined by the U.S. Centers for Disease Control and Prevention as "the body's extreme response to an infection,"[1] affects at least 1.7 million adults in the U.S. each year and kills nearly 270,000 of them.[2] The condition can be triggered when the body's immune system overreacts to a trauma or infection, and people with impaired immune systems—such as the very young, the very old, and individuals with chronic illnesses—are at a greater risk of developing it.[3] It is treated in the hospital with antibiotics and sometimes oxygen and fluid therapy. If caught in time, complete recovery is likely, although in some cases, patients may have permanent organ damage.[4]

According to a 2019 report from the National Advisory General Medical Sciences Council (NAGMSC) Working Group on Sepsis, despite decades of research on sepsis and dozens of clinical trials, no new drugs to treat the condition or technology to diagnose it—a critical step in identifying it so that it can be treated early—have been discovered.[5]

Using Mice to Study Sepsis in Humans: Lost in Translation

Mice are the animals most commonly used in sepsis research—not because they make good "models" of human sepsis but because they're cheap, plentiful, small, and docile.[6] The difficulty in reliably translating results from mice to humans is believed to be the primary cause of the failure of practically all human trials of sepsis therapies.

In 2013, the esteemed journal *Proceedings of the National Academy of Sciences* (*PNAS*) published a landmark study that had been 10 years in the making and involved the collaboration of 39 researchers from institutions across North America, including Stanford University and Harvard Medical School. Dr. Junhee Seok and his colleagues compared data obtained from hundreds of human clinical patients with results from experiments on animals to demonstrate that when it comes to serious inflammatory conditions such as sepsis, burns, and trauma, humans and mice are not similar in their genetic responses.

NIH Director Dr. Francis Collins authored an article about these results, lamenting the time and resources spent developing 150 drugs that had successfully treated sepsis in mice but failed in human clinical trials. He called this disaster "a heartbreaking loss of decades of research and billions of dollars."[7]

The *PNAS* paper reveals that in humans, many of the same genes are involved in recovery from sepsis, burns, and trauma but that it was "close to random" which mouse genes might match these profiles.[8] Collins explains it as follows:

> Mice, however, apparently use distinct sets of genes to tackle trauma, burns, and bacterial toxins—when the authors compared the activity of the human sepsis-trauma-burn genes with that of the equivalent mouse genes, there was very little

> overlap. No wonder drugs designed for the mice failed in humans: they were, in fact, treating different conditions![9]

Even before this landmark study, the criticism of mouse "models" had been documented by more than 20 peer-reviewed scientific publications. The mice used in sepsis experiments are young, inbred, and of the same age and weight, and they live in settings that are mostly free of germs (other than those of their own feces); in contrast, it is mostly infant and elderly humans, who live in a variety of unsterilized, unpredictable environments, who develop sepsis.[10,11] When experimenters induce the condition in mice, the onset of symptoms occurs within hours to days, whereas it happens within days to weeks in humans. And mice are not typically provided with the supportive therapy that human patients receive, such as fluids, vasopressors, and ventilators.[12] Another complicating factor is that, unlike humans, mice are rarely given pain relief,[13] another difference that undermines data of already questionable value, as pain affects other physiological processes.

In 2014, the late Mitchell P. Fink, who is considered "one of the most inspiring and influential leaders in the field of intensive care medicine,"[14] published an article discussing over 60 human clinical trials that had been conducted since 1982 for the evaluation of pharmacological interventions for the treatment of sepsis. Only eight of these resulted in any benefit for the patients involved, and none of them identified a cure for sepsis. Four studies resulted in further harm to the patients, while the others were of no benefit. Furthermore, Fink detailed nine specific examples of pharmacological agents that had yielded beneficial results in several animal experiments but "negative results in one or more human clinical trials."[15] He concluded that "most animal models of human sepsis are flawed" and warned that "results from these preclinical studies never should be extrapolated directly to the problem of human sepsis."

The NAGMSC Working Group on Sepsis concluded that the scientific community has substantial doubt about the usefulness of mice in enabling researchers to develop new sepsis treatments and emphasized that sepsis research funding needs a more clinical (human) focus.[16]

Inducing Sepsis in Mice With Cecal Ligation and Puncture: Abhorrently Cruel—and Bad Science

The "gold standard" method of inducing sepsis in mice is a procedure called "cecal ligation and puncture" (CLP). Experimenters cut open the animals' abdomens and puncture their intestines with a needle so that fecal matter and bacteria will leak out. The mice then endure widespread pain, with the worst symptoms in the abdomen, and eventually become so sick that they are unable to move. They experience fever, chills, diarrhea, difficulty breathing, lethargy, disorientation, septic shock (when the infection reaches their bloodstream, causing their blood pressure to plummet), and, finally, multiple organ failure and death.

Abhorrently cruel, this "best practice" method is also terrible science. First, mice's responses to CLP vary by age, sex, strain, laboratory, the size of needle used, and the size of the incision, which makes results between laboratories incomparable.[17] Second, the procedure causes the formation of an abscess, whose effects may disguise or be disguised by the effects of the sepsis itself. This means that an intervention that appears to be beneficial for sepsis may actually be beneficial only because of its effects on the abscess.

Using Other Animals in Sepsis Experimentation
Rats, dogs, cats, pigs, sheep, rabbits, horses, and primates, including baboons and macaques, have also been used in sepsis experimentation. None of these species reproduces all the physiologic features of human sepsis. The pulmonary artery pressure responses of pigs and sheep differ from those of humans, so this aspect of sepsis cannot be compared between these species. Furthermore, baboons, like mice, are less sensitive to a species of bacteria commonly used to induce sepsis in experimental settings. This is likely because animals are housed in feces-contaminated environments, allowing for the development of a level of resistance to pathogens and some interventions that is not present in the majority of humans.

Poor Ethics in Sepsis Experimentation
In 2014, researchers from the University of Alberta surveyed 77 papers on animal studies that had been published in three high-impact critical-care journals between January and June 2012 and found that the "[r]eported … ethical quality" of this research was "poor."[18] The results of the analysis speak for themselves:

> Most studies did not report monitoring the level of anesthesia during invasive procedures, even when muscle paralytics were used, nor monitoring or treatment of expected pain. When euthanasia was used, the method was often not stated, and when stated, most methods were not appropriate for the species. A sample-size calculation was rarely used, and animal numbers were often poorly described. No studies performed a systematic review to ensure that the animal research would be useful and not simple repetition. … Most studies were funded with public funds (foundation or government funding). Sepsis models less often met the composite outcome of … using anesthesia and pain control, and stating the method of euthanasia.

The authors note that the disregard for the pain and distress experienced by the animals "may confound the study results, and may thus be a reason for the poor translation of [experiments on animals] to humans." In addition, the report states, "Alternatives to animal models were almost never explicitly considered"—even though consideration of non-animal methods is required by federal law. The chronic failure of experimenters who conduct sepsis research to adhere to even the minimum standards for the use of animals in laboratories causes animals to suffer, squanders public funds, and impedes a scientifically rigorous search for a treatment for sepsis in humans.

Superior Methods for Studying Sepsis
Fortunately, researchers do not have to use animals to study and find treatments for sepsis in humans. In 2015, an expert working group consisting of veterinarians, animal technologists, and scientists issued a report on the implementation of the 3Rs (replacement, reduction, and refinement) of the use of animals in sepsis research.[19] The group noted several methods that could be used instead of animal models, such as *in vitro* cell-culture models for studying sepsis mechanisms, systems and computation biology for laying out the inflammatory processes occurring during sepsis, 3-dimensional cell-culture models for exploring human disease progression and infectious disease mechanisms, synthetic human models to recreate human disease-related cell types and tissues, and human genomic information to discover how sepsis

affects individuals differently and which groups may be more at risk. The authors state that genomic information "will complement or even replace the need for mouse models in disease discovery and drug development."

In fact, there may have already been a breakthrough in sepsis research. A few physicians have recently had impressive results by treating sepsis patients with intravenous vitamin C,[20] although more studies are needed to confirm the efficacy of this therapy. Importantly, these successes were achieved using only human patients, not mice or other animals, and many of these people were helped tremendously in the process.

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