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Murine “Model” Monotheism – an Iconoclast at the Altar of Mouse

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Abstract:

Many use mice for contemporary cardiovascular research, as we should, given the power of the genetic and other tools developed to permit rigorous mechanistic experimentation in this species. We must remain mindful, nonetheless, of the reasons results of mouse experiments may not extrapolate readily to human disease. Large gaps separate our mouse experiments from clinical conditions. The differences arise from the genetic homogeneity of inbred mouse strains used in experimentation, the restricted exposure to many infectious agents and a more monotonous microbiome than free-living rodents and humans, lack of co-morbidities, focus on youthful mice and exaggerated experimental conditions contrived to speed studies, and the lack of congruence between many experimental “models” and human cardiovascular conditions. We must and should continue to mine mouse studies for the incredibly valuable mechanistic insight they provide. Yet, we could consider more carefully as a community some of the barriers to glib extrapolation of the results of experiments in mice to our human patients.

Key words:
Murine models
Hygiene hypothesis
Microbiome
Arterial injury
Transverse aortic constriction
“These experimental results in mice suggest that X is a promising novel target for the treatment of human cardiovascular disease”

(A paraphrase of the concluding sentence of many contemporary cardiovascular research papers)

Most use mice. We must use mice for much contemporary cardiovascular experimentation. Yet, we must not succumb to “group think” about the ready translatability of our experimental results to the clinic. Although many millennia of evolution separate them from humans\(^1\), mice provide incredibly valuable tools for biomedical investigation. In particular, the availability of inbred strains and the development of ever more powerful techniques for sophisticated genetic manipulation allow “reductionist” experiments of increasing complexity, sophistication, and rigor. On the other hand, the primacy of mice as experimental animals for cardiovascular research over the last 25 years may have fostered a tendency to minimize some of the limitations of modified mice as “models” for human diseases. This commentary highlights some of the issues to which we as a community might pay more heed in interpreting and extrapolating to humans the results of our mouse experiments. I do not denigrate the use of these powerful approaches to experimental work. Rather, this essay aims to raise some of the issues that require consideration and rigorous thinking regarding the relationship of our laboratory approaches to clinical cardiovascular disease. I consider serially a number of such potential limitations.

1. Congenic Strains – An Enabling Strength, but also a Potential Weakness

We possess a repertoire of over 100 exceedingly well-characterized congenic strains of mice. Generations of brother/sister mating leads in this species leads to construction of lines of
mice with virtual genetic identity. This tactic provides a homogeneous preparation that reduces the variability in our experiments and renders them more readily reproducible. Yet, these very virtues represent a potential barrier to extrapolation to human populations. As opposed to our popular mouse strains used in cardiovascular investigation, taboo, custom, social mores, and strong biological drivers constrain consanguinity in human societies. In contrast to the genetic uniformity of inbred mice, our patients present a varied genetic palette. We know from quantitative trait locus mapping and other studies in mice that modifier genes can markedly influence the pathophysiological consequences of various genetic variants. We also have learned that studying a specified genetic alteration on different genetic backgrounds can markedly influence the phenotypic expression. Thus, while offering an enormous advantage for experimental rigor, the use of congenic strains presents *prima facie* an enormous gap between the laboratory and the clinic. Genetic variation in human populations has important health implications. For example, polymorphisms in the major histocompatibility complex likely permit human populations to survive various epidemics, as some individuals will mount an immune response to a given pathogen better than others. Thus, genetic variability likely contributes to the very survival of the human species. Experiments in congenic mice eliminate this biologically important attribute of human populations.

2. The “Hygiene Hypothesis” and the Microbiome as a Barrier to Translatability of Laboratory Experiments in Mice to Humans
Our shiny modern animal facilities tout a “specific pathogen-free” environment. We carefully protect our experimental mice from the onslaught of potentially pathogenic microorganisms that they would encounter in the field. In contrast to our contrived laboratory environment, human populations have constant exposure to varied pathogenic and commensal microbial organisms. Moreover, we have increasing recognition that the intestinal microbiome can critically influence the expression of various diseases including those that affect the cardiovascular system, in both humans and in mice. Our laboratory mice engage in coprophagia, a behavior that favors the perpetuation of a particular microbial environment in carefully caged animals. In contrast, rodents in the field, more like human populations, undergo varied and continuous exposure to microorganisms that would yield much greater heterogeneity in the microbiome that would “educate” immune responses more intensely.

These considerations reflect a variant of the “hygiene hypothesis” that microbial challenges to the immune system in more sanitary environments affect the development of the immune system. Indeed, wild rats and wild mice exhibit higher IgG and IgE concentrations in serum than rodents in the laboratory. The immune system of wild-caught rats contrasts considerably with those of laboratory animals in relation to T cell characteristics as well. Some have invoked the “hygiene hypothesis” to explain increases in allergic diseases in post-industrial societies. From our experimental perspective we need to remain mindful that the immune system of our favorite laboratory mice may vary considerably from field mice and from human populations exposed to a broader environment of microbes both pathogenic and commensal. The lack of constant challenge by a diversity of microorganisms and a more monotonous microbiome distorts the immune response in our laboratory mice. Moreover, the
continuous exposure to a variety of microorganisms “educates” human adaptive immunity, providing a palatte of memory responses that may vary substantially from primary responses. De novo responses evoked in mice with a more restricted experience with microorganisms may yield a different picture from memory responses in humans.

3. Mice Have a More Categorical Immune System Than Humans

Another challenge to the ready translatability of experiments the immune system in mice versus humans relates to the apparently less strict demarcation between functional subtypes of both innate and adaptive immune cells in humans than mice. For example, an enormous literature has focused on Th1 versus Th2, regulatory T cell (T\textsubscript{reg}) functions, and differences between B1 and B2 lymphocytes in experimental preparations. In the realm of innate immunity, the study of “M1” versus “M2” macrophages has become a common refrain. Yet, considerable evidence suggests that the strict categorization of these functional subsets of both adaptive and innate immune cells in humans has more diffuse borders and perhaps greater instability than evident in the mouse. Immunologic researchers have called attention to the differences in functional markers and stability of T\textsubscript{reg}s in mice versus humans. Moreover, the markers commonly used for “phenotyping” macrophages in mice render less clear results in humans. Interpretation of the results of manipulations of the mouse immune system for human disease should take these disparities into account more frequently.
4. Are Our “Models” Really Such?

We often engage in laboratory perturbations in mice with various genetic modifications to delineate the roles of specific mediators in human disease. Such an approach offers enormous value. Yet our manipulations, while convenient and often reproducible, may stray substantially from replicating the human situation. For example, generations of studies of arterial injury in the quest of new therapies for restenosis following clinical arterial intervention used previously normal animals. There is little reason to expect that the response to arterial injury in a previously normal vessel “models” faithfully clinical percutaneous arterial interventions, performed on atherosclerotic arteries. Many rodent arterial injury experiments use withdrawal of an over-expanded balloon, deployment of an over-expanded stent, or abrasion produced by repeated passage of a wire as the “model” injury. Yet none of these manipulations mimics clinical percutaneous arterial intervention. These differences, to which we called attention in 1992, could contribute to the huge disparity between interventions that proved effective in animal experiments but disappointing in the clinic. ¹³

In the realm of complications of atherosclerosis, many mouse papers refer to features of plaque “stability” or “instability.” Experimentally induced plaques in mice seldom if ever disrupt and cause thrombosis, rendering the use of these terms highly suspect. Many mouse studies have addressed the pathogenesis of abdominal aortic aneurysms. At least three types of manipulations can induce abdominal aortic abnormalities in mice, the combination of hyperlipidemia and angiotensin II infusion, perfusion with elastase, and peri-aortic exposure to calcium chloride. None of these manipulations faithfully replicate the human condition. The lesions induced by angiotensin II infusion in hyperlipidemic mice, widely used by our own and many other groups,
produces lesions that likely represent transmural penetrating ulcers of the aorta with peri-aortic hemorrhage, thrombosis, and organization. These lesions do not occur in the infra-renal region characteristic of human abdominal aortic aneurysms. Such examples illustrate how arterial lesions produced in mice do not undergo complications that lead to clinically important manifestations of the disease in humans.

Likewise, in studies of myocardial biology we perform transverse aortic constriction (“TAC”) in mice that often have previously normal hearts, in an attempt to “model” disease of human hearts that often have chronic rather than acute pressure overload that yields an abnormal heart before experiencing critical outflow obstruction. Likewise, our common “ischemia/reperfusion” preparations in the mouse myocardium start with a normal vasculature and myocardium rather than one diffusely affected by atherosclerosis and previous ischemic insults. Is it thus surprising that few if any of the multitudinous manipulations that have improved the outcome of experimental ischemia/reperfusion injury in mice and other animals have borne fruit in the clinic?

5. Mouse Experiments Usually Fail to Account for Comorbidities Common in Our Human Patients

The foregoing section highlighted distinctions between the normal substrate for intervention, both vascular and myocardial, in mice versus the human situation usually affected by chronic diseases of those organs. Beyond the cardiovascular system, our patients usually present with comorbidities that interlace inextricably with their cardiovascular disease. Common
concomitant conditions include renal dysfunction, diabetes mellitus, obesity, tobacco abuse, periodontal disease, and pulmonary disease. These comorbidities inevitably influence the expression of cardiovascular disease. We appreciate increasingly the crosstalk between acute and chronic inflammatory activation remote to the myocardium or atheroma and “echoes” within the cardiovascular system. \(^\text{14 \text{15 \text{16}}\) Our well-defined and reproducible mouse experiments again fail to replicate the complexity of clinical cardiovascular disease, whose course often depends upon concomitant comorbidities.

6. The Time Scales of Laboratory Investigations and Human Disease Vary Considerably (Funding Cycle versus Life Cycle)

Laboratories depend on funding cycles of a few years. Graduate students and post-doctoral fellows in common with principal investigators need to finish projects in a timely fashion to permit publications that allow them to advance to the next echelon of their professional careers or obtain the next limited cycle of grant funding to support their research. These constraints cause us to eschew the long-term experiment in favor of short-term manipulations that can generate data rapidly and meet the short-term goals imposed on us by professional ladders and funding organizations. Hippocrates famously stated, “The art is long, life is short… experiment difficult…” He was doubtless correct for his era, and his assessment of the difficulty of experiment endures. Yet the sage had no conception that by making inroads in communicable diseases and other advances both societal and medical, that the human lifespan would become a lot longer in the 21\(^{\text{st}}\) century than in classical antiquity. Cardiology practice
continues to shift towards an accumulation of the aged. As our experiments remain short, we adopt “models” of disease that do not reflect aging of the cardiovascular system.

Atherosclerotic mice commonly used in the laboratory typically have degrees of genetically- and/or dietarily-determined hyperlipidemia that caricature rather than mimic the human conditions. Such acceleration produces atherosclerotic lesions in mice in weeks not in years. Yet, we blithely expect the lesions so produced to represent the ravages of a disease that plays out over decades in humans. In our haste to complete the next manuscript, to finish the thesis, and to compete for grant funding, we tend to sweep under the rug the enormous differences in time scale and the intensity of interventions between clinical and experimental disease in our cardiovascular experimentation.

7. Human Studies Have Limitations Too

The foregoing cautionary statements regarding mouse studies raise some similar limitations inherent in many clinical trials. As the interpretation of animal experiments requires caution, so too does the design and extrapolation to broader practice of clinical investigations. For example, while our human populations included in clinical trials lack the genetic homogeneity of inbred strains, we must strive to be more inclusive of individuals from different geographic locations and ethnicities when recruiting for clinical trials. In particular, we must maximize the participation of women, a growing segment of our cardiovascular patient population. Entry criteria for clinical trials often also exclude comorbidities such as advanced renal disease or cancer, conditions commonly encountered in clinical practice. Clinical trials also usually exclude the “elderly old,” another portion of the population on the rise. The span of our
experimental studies depends on academic and grant funding cycles. The duration of clinical
trials also confronts time limitations due to the haste of the sponsor and the academic
investigators alike to conclude a study, albeit for distinct reasons. We often encounter
disappointment when extrapolating the results of smaller phase 2 studies, particularly those that
employ surrogate endpoints rather than clinical events. We must bear these limitations in mind
when evaluating human studies, as I urge for experimental work above.

Conclusions and Implications

These and doubtless other important considerations highlight the challenges of
interpreting our investigations conducted in mice. Should we therefore abandon such
experiments? That conclusion comprises a *reductio ad absurdum*. We should and must use
mouse experiments to propel our field forward. Nonetheless, we must not neglect the gaps that
yawn between these convenient and powerful animal preparations and human disease. Many
hundreds of published papers reporting cardiovascular research have conclusions that paraphrase
the introductory quotation above: that the results uncover a new target for therapy of human
disease. Few of the findings touted in these promissory statements have actually led to new
treatments proven to benefit patients. We do a disservice to our trainees and to clinical
investigative colleagues to minimize the enormous distance, effort, and expenditure of time,
energy, and resources that stand between the glib extrapolations in our research papers, and the
reality of clinical translation. While the rigor, reproducibility, and practicality of mouse
experiments provide compelling reason for their continued widespread use in cardiovascular
investigation, we could well adopt a bit more caution in our rush to extrapolate results to the clinic.

Facile statements in experimental papers regarding the translatability of the findings unwittingly demean clinical trialists who travel the long, arduous, and perilous road actually required to test hypotheses regarding the efficacy of therapeutic interventions in humans. It takes the basic science investigator seconds to dash off a sentence regarding clinical translation. It requires 7–10 years of dedicated effort for the clinical investigator to put the proposition to the test. Armed with a hypothesis, the clinical trialist must conceive, design, seek support for, start, sustain, complete, and analyze a patient study. Many basic investigators have no inkling of the many moving parts, inevitable barriers, frustrations, and enormous effort over many years required to bring a clinical trial across the finish line.

Our laboratory group tries to practice the discipline of using the term “model” cautiously. We favor the stance that animal experiments provide us with a rich opportunity to test defined hypotheses regarding pathophysiologic mechanisms under tightly controlled circumstances. This approach applies not only to mice, but also to experiments performed in other species: rats, rabbits, fish, and worms alike. I challenge trainees and colleagues with the assertion that attempts to “model” a human disease in mice virtually guarantee failure. I prefer the nostalgic term “preparation” as a more humble and perhaps realistic replacement for the word “model.” Ernest Henry Starling, a pioneer in cardiovascular physiology, realized that the “heart-lung preparation” that he developed at the dawn of contemporary cardiovascular physiology was not a model of human disease but a tool to use to gain pathophysiologic insight that provides the foundation and daily bread of contemporary hemodynamics and cardiovascular
If the term “preparation” sufficed for Starling, perhaps it could serve us well as well.

Ultimately, cardiovascular science will continue its impressive advance by combining the rigor and control of laboratory experimentation with the much muddier reality of human patients and populations: the delight and challenge we encounter in daily practice. To achieve this end, we must continue to train physician-investigators, to encourage dialogue between laboratory scientists and clinical investigators, and to apply a dose of humility and introspection to the implications of our experimental work for human disease.
Figure Legend: Mice are Mice, not “Models.”

This photograph shows mice that guard the threshold to Otoyо Shrine, one of the treasures on the Philosophers’ Path (tetsugaku no michi) in Kyoto, Japan. Appropriately, this shrine celebrates health, longevity, and mating (matrimony for us humans.) The selection of mice as guardians of this shrine has nothing to do with the premise of this commentary. Rather this statuary reflects an ancient Japanese myth that recounts how a mouse saved a suitor from dire distress. One of the guardian mice carries a scroll (high power view, left), which we might regard as a manuscript for submission to Circulation Research or a grant application. The other mouse (high power view, right) bears a sake cup that he might fill locally, as the shrine usually houses ample offerings of large tubs of sake (see Online Supplement). Perhaps the sake-bearing mouse aims to provide solace to his aggrieved scroll-bearing brother when he receives his decision letter from the journal editors or the summary statement from study section. For the cardiovascular investigator, the positioning of these statues of mice on pedestals has appeal, as this species has proven pivotal in moving cardiovascular research forward. It is the interpretation and overly simplistic extrapolations of results to the clinic of which we must remain mindful. To paraphrase Shakespeare’s Cassius: “The fault, dear colleagues, is not in the mice, but in our selves” (Julius Caesar, Act I, Scene II, Lines 140-141). Photographs taken from GoJapanGo.com.

Supplementary Figure Legend

This photograph shows the offerings of sake at Otoyо Shrine.
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None.

Conflicts of Interest

None.
References:


