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Review Article

Pros and Cons of Animal Models: Special Emphasis on Anti-arthritic Clinical Evaluation Model

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ABSTRACT

Introduction: Generally, animal models are regarded as very useful tools for studying pathophysiology and the clinical aspects of the disease and are always used as the first step for investigating a prospective new therapy. On the other hand, these models have numerous distinctions from the human condition by numerous constraints such as cost, animal size, and accessibility. **Objectives:** Nevertheless, scientists keep on depending on creature models because of the way that they can be promptly tested, biopsied, and autopsied, their hereditary and ecological background is now known and generally they serve studies that could not, in any case, be accomplished in humans. Therefore, the continuing effort for inventing new models has always positive critics and animal models will continue to have a major and meaningful place in clinical research. However, all researchers should always look into the ethical limits in the application of animal models for their experiments, utilize animals only when they are indispensable for a study, and avoid causing them pain, distress, suffering, and lasting harm. **Conclusion:** Here, we have summarized some significant *in vivo* and *in vitro* clinical models for the assessment of anti-arthritic activity of future drug candidates along with a general consideration of selection criteria and drawbacks; which is also applicable for animal models apart from arthritis. In this sense we suggest that the various models should be increasingly investigated, providing subsidies for new findings in the pathogenesis of diseases, with a rational discussion about the pros and cons of each experimental model to allow the best choice for the study in question.

Keywords: Animal Model, Human Condition, Critics, Arthritis model, *in vivo* model, *in vitro* model, Biomedical examination.

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INTRODUCTION

An animal model for biomedical examination is one in which standardizing biology or behavior can be assessed, or in which a spontaneous or actuated pathological interaction can be researched as well as the clinical findings based on one or many parameters reveal similar marvel in humans or different types of animals. American National Research Council Committee on Animal Models for Research and Aging has classified the application of creature models in biomedical exploration into five groups (Table 1).[1]

In the previous century, with an end goal to optimize the number of animals employed in clinical experiments, Russell and Burch suggested that the utilization of animals should follow the four "Rs" as demonstrated below (Figure 1).

Replacement implies subbing animals with non-creature (elective) models; Reduction, diminishing the number of creatures utilized in an investigation; Refinement, following the best quality consideration that can be given to the creature and Responsibility, genuine supervision of the whole investigation to avoid unnecessary scarification of test animals.[2]

As far as drug discovery and new moiety investigation are concerned, it is essential to check all possible aspects of the selected animal model as mentioned above to minimize the magnitude of disadvantages by utilizing the advent features of the model to the best of available research knowledge. In this regard, we have discussed the selection strategy and possible drawbacks of the animal models, which will assist in finding out an animal model for investing pharmacological profile of the investigating molecule.

Planning before selecting an animal model

Animal models have been utilized for a very long while to contemplate the etiology and pathology, simultaneously to approve the pharmacological effect of new remedial approaches. Creature models facilitate a complete investigation of inquiries

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Table 1: Principles of selecting a group for animal study.

1. Unconstrained models in which sicknesses or conditions manifested unexpectedly in animals as in people.
2. Experimental models
3. Hereditarily altered models in which infections or pathological conditions are actuated surgically/ chemically or by hereditary control, separately
4. Creatures impervious to a specific condition or ailment as negative control models
5. Vagrant models, incorporating animal models with illness obscure to human counterparts

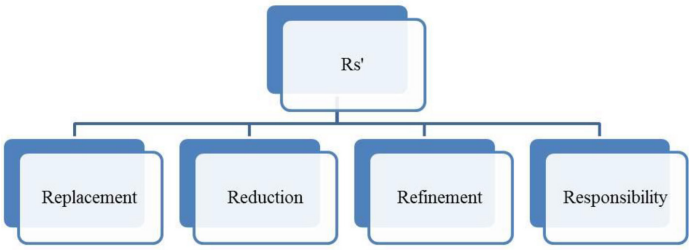

Figure 1: Crucial Rs for conducting an animal study.

that cannot be tended to in human examinations, numerous sampling at key occasions during evolution, experimentation with the least possible number of variables.[3]

Before utilizing a creature model, one should consider some important parameters like the reproducibility (extent of creatures arriving at the ideal state in a constant period), the particularity (i.e., the model ought to have the intended anomaly with no other pathological adverse events and if such complication exists, it should be both identified and reproducible) and the attainability (regardless of whether the research center has the professionals, labor, instruments, explicit skilled individual to generate or deal with the model).[4] Moreover, inciting, modulating, and regulatory mechanisms are species, strain, protocol, and model dependent. Rodents may offer a few benefits in tests in which size is an issue, for instance, imaging methods or hemodynamic estimations. Moreover, Chimpanzees and Baboons are chosen preferably to examine liquor instigated and viral hepatitis, separately to investigate hepatic fibrosis. In certain investigations, models in bigger creatures (rabbits, canines) or fascinating rodents are employed.[5]

Drawbacks of animal study

The hindrances of animal models are occasionally neglected. The clearest is that they are not human and there are huge species contrasts in genetic expression, pharmacological response, immune reaction, and metabolic rate.[6]

The selection of the inciting stimulus in a creature model depends on the assumption that the reaction will engage with components that are like those happening in the related human ailment. Such a methodology may be misleading. To start with, models are not accessible for all diseases. Second, an etiology-explicit condition probably would not be pathogenic in creatures. For instance; the hepatitis C infection does not taint rodents; rodents have a significant repugnance for alcohol and are in some way obstinate to alcohol-initiated fibrosis. Third, the reaction to a particular instigating agent may change among humans and animals.[7-8] For instance; biliary fibrosis which is gradually reformist in humans is traditionally modelized by a bile channel ligation related to high mortality and cirrhosis within half a month.[9] For the entirety of the above-mentioned statement, it appears to be improbable that any creature model can give an ideal recreation of human disease, engaging similar causative variables and pathobiological measures (counting phlogistic or immune response) to show up at indistinguishable pathology and reproducible infection results. Outrageous alert should consequently be applied for interpreting animal observations to the clinical setting.

Anti-arthritic activity evaluation models both *in vivo* and *in vitro*

Rheumatoid Joint Pain (RJP) is an immune system problem wherein the body's immune system typically secures its wellbeing by assaulting unfamiliar substances like viruses and bacteria erroneously attacks the joints. This brings about inflammation that causes the tissue that line within joints (the synovium) to thicken, bringing about pain and swelling in and around the joints.[10] The synovium makes a liquid that greases up joints and assists them with moving easily. RJP most generally influences the joints of the hands, feet, wrists, elbows, knees, and lower legs. Since RJP additionally can influence other body systems, like the respiratory or cardiovascular systems, therefore, it is also known as a systemic ailment.[11] Systemic signifies "whole body". Systemic manifestations are aggravated; as the disease progress and if the treatment does not start at the right time. Rheumatoid arthritis influences about 0.92% of the grown-up populace in India. Early pathological determination and instant treatment can generally forestall perpetual disability. The beginning of arthritis is generally frequent during middle age and ladies are affected 2.5 occasions as regularly as men. In 2013, it brought about 38,000 deaths from 28,000 deaths in 1990; the mortality rate is expanding year by year hazardously.[12]

Etiopathology of Rheumatoid arthritis

The specific cause of Rheumatoid Arthritis (RA) is as yet muddled. It is accepted that B cells, T cells, and abnormally high production of different phlogistic cytokines and de-regulation of incendiary cytokines control the pathophysiology of RA.

The present concept on etiology and pathogenesis recommends that RA happens in an immunogenetically predisposed individual with the exposure of microbial agents (like mycoplasma, Epstein-Barr infection, cytomegalovirus, or rubella) acting as trigger antigen. The common causative micro-organisms are

gonococci, *meningococci, pneumococci, staphylococci, streptococci*, *H. influenza* and gram negative bacilli.[13]

Environmental factors also affect the development, extent, and pace of progression of the infection. Albeit various pathogenic micro-organisms are embroiled, currently smoking is strongly advocated late as a significant environmental danger factor for the induction of RA in HLA-DR4-positive people.[14]

Primarily RA includes the enactment of both T and B cells. Cytokines play a significant part in the pathophysiology of RA as phlogistic cytokines, for example, TNFα, IL-1, IL-17, invigorates inflammation and morphological destruction of bone and ligament that underlie the clinical manifestation and progression of RA (Figure 2).[15] In rheumatoid arthritis patients, serum or plasma levels of cytokines might show the intensity of the disease.

There are a few variant types of RA. Adolescent RA found in young adult patients is featured by the intense onset of pyrexia and prodrome implication of ankles and knees. Pathological parameters are similar however RF is scantly present. Felty's syndrome comprises poly-articular RA related with hypersplenism, and splenomegaly and ensuing hematologic disturbances. Rheumatoid spondylitis or Ankylosing spondylitis is rheumatoid participation of the spine, especially sacroiliac joints, in adult male patients. The condition has a firm HLA-B27

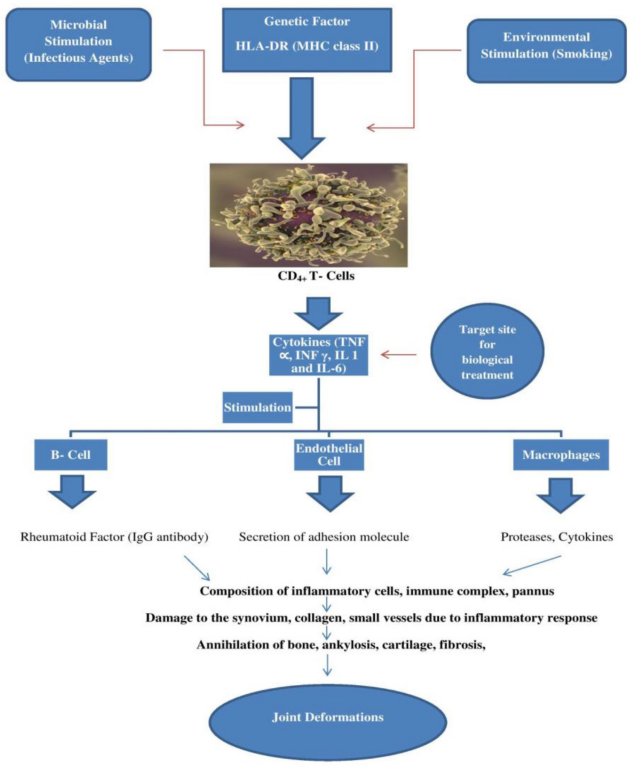

Figure 2: Flow chart of etiopathology and target site of anti-arthritic drugs.[16]

involvement and may have association with phlogistic diseases like incendiary bowel disease, foremost uveitis.[16]

Arthritis is a gradually spreading disease at present, so a new drug entity must be clinically evaluated on the best suitable animal models. In the present review, we have compiled some relevant and popular *in vivo* and *in vitro* models, which are commonly employed by researchers for the clinical investigation of therapeutic efficacy of anti-arthritic agents against arthritis and its variants.

In vivo Models

Freund's complete adjuvant-induced arthritis

The utilization of a rodent creature model to assess the sickness attributes of RA has effectively been demonstrated. A few trial models have been created in rats to examine the pathogenesis of joint arthritis and to test the expected effectiveness of hostility to anti-rheumatic medications (Figure 3).

Complete Freund's adjuvant (CFA) - initiated arthritis model is additionally considered as a model for long-term polyarthritis with manifestations that look like RA.[17]

Mycobacterium butyricum[18] or heat-killed Mycobacterium tuberculosis[19] is used as an inducing agent for the CFA model.

Arthritis advancement is genuinely estimated by body weight, joint inflammation, paw volume, mobility, step, and injuries. The biochemical estimations are erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum TNF-alpha and rheumatoid factor (RF). These parameters (Figure 2) will be elevated abnormally after induction of arthritis, and the test drug is expected to decrease the magnitude of such parameters; when compared to standard medicine.

Adjuvant-incited joint pain is a better model as compared to other exploratory animal models and is still broadly utilized in the preclinical testing of anti-arthritic medicines because CFA model help to estimate the potential of test drug on each biochemical parameter of arthritis.

Formaldehyde induced arthritis

Formaldehyde prompts a provocative response at the site of infusion, and this has been utilized for considering inflammation quantitatively in the rat foot.[20] Selye previously portrayed the impact of infused formaldehyde in the rodent foot as an "arthritic" response; he has shown that in the adrenalectomized rat both adrenocorticotrophic and cortisone hormone are viable in offending the response to formaldehyde. However, this model has been objected to by Bourne as wrong. Despite that, Different authors are upon the primary concern, that formaldehyde delivers a local inflammation. It has been observed by Setnikar, Salvaterra, and Temelcou that the edema of the rodent foot brought about by an infusion of formaldehyde can be halfway forestalled by iproniazid, phenylbutazone, and salicylamide.[21]

It is feasible to clarify the anti-phlogistic activity of the antipyretic-pain relieving class of medications based on their anti-fibrinolysin effect, and it very well may be hypothesized that the capacity of the different pain-relieving antipyretic medications capable of restraining formaldehyde-instigated inflammation in the mouse foot is because of their anti-fibrinolysin activity.[22] It means this model may not be suitable to access the anti-arthritic potential of a newborn molecule alone.

Monosodium urate crystal-induced inflammation in mice

Uric acid is the byproduct of purine nucleotide and its soluble form is typically found in plasma. Since, after saturation point uric acid can solidify. The amassing of monosodium urate (MSU) crystals in the joints is noticed to be the reason for gout that can create intense or chronic arthritis. In gout, MSU crystals invigorate the creation of fiery cytokines by the P2Y6 receptor pathway, for example, IL-6, TNF-alpha, IL-1and chemotactic factors for neutrophils, for example, S100A8/A9 and IL-8/CXCL8.[23]

The inciting agent is prepared by compounding around 4 g of uric acid dissolved and warmed in 800 ml H_2O with NaOH (9 ml/0.5N), acclimated to pH 8.9 at 60°C; cooled overnight in a cold room; washed and dried. Needle-like crystals are gotten which are suspended in sterile saline (20 mg/ml).[24]

The arthritis development is estimated by expanded paw edema; the raised concentration of lysosomal catalysts of serum, spleen, liver, and TNF-∝; high degree of lipid peroxidation in liver, plasma, and spleen, and diminished concentration of enzymic reducing agents in spleen and liver.[25]

On the other hand, this model cannot be considered a "pure anti-arthritic model" because MSU stones are also present in skin infections such as atopic skin inflammation, lichen planus, harmful epidermal necrosis, and contact dermatitis.[23]

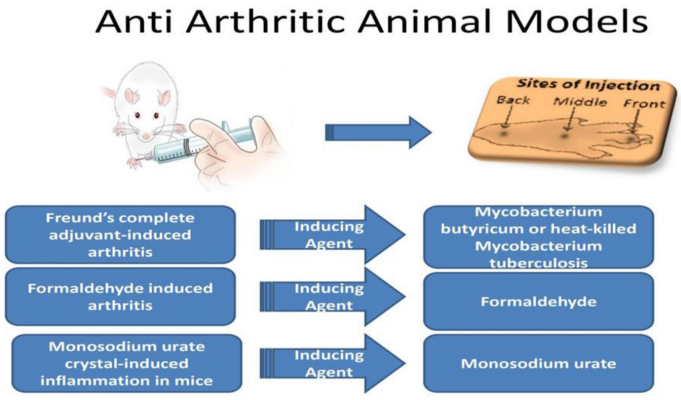

Figure 3: List of extensively used anti-arthritic animal models and application site of inducing agents (Animated representation).

In vitro Models

These models are frequently used by researchers now a day. *In vivo* models are selected based on 4 R's principle in which one "R" stands for reduction in number of test animals (Figure 1). In this context, *in vitro* models help to minimize the use of animals as much as possible to avoid unnecessary scarification of animals and if it is found that the efficacy of a test molecule can be assessed without utilizing animals.

Protein denaturation inhibition test

Common drugs are prescribed for the management of inflammatory conditions mainly RA and other infectious illnesses. They reportedly tied to plasma albumin, forestalling or repressing the heat denaturation of albumin.

Protein denaturation has been distinguished as the reason for inflammation. Signs are that when living tissues are harmed, inflammation results. This is manifested by redness, torment, heat, edema along with loss of function in the influenced region. In addition, a cascade of enzyme initiation, mediator discharge, cell migration, tissue breakdown making the protein lose its molecular configuration to become denatured. It is hence concluded that chemical agents which can prevent these progressions and restrain thermally or heat-instigated protein denaturation, have likely helpful worth as antiphlogistic agents.[26]

The reaction mixture consists of 1% bovine albumin, phosphate-buffered saline (PBS, pH 6.4), and the test drug. The reaction mixture can be modified by using trypsin, Tris-HCl buffer (pH 7.4), methanol, 0.8% (w/v) casein and 70% perchloric acid.[27]

Membrane stabilization test

Inflammation is portrayed by a natural response of tissues in the body which may include a chain reaction of cellular enzymes followed by labialization along with disruption of cell membranes. Consequently, stabilization of cell membrane could be a crucial target for antiphlogistic activity in RA. Steroidal and non-steroidal drugs actuate anti-phlogistic impacts by membrane stabilization.[28]

The assay reaction blend contains 0.15 M PBS (pH 7.4), hypotonic saline solution (0.25% NaCl), human red blood cell suspension [10% v/v] in normal saline, and a test sample.[29]

Proteinase inhibitory test

Lysosomal enzymes are engaged with the degradation of underlying macro-molecules in ligament proteoglycans and connective tissue. They are additionally capable of annihilating extracellular activities and may take part in intervening tissue injury in RA. The reaction combination contains trypsin, 25 mM tris-HCI buffer (pH 7.4), and a test drug.[30-31]

Monosodium urate crystal-PMNL cell Interaction test

Gouty joint inflammation is an intense incendiary disease manifested by an increased level in serum urate concentration and accumulates MSU stones in and around the joints of extremities with serious agony. An enormous number of studies have featured the significance of both humoral and cellular inflammatory mediator systems in the pathogenesis of the response to urate stones. Specifically, it appears to be likely that the release of cytokines, such as IL-1, IL-8, and TNF-alpha by macrophages/monocytes upon phagocytosis of urate crystals assumes a focal part in the initiation and proliferation of the inflammatory response.

Human PMNL (poly morpho nuclear leukocytes) cell suspension and monosodium urate stones are utilized for the *in vitro* examination of anti-arthritic activity.[32]

Hyaluronidase test

Hyaluronate naturally occurs within the ligament and synovial liquid. Its rheological attributes are associated with the principle function of synovial liquid to serve as a scrounger for free radicals, grease, for the regulation of cytological activities like protein binding, and serving as a space filler to permit the joint to remain open. During the progression of osteoarthritis; the endogenous hyaluronic acid (HA) in the joint is depolymerized, which therefore reduces the mechanical and viscoelastic attributes of the synovial liquid in the affected joint.[33]

Hyaluronidase is a term applied to a bunch of different enzymes that degrade HA, a high atomic weight glycosaminoglycan of the extracellular network. This enzyme is employed to conduct the bioassay of anti-arthritic activity by assessing the magnitude of HA degradation. An improved ELISA-like tool is being developed, through which hyaluronidase action can easily be detected in most biological samples.[34]

Hyaluronidase assay test is taken as one the best *in vitro* models for investigating anti-arthritic activity because HA is an endogenous substance and serves as a genuine pathophysiological indicator of RA.

All described *in vitro* models use chemical or biochemical markers to confirm the anti-arthritic activity of the test molecule. Such markers are very sensitive to the working atmosphere and are prepared under extremely controlled conditions, even with a slight change in mixture quantity or pH the test result would be affected to a greater extent.

CONCLUSION

Drug discovery starts with the aid of animals to assess the preliminary medicine profile. The benefits of animal research to the understanding of the human disease are without question. Virtually every clinical accomplishment of the most recent century depended straightforwardly or by implication on research with animals. In these past applications, animal models both complemented and coordinated clinical exploration. Creature models permitted clinical perceptions and speculations to be investigated in incredible profundity, while novel discoveries inferred in creature models were meant the clinical studies. Because of this association, animal research regularly advances all the while with clinical exploration, and both continually change as information about a specific infection or ailment obtained from one model is applied to the next.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RJP: Rheumatoid Joint Pain; RA: Rheumatoid Arthritis; **CFA:** Complete Freund's adjuvant; **TNF:** Tumor Necrosis Factor; **MSU:** Mono-sodium Urate; **IL:** Interleukins; **PBS:** Phosphate Buffer Saline; **HA:** Hyaluronic Acid; **ELISA:** Enzyme Linked Immuno-sorbent Assay; **PMNL:** Poly Morpho Nuclear Leukocytes; **HLA-DR:** Human Leukocyte Antigen – DR isotype; **MHC:** Major Histocompatibility Complex.

SUMMARY

Animal studies lay the foundation for drug discovery. It is the first step to searching for and scientifically establishing the therapeutic magnitude of a simple molecule. So, the significance of animal studies is an unquestionable and unavoidable step during drug discovery. This review article acknowledges the contribution and possible demerits of the creature model. Moreover, the article talks about selection criteria and crucial key points, which must be considered before selecting the pre-clinical study model. We encompass commonly used anti-arthritic *in vivo* as well as *in vitro* study models along with possible advantages and the grey areas of the test models. Lastly, I must say that improvements are essential in every segment of drug discovery, such as proper utilization of animal models so that clinical studies can be conducted more efficiently to get a potential new drug candidate, because the success of a drug depends on its intended therapeutic effects for the well being of humanity.

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