Journal of Young Pharmacists, 2024; 16(3):391-399.
https://www.jyoungpharm.org

Review Article

Animal Models: Catalysts for Advancing Topical Treatments in Rheumatoid Arthritis

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ABSTRACT

Rheumatoid Arthritis (RA) is a chronic autoimmune condition causing joint inflammation and damage. Despite treatment progress, effective topical therapies pose challenges. Animal models, like Collagen-Induced Arthritis (CIA) and Adjuvant-Induced Arthritis (AIA), mimic RA pathogenesis, aiding preclinical evaluation of topical treatments. By studying these models, experts can assess the efficacy, safety and pharmacokinetics of novel topical treatments, thereby accelerating their translation into clinical practice. Optimizing drug delivery, bioavailability and minimizing side effects are critical in formulation development. Animal studies refine parameters such as vehicle selection, drug concentration and application frequency for maximal therapeutic benefit. These models also deepen understanding of RA mechanisms, informing targeted topical therapy design against specific inflammatory mediators or immune cells. Lastly, animal models accelerate topical RA treatment development, providing vital data and mechanistic insights. Leveraging these models enhances formulation safety and efficacy, potentially improving patient outcomes.

Keywords: Animal models, Autoimmune diseases, Rheumatoid arthritis, Topical administration.

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Received: 29-02-2024;
Revised: 20-04-2024;
Accepted: 14-05-2024.

INTRODUCTION

Rheumatoid Arthritis (RA) stands as a significant challenge in the landscape of autoimmune diseases, characterized by chronic inflammation of the synovial joints, leading to joint damage, functional disability and systemic complications. Despite advancements in systemic therapies, the search for effective and safer treatment options remains crucial. In this context, the exploration of innovative topical formulations appears to be a potential area, aiming to enhance local drug delivery to inflamed joints while minimizing systemic side effects.[1,2] Conventional systemic therapies, including Disease-Modifying Anti-Rheumatic Drugs (DMARDs) and biologics, exhibit considerable efficacy in managing RA symptoms. However, their systemic administration often requires dose-limiting adverse effects and may not adequately target localized joint inflammation. Novel topical formulations offer an attractive alternative by facilitating targeted drug delivery to affected joints, thereby potentially optimizing therapeutic outcomes while mitigating systemic toxicity.[3] Yet, the development of topical formulations for RA presents multifaceted challenges. Overcoming the formidable barrier posed by the skin to ensure adequate drug penetration into the synovial tissue represents a pivotal concern. Additionally, achieving optimal drug concentrations at the target site necessitates precise formulation optimization to balance therapeutic efficacy with systemic absorption kinetics. Despite these challenges, the potential benefits of topical formulations, including improved patient compliance and reduced systemic toxicity, make them a promising avenue for RA treatment.[4,5]

Animal models are essential tools in elucidating RA pathophysiology and evaluating novel therapeutic interventions, including topical formulations. By inducing RA-like manifestations in animals, researchers can simulate disease progression and assess the efficacy, safety and pharmacokinetic profiles of topical treatments. For example, preclinical studies utilizing murine models of Collagen-Induced Arthritis (CIA) have demonstrated the efficacy of topical anti-inflammatory agents in ameliorating joint inflammation and preventing cartilage degradation. Similarly, investigations employing rodent models of Adjuvant-Induced Arthritis (AIA) have elucidated the pharmacokinetic profiles and optimal dosing regimens of topical corticosteroid formulations.[6-8] To summarise, the development of novel topical formulations represents a promising avenue in RA therapeutics, offering the potential for enhanced local drug delivery and improved therapeutic outcomes. Leveraging animal models enables rigorous preclinical evaluation of these formulations, facilitating their translation from bench to bedside. By overcoming the challenges inherent in topical drug delivery and harnessing the molecular insights gathered from animal

DOI: 10.5530/jyp.2024.16.51

studies, researchers are poised to reform RA treatment paradigms and, eventually, reduce the impact of this fatal illness.

ANIMAL MODELS OF RHEUMATOID ARTHRITIS

Collagen-Induced Arthritis (CIA)

Creating a Collagen-Induced Arthritis (CIA animal model for RA) involves several steps, precisely executed to induce an autoimmune response mimicking human RA pathology.

Selection of animal model

CIA is commonly induced in mice or rats due to their genetic similarity to humans and the availability of various genetic strains. DBA/1J mice are widely used due to their susceptibility to collagen-induced arthritis.

Preparation of collagen solution

Type II Collagen (CII) extracted from cartilage is commonly used to induce arthritis. The CII solution is prepared by dissolving bovine or chicken collagen in acetic acid to a concentration of 2-4 mg/mL and emulsifying with an adjuvant such as Complete Freund's adjuvant (CFA).

Immunization

Mice are immunized with the CII emulsion by intradermal injection, typically at the base of the tail or hind limb footpad. Each mouse receives a standardized dose of CII, usually 100-200 µg, mixed with an equal volume of CFA.

Booster injection

Approximately 21 days after the initial immunization, a booster injection of CII emulsified in incomplete Freund's Adjuvant (IFA) is administered to enhance the immune response.

Clinical assessment

Disease progression is monitored by evaluating clinical symptoms such as joint swelling, erythema and loss of mobility. Scoring systems are often employed to quantify arthritis severity, with higher scores indicating more severe disease.

Histological analysis

At the desired endpoint, mice are euthanized and joints (typically hind limbs) are harvested for histological examination. Joint tissues are fixed, decalcified, sectioned and stained to assess inflammation, synovial hyperplasia, cartilage destruction and bone erosion.

Data analysis

Data collected from clinical assessments and histological analyses are statistically analysed to compare disease severity between experimental groups and controls. Parameters such as arthritis incidence, severity scores and histopathological scores are quantified and compared.[9-11]

Adjuvant-Induced Arthritis (AIA)

Several procedures are required to create an Adjuvant-Induced Arthritis (AIA) animal model for RA. Firstly, rodents, commonly rats or mice, are selected for their genetic similarity to humans and susceptibility to arthritis induction. Typically, Lewis rats are chosen for AIA due to their susceptibility. The arthritis induction is achieved by preparing Complete Freund's Adjuvant (CFA), which involves mixing heat-killed *Mycobacterium tuberculosis* in mineral oil to form an emulsion. Subsequently, rats are immunized with CFA via subcutaneous injection, usually at the base of the tail or hind limb footpad, with each rat receiving a standardized dose of CFA. Disease progression is monitored through clinical assessments, evaluating symptoms like joint swelling, erythema and loss of mobility. Scoring systems quantify arthritis severity. Rats are humanely euthanized at the end of the study and their joint tissues, generally from their hind legs, are collected for analysis under the microscope. These tissues undergo fixation, decalcification, sectioning and staining to assess inflammation, synovial hyperplasia, cartilage destruction and bone erosion. Data from clinical evaluations and histological investigations are statistically analysed to evaluate disease severity across experimental and control groups, with characteristics such as arthritis incidence, severity ratings and histopathology scores measured and compared. This comprehensive methodology enables the creation of an AIA model for studying RA pathogenesis and potential therapeutic interventions.[12-14]

K/BxN Serum Transfer Model

K/BxN Serum Transfer Model for RA involves specific steps aimed at inducing arthritis-like symptoms through the passive transfer of arthritogenic antibodies. Initially, donor mice of the K/BxN strain, known for spontaneously developing arthritis-like symptoms, are selected due to their high levels of arthritogenic antibodies, particularly targeting Glucose-6-Phosphate Isomerase (GPI). Serum containing these antibodies, obtained through centrifugation of blood collected from K/BxN mice, is then prepared. Recipient mice, usually wild-type or genetically modified strains chosen for their susceptibility to arthritis induction, are prepared for serum transfer by often receiving a sublethal dose of Lipopolysaccharide (LPS) to enhance joint tissue susceptibility to antibody-mediated inflammation. Subsequently, recipient mice are intravenously injected with the K/BxN serum, wherein the arthritogenic antibodies bind to GPI in joint tissues, triggering an inflammatory response leading to arthritis-like symptoms. Disease progression in recipient mice is monitored through clinical assessments, including evaluating joint swelling, erythema and paw thickness, with scoring systems employed to quantify arthritis severity. At the endpoint, recipient mice are euthanized and joint tissues are harvested

for histological examination to assess inflammation, synovial hyperplasia, cartilage destruction and bone erosion. Data collected from clinical assessments and histological analyses are then statistically analysed to compare disease severity between experimental groups and controls, with parameters such as arthritis incidence, severity scores and histopathological scores quantified and compared. This methodology provides a valuable tool for studying RA pathogenesis and evaluating potential therapeutic interventions.[8,12,15]

TNF-α Transgenic Mice

Generating TNF-α Transgenic Mice for RA involves designing a transgenic construct containing the human TNF-α gene under a tissue-specific or constitutive promoter, such as the human Cytomegalovirus (CMV) promoter or tissue-specific promoters like the Collagen type II (Col2a1) promoter for joint-specific overexpression. This construct is then microinjected into fertilized mouse embryos at the pronuclear stage and implanted into pseudo-pregnant female mice. Offspring carrying the transgene are identified through Polymerase Chain Reaction (PCR) analysis of tail biopsies or screening for reporter gene expression if a reporter gene is co-expressed with TNF-α. Further characterization confirms TNF-α overexpression, typically through quantitative PCR analysis of TNF-α mRNA levels or by measuring TNF-α protein levels using techniques like Enzyme-Linked Immunosorbent Assay (ELISA) or immunoblotting. Subsequently, transgenic mice are monitored for arthritis-like symptoms, including joint swelling, erythema and loss of mobility, with clinical scoring systems used to quantify arthritis severity. Upon euthanasia, joint tissues are harvested for histological examination to assess inflammation, synovial hyperplasia, cartilage destruction and bone erosion. Data from clinical assessments and histological analyses are then statistically analysed to compare disease severity between transgenic mice and controls. This methodology facilitates the study of RA pathogenesis and potential therapeutic interventions.[16-20]

METHODOLOGY IN ANIMAL STUDIES

Selection and Characterization of Animal Models

The selection of appropriate animal models is fundamental to accurately recapitulate the pathophysiology of RA and predict treatment outcomes. Commonly used animal models include Collagen-Induced Arthritis (CIA), Adjuvant-Induced Arthritis (AIA) and transgenic mice overexpressing pro-inflammatory cytokines such as Tumour Necrosis Factor-alpha (TNF-α).[6] Characterization of animal models involves inducing arthritis through various methods, such as immunization with type II collagen or injection of adjuvants and assessing disease severity using clinical, histological and molecular parameters. For example, in CIA models, arthritis severity is evaluated based on paw swelling, joint tenderness and histopathological scoring of synovial inflammation and cartilage destruction.[21,11]

Formulation Development and Optimization

These potent medications often induce unintended effects that significantly reduce patient adherence. Moreover, traditional nonsteroidal anti-inflammatory formulations have several problems, including limited solubility and permeability, poor bioavailability, breakdown by gastrointestinal enzymes, dietary interactions and toxicity. To circumvent these limitations, researchers have resorted to the topical route of medication delivery, which has higher patient compliance and avoids the first past effect associated with conventional oral administration. Furthermore, nanosized carriers such as liposomes, nanoemulsions, niosomes, ethosomes, solid lipid nanoparticles and transferosomes have been developed to improve medication absorption through epidermal layers and delivery to the site of inflammation. These drug delivery systems are non-toxic and have high drug encapsulation efficiency and they also provide sustained release of drug. Lipophilic nanocarriers and vesicular systems show great potential for selective drug delivery to inflamed barriers. For example, liposomal formulations can improve the solubility and stability of hydrophobic drugs while facilitating their uptake by inflamed synovial tissue. Optimization of formulations involves iterative refinement based on preclinical data, including *in vitro* release kinetics, skin permeation studies and stability assessments.[3,5]

Preclinical Evaluation Techniques

Preclinical evaluation of topical treatments in animal models encompasses a range of techniques to assess safety, efficacy and pharmacokinetic properties. *In vivo* studies involve administering formulations to arthritic animals and monitoring clinical parameters such as joint swelling, mobility and pain. Histological analysis is performed to evaluate tissue inflammation, cartilage degradation and immune cell infiltration, providing insights into treatment effects on joint pathology. Biomarker analysis, including pro-inflammatory cytokines and matrix metalloproteinases, offers additional mechanistic insights into disease progression and treatment responses. Pharmacokinetic studies measure drug absorption, distribution, metabolism and excretion to optimize dosing regimens and predict human pharmacokinetics. Techniques such as microdialysis and imaging modalities like Positron Emission Tomography (PET) can provide spatial and temporal information on drug distribution and pharmacodynamics.[7,22]

Integration of Data and Interpretation

Integrating data from various preclinical evaluation techniques allows for a comprehensive understanding of treatment effects and mechanisms of action. Correlation of clinical, histological and molecular endpoints enables researchers to assess treatment efficacy and safety. Statistical analysis of preclinical data is essential for quantifying treatment effects and determining statistical significance. Methods such as analysis of variance (ANOVA) and

non-parametric tests are commonly used to compare multiple treatment groups and assess differences in outcome measures.[23,24]

Post-Animal Study Evaluation Parameters

Clinical Efficacy

Clinical efficacy in the context of post-animal treatment (preclinical evaluation) of RA refers to the effectiveness of therapeutic interventions in mitigating disease severity, arthritis severity scores and functional disability. The evaluation encompasses several key aspects like:

Disease severity assessment

This involves assessing improvements in clinical manifestations of RA, such as joint swelling, erythema and mobility impairment. Therapeutic interventions aim to alleviate these symptoms, leading to reduced inflammation and improved joint function. Quantitative measurements of joint swelling and erythema, along with subjective evaluations of pain and stiffness, provide valuable insights into the treatment's efficacy in mitigating RA disease severity.[25-27]

Arthritis severity scores

Standardized scoring systems, including the Clinical Disease Activity Index (CDAI) or Disease Activity Score (DAS), are utilized to quantify arthritis severity objectively. These scoring systems incorporate various clinical parameters, including swollen and tender joint counts, patient and physician global assessments and acute-phase reactants. Reductions in arthritis severity scores following treatment administration indicate the therapeutic efficacy of the intervention in modulating RA disease activity and inflammation.[28]

Functional Disability Evaluation: Improvements in functional capacity and quality of life are assessed using validated instruments such as the Health Assessment Questionnaire (HAQ). The HAQ evaluates the impact of RA on activities of daily living, including dressing, grooming and walking. Reductions in HAQ scores post-treatment reflect enhancements in functional abilities and overall well-being, indicating the clinical efficacy of the therapeutic intervention in mitigating RA-related functional disability.[29,30]

Biomarker Analysis

Biomarker analysis plays a crucial role in evaluating treatment response and disease progression in RA. In the context of preclinical evaluation post-animal treatment, two key categories of biomarkers are commonly assessed:

Inflammatory Markers

These biomarkers provide insights into the inflammatory processes underlying RA pathogenesis and response to treatment. Measuring serum levels of key inflammatory cytokines, such as Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-6 (IL-6), allows researchers to gauge the extent of inflammation and monitor changes in response to therapeutic interventions.[31] Additionally, acute-phase reactants like C-Reactive Protein (CRP) and Erythrocyte Sedimentation Rate (ESR) are indicative of systemic inflammation and are often used as surrogate markers for disease activity. Reductions in the levels of these inflammatory markers following treatment administration suggest a decrease in RA disease activity and inflammation, reflecting a favorable treatment response.[32]

Autoantibodies

RA-specific autoantibodies play a pivotal role in disease diagnosis, prognosis and monitoring. Rheumatoid Factor (RF) and Anti-Citrullinated Protein Antibodies (ACPAs) are two prominent autoantibodies associated with RA. RF is directed against the Fc portion of Immunoglobulin G (IgG), while ACPAs target citrullinated peptides derived from various proteins. Elevated levels of RF and ACPAs are strongly correlated with RA disease activity and progression, as well as increased joint damage. Therefore, assessing changes in RF and ACPA levels post-treatment provides valuable insights into treatment efficacy and disease modification. Reductions in RF and ACPA levels following treatment administration indicate suppression of autoantibody production and may correlate with improvements in disease.[33]

Histopathological Evaluation

Histopathological evaluation plays a pivotal role in assessing disease severity and tissue damage in RA. This evaluation involves:

Synovial inflammation

Joint tissues are analyzed for signs of inflammation, including synovial hyperplasia, pannus formation and leukocyte infiltration. Histological staining techniques such as Hematoxylin and Eosin (H&E) staining allow visualization of cellular infiltrates and structural changes in the synovium. Synovial hyperplasia, characterized by the thickening of the synovial lining due to increased cell proliferation, is a characteristic feature of RA pathology.[34] Pannus formation, the invasion of inflammatory tissue into the joint space, leads to cartilage destruction and joint deformity. Leukocyte infiltration, particularly by lymphocytes and macrophages, perpetuates the inflammatory response in RA joints.

Cartilage and bone integrity: Cartilage degradation, bone erosion and joint destruction are assessed through histomorphometric analysis and imaging modalities such as micro-Computed Tomography (micro-CT).[35] Histomorphometric analysis quantifies changes in cartilage thickness, chondrocyte density and proteoglycan content, providing insights into cartilage degradation. Bone erosion, characterized by the loss of bone tissue at the joint margins, is visualized and quantified using micro-CT

imaging. This technique allows for the three-dimensional assessment of bone architecture and the precise measurement of erosions, facilitating the evaluation of disease progression and treatment efficacy in RA.[36]

Pharmacokinetics (PK) and Pharmacodynamics (PD)

Pharmacokinetics (PK) and Pharmacodynamics (PD) evaluation is essential for understanding the behavior and therapeutic effects of drugs in RA treatment. This evaluation involves:

Drug levels

Quantifying drug concentrations in serum and target tissues to determine PK parameters such as bioavailability, distribution and elimination kinetics. PK studies assess how drugs are absorbed, metabolized and excreted by the body. By measuring drug levels over time, researchers can determine the optimal dosing regimen and ensure therapeutic concentrations are achieved to effectively manage RA symptoms.[37]

Molecular pathways

Investigating the impact of the therapeutic candidate on relevant molecular pathways implicated in RA pathogenesis, including cytokine signaling, immune cell activation and tissue remodeling. PD studies elucidate how drugs modulate specific molecular targets and biological pathways associated with RA. For example, therapeutic agents targeting Tumor Necrosis Factor-alpha (TNF-α) or Interleukin-6 (IL-6) pathways can inhibit pro-inflammatory cytokine production and attenuate immune cell activation, leading to reduced inflammation and joint damage in RA patients. Additionally, drugs may influence tissue remodeling processes such as synovial hyperplasia and cartilage degradation, thereby preserving joint integrity and function.[14,16]

Safety Assessment

Safety assessment is critical in evaluating the potential risks associated with therapeutic interventions for RA. This assessment involves:

Toxicity profiles

Comprehensive toxicology studies are conducted to evaluate the potential adverse effects of the treatment on vital organs and physiological systems. These studies assess the toxicity profile of the drug, including its effects on organ function, hematological parameters and systemic toxicity. Commonly evaluated organs include the liver, kidneys, heart and lungs, as well as hematopoietic and immune systems. By identifying potential toxicities early in the drug development process, researchers can mitigate risks and ensure patient safety during clinical trials and subsequent use;

Immunogenicity

Monitoring the development of Anti-Drug Antibodies (ADAs) and immune-related adverse events is essential to assess the immunogenic potential of the therapeutic candidate. ADAs can form in response to biologic therapies, leading to reduced drug efficacy, infusion reactions, or autoimmune responses. Immunogenicity studies evaluate the incidence and titers of ADAs in treated individuals and assess their impact on treatment outcomes and safety. Additionally, immune-related adverse events, such as hypersensitivity reactions, cytokine release syndrome, or autoimmune phenomena, are closely monitored to identify potential safety concerns associated with the therapeutic intervention.[38,39]

Cellular and Molecular Mechanisms

Elucidating the underlying mechanisms of action of the treatment through *in vitro* studies and *ex vivo* analyses of immune cell function, cytokine production and tissue-specific responses. *In vitro* studies involve culturing immune cells, such as macrophages, T cells and B cells, in the presence of the therapeutic agent to assess its impact on cell activation, proliferation and cytokine secretion. These studies provide insights into the direct effects of the treatment on immune cell function and cytokine signaling pathways implicated in RA pathogenesis.[37] Additionally, *ex vivo* analyses of joint tissues from RA patients or animal models allow researchers to investigate tissue-specific responses to the treatment, including changes in synovial inflammation, cartilage degradation and bone remodeling.[40] By elucidating the cellular and molecular mechanisms underlying treatment efficacy, researchers can identify potential therapeutic targets and optimize treatment strategies for RA management.[41]

Efficacy of Topical Anti-inflammatory Agents in CIA Model

In a study by Zhang W. *et al.* (2018), the efficacy of topical anti-inflammatory agents was evaluated in a Collagen-Induced Arthritis (CIA) model. The purpose of this preclinical study was to evaluate the therapeutic potential of topical therapies for reducing joint inflammation and avoiding cartilage deterioration, both of which are characteristics of RA pathogenesis. Through rigorous experimental design and comprehensive outcome measures, including clinical scoring of arthritis severity and histological analysis of joint tissues, the study demonstrated promising results. Topical administration of anti-inflammatory agents effectively reduced joint inflammation and protected against cartilage destruction in CIA mice, highlighting the potential of topical therapies as a targeted approach for RA management.[39]

Pharmacokinetics of Topical Corticosteroids in AIA Model

Another insightful example comes from study by Smolen J. (2020), which focused on the pharmacokinetics of topical

corticosteroids in an Adjuvant-Induced Arthritis (AIA) model. This preclinical investigation aimed to elucidate the absorption, distribution, metabolism and excretion of topical corticosteroids following local administration in arthritic animals. By employing sophisticated pharmacokinetic techniques, such as microdialysis and mass spectrometry, the study provided critical insights into the pharmacokinetic profiles of topical corticosteroids in AIA rats. The findings revealed important parameters influencing drug bioavailability and tissue distribution, informing optimal dosing regimens and formulation strategies for topical corticosteroids in RA treatment.[42]

Some other examples are briefly explored in Table 1.

Topical formulation development for RA requires careful consideration of several key factors to ensure optimal therapeutic outcomes while minimizing systemic side effects.

Drug Delivery Mechanisms

Effective drug delivery mechanisms are critical for delivering Active Pharmaceutical Ingredients (APIs) to the inflamed joints in RA while minimizing systemic exposure. Various delivery systems, such as liposomes, nanoparticles and hydrogels, offer distinct advantages in enhancing drug penetration into the affected tissues. Liposomal formulations, for instance, encapsulate APIs within lipid bilayers, improving their solubility and stability while

Table 1: A comprehensive overview of various studies on topical treatments for RA, highlighting the diversity of methodologies, formulations and model systems utilized in preclinical research.

Model Used	Formulation Used	Methodology	Analysis	Conclusion	References
Collagen-Induced Arthritis (CIA).	Topical Anti-inflammatory Agents.	CIA was induced in mice. Topical anti-inflammatory agents were applied and arthritis severity was assessed.	Significant reduction in joint inflammation and cartilage degradation compared to controls.	Topical agents show promise as targeted therapy for RA.	21
Adjuvant-Induced Arthritis (AIA).	Topical Corticosteroids.	AIA was induced in rats. Topical corticosteroids were administered and pharmacokinetic parameters were evaluated.	Revealed important pharmacokinetic parameters influencing drug bioavailability and tissue distribution.	Insights for optimizing drug delivery and enhancing efficacy in RA.	43
Collagen-Induced Arthritis (CIA).	Herbal Formulations.	CIA was induced in mice. Herbal formulations were administered and arthritis severity was assessed.	Significant reduction in joint inflammation, cartilage degradation and pro-inflammatory cytokine levels.	Herbal formulations may serve as complementary therapies for RA.	44
TNF-α Transgenic Mice.	Liposomal DMARD Formulation.	TNF-α transgenic mice were treated with liposomal DMARDs. Disease progression was monitored.	Effective suppression of joint inflammation and disease progression compared to conventional DMARDs.	Liposomal DMARDs hold promise for targeting inflammation in RA.	45
Collagen-Induced Arthritis (CIA).	Nanoparticles	CIA was induced in mice. NSAID-loaded nanoparticles were administered and arthritis severity was assessed.	Enhanced therapeutic efficacy and reduced systemic toxicity compared to free NSAIDs.	Nanoparticle delivery enhances NSAID efficacy with fewer side effects.	46
Adjuvant-Induced Arthritis (AIA).	Hydrogels	AIA was induced in rats. Hydrogel-based formulations of anti-inflammatory agents were administered.	Sustained release of therapeutic agents, resulting in prolonged anti-arthritic effects.	Hydrogel formulations offer sustained relief from arthritis symptoms.	47

Model Used	Formulation Used	Methodology	Analysis	Conclusion	References
K/BxN Serum Transfer Model.	Transdermal Patches.	Arthritogenic serum was transferred to recipient mice. Biologic-loaded transdermal patches were applied.	Efficient delivery of biologics, leading to significant reduction in arthritis severity.	Transdermal patches provide a promising delivery platform for biologics.	15
Collagen-Induced Arthritis (CIA).	Liposomal Herbal Extracts.	CIA was induced in mice. Herbal extracts were encapsulated in liposomes and administered to arthritic mice.	Liposomal delivery enhanced bioavailability and therapeutic efficacy of herbal extracts.	Liposomal encapsulation improves the therapeutic potential of herbal extracts.	48
Adjuvant-Induced Arthritis (AIA).	Gold Nanoparticles.	AIA was induced in rats. Anti-inflammatory drugs were conjugated to gold nanoparticles and administered.	Targeted delivery of drugs to inflamed joints, resulting in potent anti-arthritic effects.	Gold nanoparticles offer a promising platform for targeted drug delivery in RA.	49
Collagen-Induced Arthritis (CIA).	Dendrimer-based Formulations.	CIA was induced in mice. Anti-inflammatory drugs were encapsulated in dendrimers and administered.	Enhanced drug stability and prolonged retention in joints, leading to improved therapeutic outcomes.	Dendrimer-based formulations hold potential for RA treatment.	50
TNF-α Transgenic Mice.	Microneedle Patches.	TNF-α transgenic mice were treated with microneedle patches loaded with anti-inflammatory agents.	Efficient and painless delivery of drugs, resulting in significant reduction in arthritis severity.	Microneedle patches offer a convenient and effective delivery method for RA treatment.	51

facilitating their uptake by synovial tissue.[52] Nanoparticle-based delivery systems offer sustained release kinetics, prolonging drug retention at the site of action. By tailoring the formulation to exploit specific drug delivery mechanisms, researchers can enhance the bioavailability and therapeutic efficacy of topical treatments for RA.

Analytical Techniques for Bioavailability Enhancement

Analytical techniques such as microdialysis and mass spectrometry play a pivotal role in optimizing drug delivery and bioavailability enhancement. These techniques enable precise measurement of drug concentrations in tissues, facilitating pharmacokinetic studies and formulation optimization. Microdialysis, for example, allows for real-time sampling of interstitial fluid, providing insights into drug distribution and metabolism.[53] By understanding the pharmacokinetic behavior of topical formulations, researchers can design formulations that maximize drug bioavailability at the target site while minimizing systemic exposure and off-target effects.

Minimization of Systemic Side Effects

Minimizing systemic side effects is essential for improving patient safety and tolerability of topical formulations for RA. Strategies for minimizing systemic exposure include optimizing drug release kinetics, selecting appropriate excipients and incorporating targeted delivery mechanisms. For instance, by ensuring that the majority of the API remains localized to the site of inflammation, systemic side effects can be minimized without compromising therapeutic efficacy.[52] Additionally, rigorous preclinical evaluation using animal models can help identify potential safety concerns early in the development process, enabling researchers to refine formulations and mitigate any adverse effects before advancing to clinical trials.

Challenges and Future Directions

Despite the advancements in methodology, challenges remain in translating preclinical findings to clinical practice. Variability in disease presentation and treatment responses among animal models and humans underscores the importance of careful interpretation and validation of preclinical data. Future directions

in methodology include the development of more predictive animal models that better recapitulate the heterogeneity and complexity of human RA. Integration of advanced imaging techniques and biomarkers into preclinical studies holds promise for improving the predictive value of animal models and accelerating the translation of novel topical treatments from bench to bedside. Methodology in animal studies is essential for advancing topical treatments for RA. Through careful selection and characterization of animal models, rigorous formulation development and optimization and comprehensive preclinical evaluation techniques, researchers can generate robust preclinical data to inform clinical trial design and regulatory approval of novel therapies. By addressing the challenges and embracing innovative approaches, the field is poised to revolutionize RA treatment paradigms and improve outcomes for patients.

CONCLUSION

Rheumatoid arthritis (RA) remains a significant challenge in autoimmune disease management, marked by chronic joint inflammation, functional disability, and systemic complications. Systemic therapies like DMARDs and biologics are effective but often have dose-limiting side effects and fail to sufficiently target localized joint inflammation. Innovative topical formulations present a promising alternative, aiming to deliver drugs directly to inflamed joints, thus enhancing therapeutic outcomes while minimizing systemic toxicity. Animal models such as collagen-induced arthritis (CIA), adjuvant-induced arthritis (AIA), and TNF-α transgenic mice are essential for preclinical evaluation. These models elucidate RA pathophysiology, evaluate novel therapies, and optimize drug formulations. Preclinical studies have shown that topical anti-inflammatory agents can reduce joint inflammation and prevent cartilage degradation in RA models. Despite their potential, topical formulations face challenges like ensuring adequate skin penetration and achieving optimal drug concentrations at target sites. Advances in drug delivery systems and formulation optimization, including nanosized carriers, offer promising solutions to these issues. Future RA treatment should integrate advanced imaging techniques and biomarkers into preclinical studies to enhance the predictive value of animal models and expedite the translation of novel therapies from bench to bedside. Addressing these challenges with innovative approaches could revolutionize RA treatment paradigms, ultimately improving patient outcomes and quality of life.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ACPAs: Anti-citrullinated protein antibodies; **ADAs:** Anti-drug antibodies; **AIA:** Adjuvant-induced arthritis; **ANOVA:** Analysis of variance; **APIs:** Active pharmaceutical ingredients; **CDAI:** Clinical disease activity index; **CFA:** Complete Freund's adjuvant; **CIA:** Collagen-induced arthritis; **CII:** Type II collagen; **CMV:** Cytomegalovirus; **CRP:** C-reactive protein; **DAS:** Disease activity score; **DMARDs:** Disease modifying anti-rheumatic drugs; **ELISA:** Enzyme-linked immunosorbent assay; **ESR:** Erythrocyte sedimentation rate; **GPI:** Glucose-6-phosphate isomerase; **HAQ:** Health assessment questionnaire; **IL-6:** Interleukin-6; **LPS:** Lipopolysaccharide; **PCR:** Polymerase chain reaction; **RA:** Rheumatoid arthritis; **TNF-α:** Tumour necrosis factor-α.

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Cite this article: Kumari M, Gohil D, Sadhu P, Talele C, Shah N, Shah N. Animal Models: Catalysts for Advancing Topical Treatments in Rheumatoid Arthritis. J Young Pharm. 2024;16(3):391-9.