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Research Article

FIMD based model validation approaches for Rheumatoid arthritis and associated cardiovascular complications: an attempt towards translational competence

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Abstract

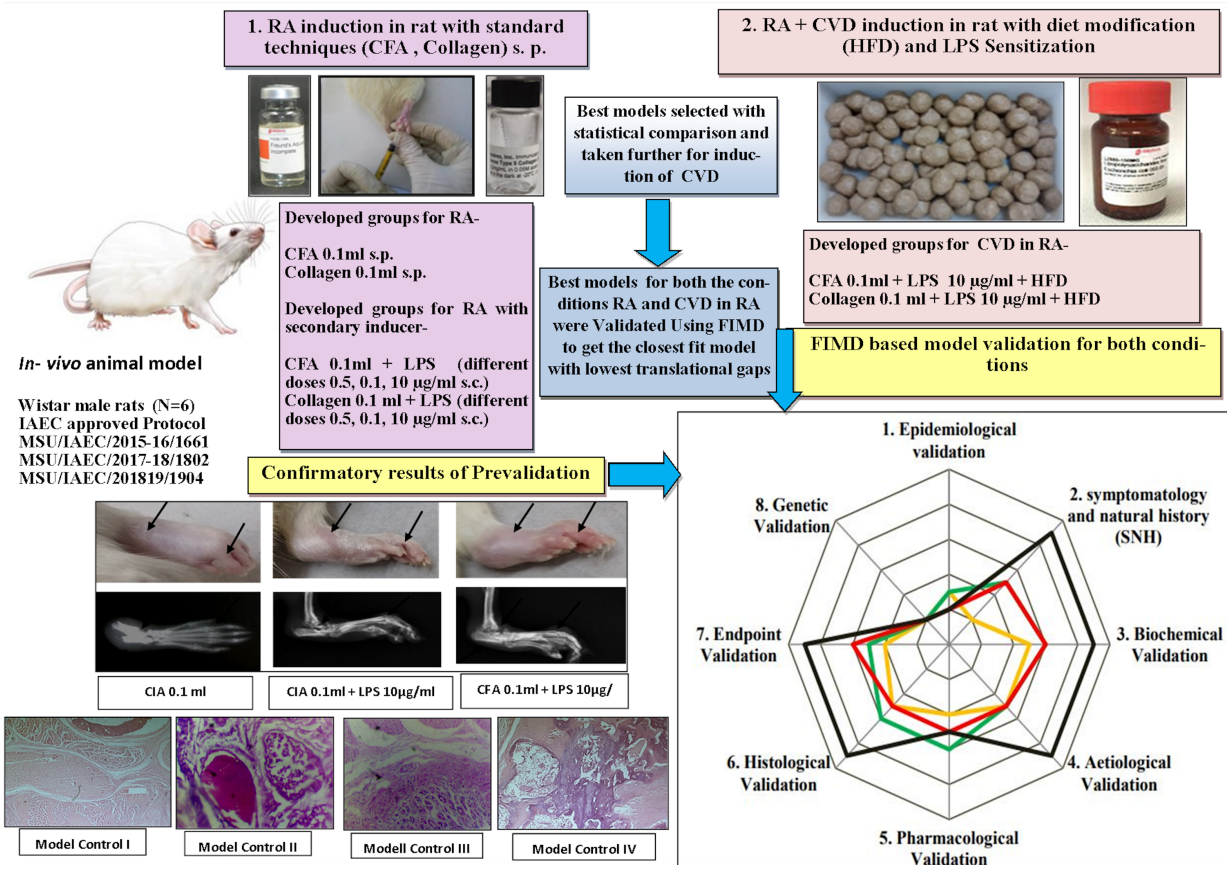
Introduction: The present *in-vivo* experiment was designed to validate newly developed rat models for Rheumatoid Arthritis (RA) as well as cardiovascular complications in RA for translational competence of disease with clinical situation. The FIMD (Framework to Identify Models for Disease) method based on etiology, pathophysiology, symptomatology, and response to therapeutic interventions was used to compare these models sensitized with primary (CFA, bovine collagen type II) and secondary inducing agents (high fat diet, lipopolysaccharide) in different combinations.

Material and Methods: Twenty-four Wistar male rats (four groups/n=6) were taken after prevalidation, where a large dataset was analyzed by using statistical methods (one way ANOVA, repeated Measure ANOVA). Among these groups, two best models from RA representative groups and two best models from cardiovascular complications in RA generated groups were taken for final comparison and validation by the same weight score method for obtaining a percentage similarity and validity score using a radar plot based on FIMD to get the best suited model.

Results and Discussion: The findings of this study on the basis of FIMD showed that the collagen (0.1 mL) +lipopolysaccharide (10 µg/mL) induced model is closely fit for preclinical events of RA, with the highest validity score (82%) among all groups and the collagen (0.1 mL)+ lipopolysaccharide (10 µg/mL)+HFD represents a validated model (95%) for co-morbid cardiovascular complications in RA

Conclusion: The developed and validated models were a fresh attempt using different inducers sequentially to culminate the emerging issue of extra organ manifestation in existing RA via a similar pathway, which can be a contributor to future research in drug discoveries in pharmacology.

Graphical abstract



Keywords

adjuvant (CFA), collagen, high fat diet, lipopolysaccharide, rheumatoid arthritis, validation

Introduction

The use of animal models as a small instrumental replica of human diseases is a basic biomedical tool. Here the model (rat/mouse/rabbit/guinea pig/dog/monkey) in pharmacology is something that mimics the human physiology and pathological conditions at certain levels (Lilley et al. 2020). In any research, the concept, findings, and conclusions should be able to generate the same results on recreating the model fundamentals with all variables (Dobbin et al. 2016), which is considered validity. An animal model is considered to be valid if it resembles the human conditions adequately and contributes valuable information to our knowledge of biology and medicine, including the discovery and development of new drugs to match preclinical and clinical events (Sams-Dodd 2006).

The present investigation was developed to create a rat model that can represent RA as well as the extra organ manifestations of RA. In this line of search, many studies were conducted to provide insight about RA and associated manifestations, but none of them is specific representation of cardiovascular complications in RA (Cassotta et al. 2020; Hong et al. 2020). To provide a scientifically proven solution, the current study was designed to get to the bottom of two major issues: 1) model resemblance with human disease by developing and validating experimental rat models for two situations; i) Rheumatoid Arthritis using Complete Freund's Adjuvant (CFA) and Bovine collagen type-II as primary inducing agents with lipopolysaccharide (LPS) as secondary inducing agent to get complete insight into etiology, progression, and maximum human resemblance. ii) Rheumatoid Arthritis with cardiovascular complications using a high fat diet (HFD) and LPS (Dubey and Chorawala 2015) in existing CFA and collagen models for combining RA with cardiovascular complications via metabolic dysbiosis and to minimize the translational gap between preclinical and clinical findings.

Multiple models were developed and compared to select the best models for both of these situations, applying traditional biostatistics in pharmacology, which shows the selectivity of four models. Furthermore, the FIMD method (Ferreira et al. 2019) was adopted for model validation, which provides standardization, integration, and the facility of model comparison on eight domains; epidemiology, symptomatology and natural history criteria (SNH) matching with human disease onset, biochemical validation, pharmacological validation, histological validations, endpoint validation and genetic validation to provide the internal and external endpoints (Ferreira et al. 2020).

Model validation in preclinical research

Validity indicates how refined your research is, in terms of proving the correctness of your hypothesis. Hindrances in model resemblance to human etiology and symptomatology are affected with the common laboratory limitation and the improper selection of a hypothesis which is based only on predictive data (Scannell et al. 2022). If a model selected is unable to prove modality and reproducibility, it can fail to level the gap between preclinical and clinical trials which is the most prominent reason of this defeat. The flawed preclinical data without a validation tool are inadequate to generate the sufficient data for further proceeding. These concepts of model validation are based on:

Predictive-validity – The basic target of predictive validity is to check to what extent the demonstrated model, in particular species, replicates the human disease condition. The predictive validity is based on the evaluation of the end points (parameters) based on statistical tools in terms of reliability and relevance. Reliability is assessed by calculating the inter-laboratory reproducibility and intra laboratory repeatability;

Face validity – Face validity is logical validity, which is primarily the theoretical consideration of the procedure as to what extent it is similar to the set hypothesis (Silverman et al. 2020);

Constrict validity and target validity – The target under investigation at the time of recreation should have a similar role in the disease model as in the clinical situation (Tadenev and Burgess 2019).

The basic validation strategies were given by Frank Sams-Dodd (2006), Wilner et al. (2011), and Tinneke Denayer et al. (2014), in which model selection is based only on external validity criteria such as species, strain, complexicity level and duration of treatment for an animal selected for studies.

These basics can be used to avoid the lower-level faults in conceptualization of a model. However, an animal model should also replicate the internal validity (symptomatology, disease-specific criteria, subjectivity and reproducibility), which is a prime requisite for the resemblance of clinical conditions. On the other side, if they are adequately designed and conducted, animal models can contribute valuable information to our knowledge of biology and medicine, including the discovery and development of new drugs (Deore et al. 2019).

On the basis of all the above-discussed points, the sequences in this study were conceptualized on two problem statements mentioned in relevance to RA model development.

The first and foremost challenge was to develop an animal model which is similitude to clinical (human like) complications arisen in RA, which was done by:

a) Comparing primary inducing agents CFA and Collagen

b) Comparing secondary inducing agent LPS with primary inducing agent (CFA and Collagen) in model developed for RA.

The challenge in model development was to take one more step in relation to complexity of diseases as incorporation of clinical co-morbid conditions (RA along with CVD). In attempted preclinical model development, cardiovascular complications were induced via the incorporation of one more secondary inducing agent; high-fat diet with CFA, collagen and LPS model to link the metabolic dysbiosis with already immune compromised animals to produce metabolic insults to crosslink inflammation and immune responses.

Materials and Methods

Animals

All experiments were carried out on male wistar rats (150-200 g), obtained from registered breeders of experimental animals. Animals were housed in well-controlled conditions of temperature (22±2°C), humidity (55±5%), and a 12-hrs light-dark cycle with free access to a conventional laboratory diet in all groups and high fat diet (HFD) in specific groups with purified water *ad libitum*.

All the mentioned studies were approved by the Institutional Animal Ethics Committee (IAEC), Pharmacy Department. Faculty of Pharmacy, The M. S. University of Baroda, vide Minutes MSU/IAEC/2015-16/1661 dated 30/12/2016, MSU/IAEC/2018-19/1802 dated 29/12/2018 and MSU/IAEC/2019-20/1904 dated 21/08/2019 in accordance with the guidance of the Committee for the Control and Supervision of Experiments on Animals (CCSEA) and The Prevention of Cruelty to Animals act (PCA), 1960, Department of Animal Husbandry and Dairying, Ministry of Fisheries Animal Husbandry and Dairying Government of India (DAHDMoFAH&D).

Experimental methods

This experimental research was framed on the basis of the results obtained with the following studies:

Study design I – Development of animal models for replication of Rheumatoid arthritis, where RA models were developed using CFA alone, CFA with LPS, collagen alone, and collagen with LPS.

Study design II – Development of animal models for replication of RA and CV complications using high fat diet with selected models from RA developed groups.

Methodology for Study design I

Objective one fulfilled by using two separate inducers considered as primary stimulants in different groups of animals:

CFA (Complete Freund's Adjuvant (CFA - each mL contains 1 mg heat killed and dried *Mycobacterium tuberculosis* in 0.85 mL paraffin oil and 0.15 mL mannide monooleate) purchased from Sigma-Aldrich, Bangalore, India, was used to sensitize the animals on day 0 where 0.1 mL of CFA was injected by sub planter route on the left hind paw of rats to induce RA (Kollias et al. 2011). The total observation period for this study was 28 days.

Bovine Collagen (Type II) was purchased from Chondrex Inc Redmond, WA, USA, supplied by Krishgen Biosystem Mumbai, India. It was used in different sets of animals, where premix of CFA and collagen (1:1) (Miyoshi and Liu 2024) was used to induce RA in rats. On day 0, animals were sensitized with injecting this premix by sub planter route on the left hind paw. In this experiment, the study period was framed for 42 days.

To further stimulate the disease severity, lipopolysaccharide (cell wall components of the gram negative bacteria *Escherichia coli* O111:B4) purchased from Sigma-Aldrich, Bangalore, India, was used as a secondary inducer. Here, LPS was dissolved in saline to get the desired concentrations and given to animals via the subcutaneous route to prevent sepsis-like conditions. LPS sensitization was done from days 14 to 28 in 0.1 mL dose by subcutaneous route in rats pre challenged with CFA as an inducing agent in concentrations of 0.1, 0.5, and 10 µg/mL. Sensitization was started after the first sign of immunological intervention as evidenced by increased neutrophil and lymphocytes in hematological investigations on day 12 of the study. In collagen-induced arthritis groups also, the rats were sensitized with LPS (0.5, 0.1, and 10 µg/mL doses from day 14 to 42 by subcutaneous route (See Supplement I for detailed procedure).

Methodology for study design II

The final selected groups from study 1 - group Va (CFA+LPS10 µg/mL) and group Vb (CIA 0.1 mL+LPS 10 µg/mL) were carried out for further assessment here.

All the animals selected in this protocol were fed with modified high fat diet (Dubey and Chorawala 2015) throughout the study period for creating atherogenic environment in animals. Lard rich diet (30%) was used in this study to activate the metabolic dysbiosis with the other expects of inflammation to link it with atherogenic episodes (See Supplement file I for further details about the models).

CFA and CIA were used here also for primary induction, whereas for secondary sensitization, LPS and HFD were used. The two groups mentioned above were compared with the following group of animals; group VI (CFA 0.1 mL+HFD), which received CFA 0.1 mL on day 0 along with HFD given for 28 days. Group VII (CFA 0.1 mL+HFD+LPS 10 µg/mL) was sensitized with CFA (0.1 mL) on day 0 and further sensitized with LPS (10 µg/mL), and HFD was given to the animals for 28 days. Group VIII (CIA 0.1 mL+HFD) received CIA (0.1 mL) on day 0 with further feeding with HFD for 42 days and group IX (CIA 0.1 mL+HFD+LPS 10 µg/mL) received CIA (0.1 mL) on day 0 and further sensitized with LPS (10 µg/mL) on day 14 till day 42, and animals were fed with HFD throughout the study period (42 days). All the parameters for assessment of RA were performed at different time points for 28 days in CFA groups and 42 days in CIA groups, respectively**.** All the developed groups are initially summarized in Fig. 1.

Notation: For better understanding in succeeding sections, the final compared groups will be denoted as - group Va (CFA 0.1 mL+LPS 10 µg/mL) as Model I, group Vb (CIA 0.1 mL+LPS 10 µg/mL) as Model II (Representing RA models), group VII (CFA 0.1 mL+HFD+LPS 10 µg/mL) as Model III and group IX (CIA 0.1 mL+HFD+LPS 10 µg/mL) as Model IV (representing RA along with CVD).

Evaluation parameters for prevalidation

The estimated parameters were analyzed for prevalidation on the basis of observational outcomes of physical estimations (Paw volume, Arthritic index, Arthritic score), biochemical estimations (Rheumatoid Factor (RF), ESR, C-RP, IL-6, TNF-α, CBC, Homocysteine and Anti-CCP, Triglyceride levels, Cholesterol, HDL, LDL levels, atherogenic index) and TLR-4 activation (Luo et al. 2020) by the statistical data. Moreover, pilot studies for estimation of dose response and variability of different inducing agents (CFA, collagen, LPS, HFD) were performed with exploratory experiments (treatment responses, hypothesis correction via *p* value correction using *post hoc* tests in ANOVA) (Barnett et al. 2022) and photography, X-Ray and histopathology (bone, vastus medialis and biceps femoris muscle) were made as confirmatory tests for evaluating the development of the model in both the groups sensitized with CFA and collagen with and without LPS and HFD addition.

Methods for final model validation

As these statistical data are predictive values, and the outcome is insufficient to give a model that can be called validated, so among all nine groups, four groups: Va (CFA 0.1 mL+LPS 10 µg/ml), Vb (CIA 0.1 ml+LPS 10 µg/ml), VII (CFA 0.1 ml+HFD+LPS 10 µg/ml) and IX (CIA 0.1 mL+HFD+LPS 10 µg/mL) were carried forward for external validity measures.

Here, a decision regarding the selection of an optimum/appropriate dose of LPS (among three concentrations of 0.1, 0.5 and 10 µg/mL) was made by processing the obtained data for significance using ANOVA as primary evaluation tool for pharmacological comparison. The selected models were carried forward for validation based on the Framework to Identify Models of Disease (FIMD) given by Guilherme S. Ferreira et al. (2020).

Model validation methods

Framework to identify models for disease

This is a questionnaire-based validation system in which models can be compared and evaluated for eight domains; epidemiology, symptomatology and natural history – SNH, biochemical validation, aetiological validation, pharmacological validation, histological validation, endpoint validation, and genetic validation.

These domains were firstly defined in a questionnaire, and the questions were framed according to disease severity and progression using the mentioned domains. Each domain was given the same weighing score. Questions (Table 1) were also answered separately for all subsections on the basis of the five answer patterns suggested (yes, yes completely, yes partially, unclear, and no).

After answering the questions with suitable grades, the summation of all the scores of sections and subsections of the individual domain was done to get the final score, and it was subtracted from the actual domain score to get ratio for plotting the radar chart, which is one of the comparative tools for analyzing multivariate data (See Supplement II for questionnaire and details of calculation steps provided in supplement III and IV).

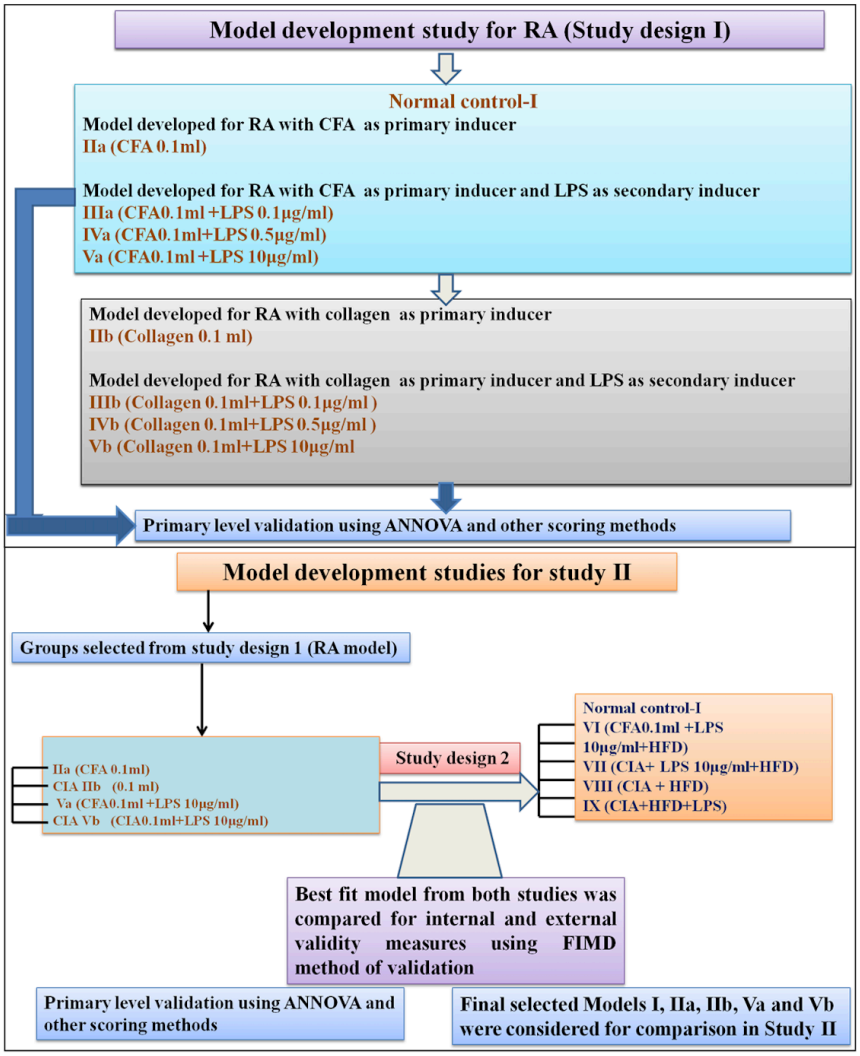


Figure 1. Study designs for development, selection and validation of models for RA and CVD in RA. ***Note:*** In this figure, the flow of model development is depicted where: Step I – Induction of RA in rats using CFA and collagen as primary inducers with and without LPS as secondary inducer was performed; Step II – Selection of models for RA was made on the basis of results of ANOVA used as pre validation tool; Step III – Here as depicted in flow chart, selected groups from RA induced models were further developed using CFA and collagen as primary inducers with LPS and HFD as secondary inducers to develop cardiovascular complications in existing RA; Step IV – models for cardiovascular complications in RA were further analyzed using prevalidation tool to select best fit model for CVD in RA; Step V – Same weight score method was applied to validate the models on the basis of FIMD method to compare and validate the developed models for RA and Cardiovascular complications in RA for human resemblance.

The framework was adopted and the questions were framed using the reference of the framework, but some extra points were added according to the need of the study. The questions were more focused on the Rheumatoid Arthritis and cardiovascular complications in the selected models with questions of clinical relevance (this is a sample questionnaire, See Supplement III and IV for detail).

Statistical analysis

Models with both situations (RA and cardiovascular complications in RA) were selected by prevalidation using statistical methods (one way ANOVA and Repeated measure ANOVA) using GraphPad Prism software, where values are expressed as Mean±SEM. Boneferroni's, Dunnett's, and Tukey's *post hoc* tests were used for comparisons between each group. Significant values were compared at *p<0.05, **p<0.01, ***p<0.001 (all the details are attached as Supplement file I).

The data obtained by ANOVA were considered as primary data and further compared by same weight score method of FIMD where all the domains were scored equally with subsections to make total score 100 to get a ratio which was used to interpret the radar plot. Here, Microsoft excel was used to generate a 2D radar plot, and the values (calculated ratio) for each domain were analyzed. The radar chart gives each domain an axis, and we can compare the models by putting the ratio obtained after giving a suitable score to each question of each domain and calculating it through the steps mentioned in the Supplement. To check the similar domains in models, similarity factors and uncertainty factors were also calculated which can account for improvements in experimental design.

The final scores were calculated by putting these values in radar plot and compared to check the levels of validation by each domain assigned. If the final percentage of the model lies between 0 and 40%, the model is considered *insufficiently validated*. If model scores are 41-60%, then it is considered *slightly validated*. If the model scores within 61-80%, it is *moderately validated,* and if the scores are within 81-100%, it is *highly validated*.

Table 1. Questionnaire for comparison of models developed for cardiovascular complications in RA

Questions for RA with CVD Model	SW
1. Epidemiology	12.5
1.1 Nature of population (Inbred/Outbred)	4.16
1.2 Is the model able to simulate the disease in the relevant age groups (juvenile, adult or aging)	4.16
1.3 Is the model able to simulate the disease in different genders	4.16
2. Symptomatology and natural history	12.5
2.1 Is model able to mimic human disease symptoms, if so which one	4.16
2.1.1 Inflammatory markers	1.38
2.1.2 Immunological markers	1.38
2.1.3 Crosslinking markers for cardiovascular complications	1.38
2.2 Natural History criteria matching with human disease onset	4.16
2.2.1 Time of onset	0.83
2.2.2 Disease progression	0.83
2.2.3 Duration of symptoms	0.83
2.2.4 Metabolic dysbiosis	0.83
2.2.5 GUT infiltration	0.83
2.3 Co-Morbid Conditions replicated in model similar to human conditions? if yes which one	4.16
2.3.1 Secondary lesions	1.04
2.3.2 Overlap syndrome (Digestion of digits)	1.04
2.3.3 Extra organ manifestation	1.04
2.3.4 Steatosis	1.04
3. Biochemical validation	12.5
3.1 Pharmacodynamic biomarkers mimic the pathophysiology of the human disease	4.16
3.1.1 Increased inflammatory markers (CRP, ESR, Arthritic Index)	1.38
3.1.2 Increased immunological markers (TNF-α, IL-6, ACCP, Hyc)	1.38
3.1.3 Increased atherogenic markers (TG, TC, Cholesterol, Atherosclerotic index)	1.38
3.2 Do these PD markers behave similarly to human?	4.16
3.3 Known prognostic markers related to pathophysiology of the disease	4.16
3.3.1 Walking disability	0.69
3.3.2 Symmetric progression of disease	0.69
3.3.3 Increase in secretions	0.69
3.3.4 Nodule formation	0.69
3.3.5 Obesity	0.69
3.3.6 Metabolic dysbiosis	0.69
4. Aetiological validation	12.5
4.1 Is the aetiology of the disease similar to human for Rheumatoid Arthritis? If yes which one	6.25
4.1.1 Cytokine activation (TNF-α, IL-6)	1.25
4.1.2 Cell infiltration (ACCP generation and Hyc activation)	1.25
4.1.3 Fibre length of vistus medialis	1.25
4.1.4 Fibre length of biceps femoris	1.25
4.1.5 Radiographic changes	1.25
4.2 Is the aetiology of the disease similar to human for RA and co-morbid conditions? If yes which one	6.25
4.2.1 Disability	1.56
4.2.2 Metabolic dysbiosis (Obesity, TG, TC, LDL, HDL and fat accumulation in stool)	1.56
4.2.3 Receptor activation (TLR-4, NLRP-3)	1.56
4.2.4 Extra organ manifestation	1.56
5. Pharmacological validation	12.5
5.1 Are effective drugs in humans also effective in this model?	4.16
5.1.1 Which one (Methotrexate)	
5.2 Are ineffective drugs also ineffective in this model?	4.16
5.3 Have drugs with different mechanisms of action and acting on different pathway been tested in this model?	4.16
5.3.1 Test drug 1	1.38
5.3.2 Test drug 2	1.38
5.3.3 Test drug 3	1.38
6. Histological validation	12.5
6.1 Do the histopathological structures in relevant tissues resemble the ones found in humans? If yes which one	4.16
6.1.1 Histopathology of bone	4.16
6.1.2 Histopathology of heart	4.16
6.1.3 Histopathology of vistus medialis and biceps femoris muscle	4.16
7. Endpoint validation	12.5
7.1 Are the endpoints used in preclinical studies are same or translatable to the clinical endpoints?	6.25
7.1.1 Radiographic changes	2.08
7.1.2 Perceptive changes	2.08
7.1.3 Cellular infiltration in histopathology	2.08
7.2 Are the methods used to assess preclinical endpoints comparable to the ones used to assess related clinical	6.25
7.2.1 Paw volume (pain and stiffness)	1.04
7.2.2 Walking disability	1.04
7.2.3 Symmetric progression	1.04
7.2.4 Severity of disease	1.04
7.2.5 Atherogenic biochemical markers (Lipid Profile)	1.04
7.2.6 Obesity and metabolic dysbiosis	1.04
8. Genetic validation	12.5
8.1 Does this species also have orthologous genes and /or proteins involved in the human disease	4.16
8.2 If so, are the relevant genetic mutations or alterations also present in the orthologous genes/proteins	4.16
8.3 If so, is the expression of such orthologous genes and/ or proteins similar to the human condition?	4.16

Note: SW – same weight score.

Results and Discussion

Results of model validation for RA

Models developed for RA were compared here to select the best model with a higher validity score, where Model I (CFA 0.1 mL+LPS 10 µg/mL) secured a moderately validated score 64% with the highest uncertainty factor of 36% and Model II (collagen 0.1 mL+LPS 10 µg/mL) secured s highly validated score of 82%, which shows the maximum clinical resemblance for RA with clinical situations. Moreover, the uncertainty factor for this model was 18%, which gives the accounts of symptomatology, biochemical estimation histological data and endpoint results domain, which are not common on the radar plot. Model II (collagen 0.1 mL+LPS 10 µg/mL) highly resembles human RA-like conditions and these validity scores suggest its validity in terms of severity and disease progression. The similarity factors were also calculated between these two models, which shows the points in domains where both the models have 29% similar representations of RA, which includes epidemiological, genetic and aetiological validation domains and they are similar throughout the model development with all the inducers (Figure 2).

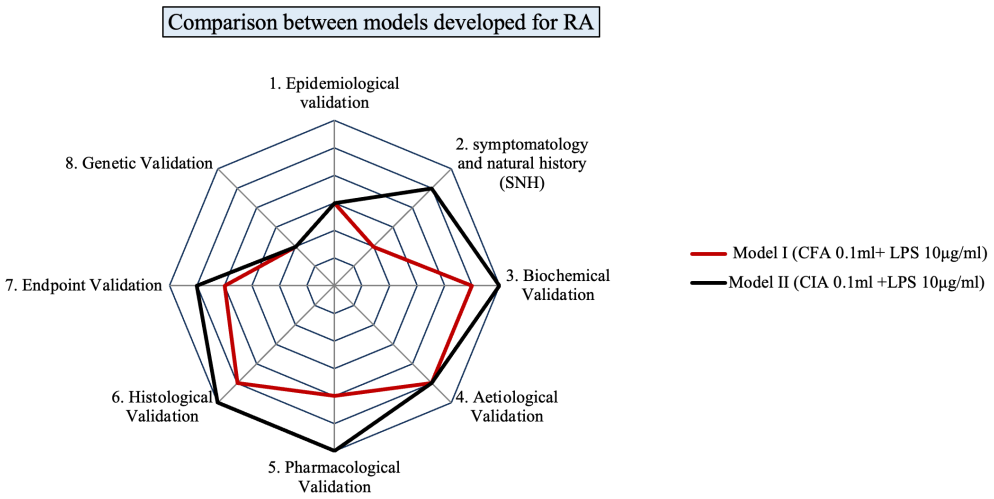


Figure 2. Radar plot for comparison between models developed for Rheumatoid arthritis.

Results of model validation among RA models and CVD in RA models

The statistical data showed that there is a potential of developing cardiovascular complication in comparative groups; model I (CFA 0.1 mL+LPS 10 µg/mL), model II (CIA 0.1 mL+LPS 10 µg/ml), model III (CFA 0.1 mL+LPS 10 µg/mL+HFD), and model IV (collagen 0.1 mL+LPS 10 µg/mL+HFD), which were compared for optimizing the interconnecting parameters responsible for progression of cardiovascular complications in existing RA. In final comparison, model IV (collagen 0.1 mL+LPS 10 µg/mL+HFD) secured a higher validation score of 95%, and the uncertainty factor was 5%, which proves that model IV has maximum resemblance with clinical situations. All these four models have some similar domains as the similarity factor among all the groups is 18% (Figure 3, Table 2).

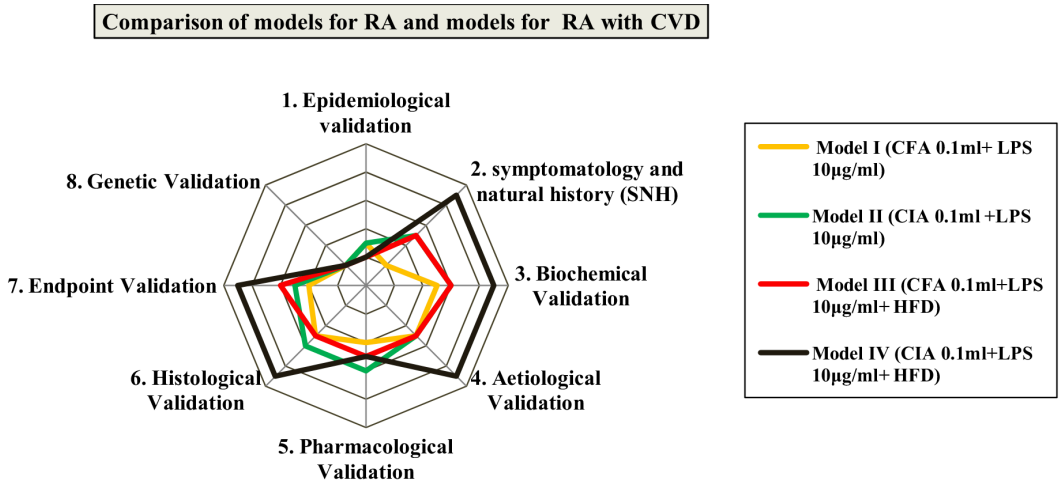


Figure 3. Radar plot for comparison between models developed for Rheumatoid arthritis and models developed for cardiovascular complications in RA.

Table 2. Score of same weight score obtained as ratio by FIMD questionnaire of RA models and CVD in RA models

Validation	Model I (CFA 0.1 mL+ LPS 10 µg/mL)	Model II (Collagen 0.1 mL+ LPS 10 µg/mL)	Model III (CFA 0.1 mL+ LPS 10 µg/mL+HFD)	Model IV (Collagen 0.1 mL+ LPS 10 µg/mL+HFD)
1. Epidemiological validation	0.3	0.3	0.2	0.2
2. symptomatology and natural history (SNH)	0.2	0.5	0.5	0.9
3. Biochemical Validation	0.5	0.6	0.6	0.9
4. Aetiological Validation	0.5	0.5	0.5	0.9
5. Pharmacological Validation	0.4	0.6	0.5	0.5
6. Histological Validation	0.5	0.6	0.5	0.9
7. Endpoint Validation	0.4	0.5	0.6	0.9
8. Genetic Validation	0.2	0.2	0.2	0.2
Interpretations from radar values				
Uncertainty Factor	36 %	18 %	12 %	5 %
Similarity Factor	18 %			
Validation Score	64 %	82 %	88 %	95 %

Results of model validation for best fit with human disease resemblance

The final comparison was done for optimizing a single model which can mimic the clinical symptoms of cardiovascular complications in patients suffering from RA. The objective was completed by comparison between the representative model of RA (collagen 0.1 mL+LPS 10 µg/mL) with representative model of RA with cardiovascular complications (collagen 0.1 mL+LPS 10 µg/mL+HFD) and the models represent 5% of similarities, which show the interconnection between both the diseases in terms of disease progression (Figure 4).

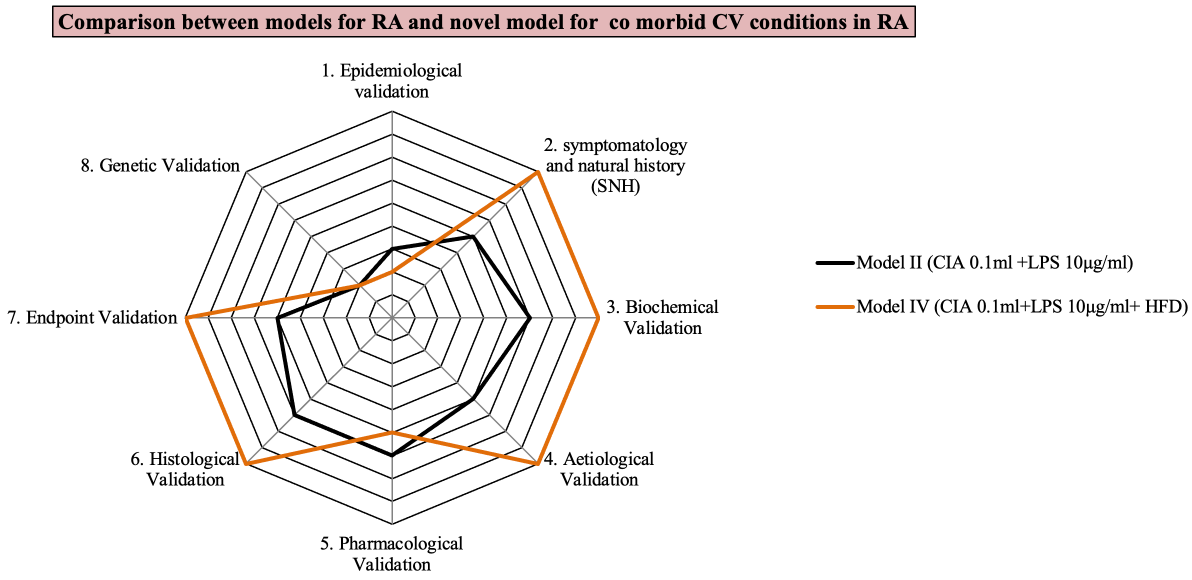


Figure 4. Radar plot for final comparison between Rheumatoid arthritis and models developed for cardiovascular complications in Rheumatoid arthritis for best fit with human disease resemblance.

Conclusion

On the basis of radar plot estimation, the comparison and model validation showed that in RA models – CFA with LPS (10 µg/mL) – have potential to represent RA but the translational competence of this model is moderate; disease symptoms are not created as in human situations. Whereas the groups sensitized with collagen with LPS (10 µg/mL) proved to give higher impressions of RA with higher similar factors in terms of resemblance of human deformities as secondary lesions and deformed bones in radiographic analysis, and these data were further confirmed with histopathological analysis.

In RA with CVD complications, models developed with HFD, CIA and LPS (10 µg/mL), when compared with other groups, were found to give higher interconnecting links of cardiovascular complications with RA as these models showed the high elevation in prevalidation parameters evaluated for RA (Anti-CCP, Paw volume, ESR, CRP TNF-α, IL-6, X-ray, histopathology of bone) and prevalidation parameters evaluated for CVD (Homocysteine levels, NF-κB, TLR-4, TG, TC, HDL, LDL, histopathology of vastus medialis and biceps femoris muscle).

On the basis of the above interpretations, we can conclude that model II (collagen 0.1 mL+LPS 10 µg/mL) is a highly validated model for Rheumatoid Arthritis with maximum human resemblance to inflammatory and immune component involvements. Another conclusion is that LPS in a 10 µg/ml dose can be used along with CFA and collagen to induce RA in animals. In the final comparison, we can draw the conclusion that HFD and LPS can be incorporated with collagen to develop atherogenic conditions in RA, as model IV (collagen 0.1 mL+LPS 10 µg/mL+HFD) is proved to be a highly validated and optimized model for cardiovascular complications in stable RA with the maximum insight into the clinical situation on the basis of the FIMD validation method.

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary information.

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Supplementary material 1

Details of study design I and II with preliminary (prevalidation) results

Authors: Trupti Dubey, Kirti V. Patel
Data type: pdf

Link: https://rrpharmacology.ru/index.php/journal/article/view/446/506

Supplementary material 2

Questionnaire details for comparison and validation of models for RA and CVD in RA

Authors: Trupti Dubey, Kirti V. Patel
Data type: pdf

Link: https://rrpharmacology.ru/index.php/journal/article/view/446/507

Supplementary material 3

FIMD domain wise calculations for model validation

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Data type: pdf

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Supplementary material 4

Final FIMD validation calculations for radar plot estimation and comparison to select best model for CVD in RA

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