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The cover page contains a figure from the article of Prof. Prabha Garg

EDITORIAL

The field of computer vision has been revolutionized by convolutional neural network (CNN). CNN employs the deep learning architecture and finds use in image recognition and pixel processing. The first article of this issue by Sharma and Garg goes beyond this conventional usage and presents diverse applications of CNN in the pharmaceutical industry. These range from optimization of drug design in terms of activity and specificity, screening of chemical libraries, monitoring drug-target interaction, etc. The authors present the basic premise of CNN and explain the concept of hierarchy in image recognition and processing. In drug design and discovery, CNN considers the spatial arrangement of the drug molecule or its target as the input and predicts information about novel high affinity binders as the output. Following training, the CNN model can be used to predict the behaviour of databases with components similar to the training dataset. Several such models have been described by the authors. The use of CNN in predicting pharmacokinetic and pharmacodynamic aspects of drugs using molecular images and in designing personalized therapy regimens has been presented. Diagnosis of diseases such as diabetic retinopathy, lymph node metastasis, tuberculosis and others using data obtained from imaging techniques by CNN is also possible. A few such examples have been presented. In addition, the application of CNN in predicting drug-drug interaction, drug safety and adverse reactions, pharmaceutical manufacturing and formulation design have also been highlighted.

The second article by Bharti, Chabra, Kondal, Ghai and Bansal is a meta-analysis of the efficacy of pharmacological and non-pharmacological agents for treatment of fibromyalgia, a chronic and multifocal pain condition whose etiology remains unclear. A combination of genetic and non-genetic factors seems to be important in the change in pain awareness. Interventions used at present mitigate the symptoms of the disease at best. However, the effectiveness of pharmacological and non-pharmacological interventions in fibromyalgia remains ambiguous. The authors carried out a comprehensive search of e-databases and analyzed randomized clinical trials (RCTs) in three categories of intervention and control. The studies were spread across several countries, with two of the being multi-centric. Both pharmacological and non-pharmacological interventions demonstrated no significant difference compared to treatment as usual. However, multi-component therapy showed significant improvement. The authors analyzed health status and depression in patients as secondary outcomes. The purpose of this meta-analysis was to provide support for informed decision-making by the clinician based on updated synopsis of available published data. The authors conclude that efficacy of non-pharmacological interventions and multi-component therapy can only be arrived at subject to availability of further high-quality data.

The third article in this issue by Dasgupta, Bajpayee, Pophali, Kadu, Jain and Misra discusses the efficacy of peptide therapeutics in search of a treatment strategy against Alzheimer's disease. Aggregation of A-beta42 and hyperphosphorylation of tau protein have been associated with disease progression. The authors discuss the challenges associated with conventional drug design protocols for inhibiting aggregation of Abeta and present peptides as an alternate approach to be explored in this area. As peptides composed of natural amino acids are substrates for endogenous proteases, synthetic foldamers based on unnatural amino acids have been highlighted. Such options include scaffolds based on beta- and gamma-amino acids and aromatic oligoamides. The authors conclude that structures based on such foldamers hold promise in designing therapeutic candidates in Alzheimer's disease and other untreatable conditions.

On behalf of the editorial team, I invite you all to read this edition and provide your valuable feedback.

Review Article

Convolutional Neural Network: Potential applications in the pharmaceutical sector

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In the past ten years, convolutional neural networks (CNN) have radically altered the landscape of computer vision and image processing and have attained cutting-edge performance in comparison to the traditional machine learning algorithms. Due to their ability to automatically learn hierarchical representations from raw input, CNNs have become a powerful tool for analyzing complex information in a wide variety of domains, from object recognition, classification, detection, and medical image analysis, to autonomous driving. The rising popularity has led to their incorporation into the pharmaceutical industry to evaluate massive chemical libraries, anticipate drug-target interactions, optimize drug design, and find innovative drug candidates that are both effective and specific. In this article, a broad overview of CNN's applications is provided in different pharmaceutical-associated domains.

Introduction

Convolutional neural networks (CNNs), deep learning (DL), and multilayer neural networks based on neuroscience findings have revolutionized the field of computer vision in recent years, achieving state-of-the-art results in tasks such as image classification, object detection, and segmentation.[1] Before CNNs, artificial neural networks (ANNs) required the use of laborious, manual feature extraction techniques to identify objects in images or perform classification. CNN, an extended version of ANN, offers a more scalable method for object recognition and image classification by extracting features from grid-like datasets, implementing matrix multiplication and other concepts from linear algebra to find patterns and excel at analyzing inputs such as images, text, speech, audio or video, distinguishing them from other neural networks.[2] CNNs process the images by assigning weights and biases to several aspects of the input images using four fundamental processes: convolution, inclusion of nonlinearity, pooling, and classification.[3] Figure 1 is an illustration of the architecture of CNN.

The convolutional layer (CL) is CNN's main building unit where most computing occurs. It requires input data (a color image composed of a 3D pixel matrix where an image's three dimensions — height, width, and depth — correspond to RGB (red, green, blue)), a filter, and a feature map.[4] A filter, also referred to as a feature detector or kernel, is a two-dimensional array (n * n) of weights that determines the receptive field size. A filter traverses through the image to check for the feature by calculating a dot product between the filter weights and input pixels and carries out various operations (blurring, sharpening, edge detection, or detecting an explicit feature) to capture the temporal and spatial dependencies of the image.[5] The dot product is then fed into an output array followed by a filter shift using a step (stride). The procedure is repeated till the filter has swept across the entire image. The final filter data, i.e., a series of dot products, generates a feature map (2D array of output values), also known as an activation map or convolved feature. This entire process is called convolution. After each convolution operation, CNNs add nonlinearity to the feature map using rectified linear unit (ReLU) transformation.[6] ReLU, the most popular activation function with biological stimulation, transforms each neuron’s output and maps it to the highest possible value or zero if negative.[7] The first

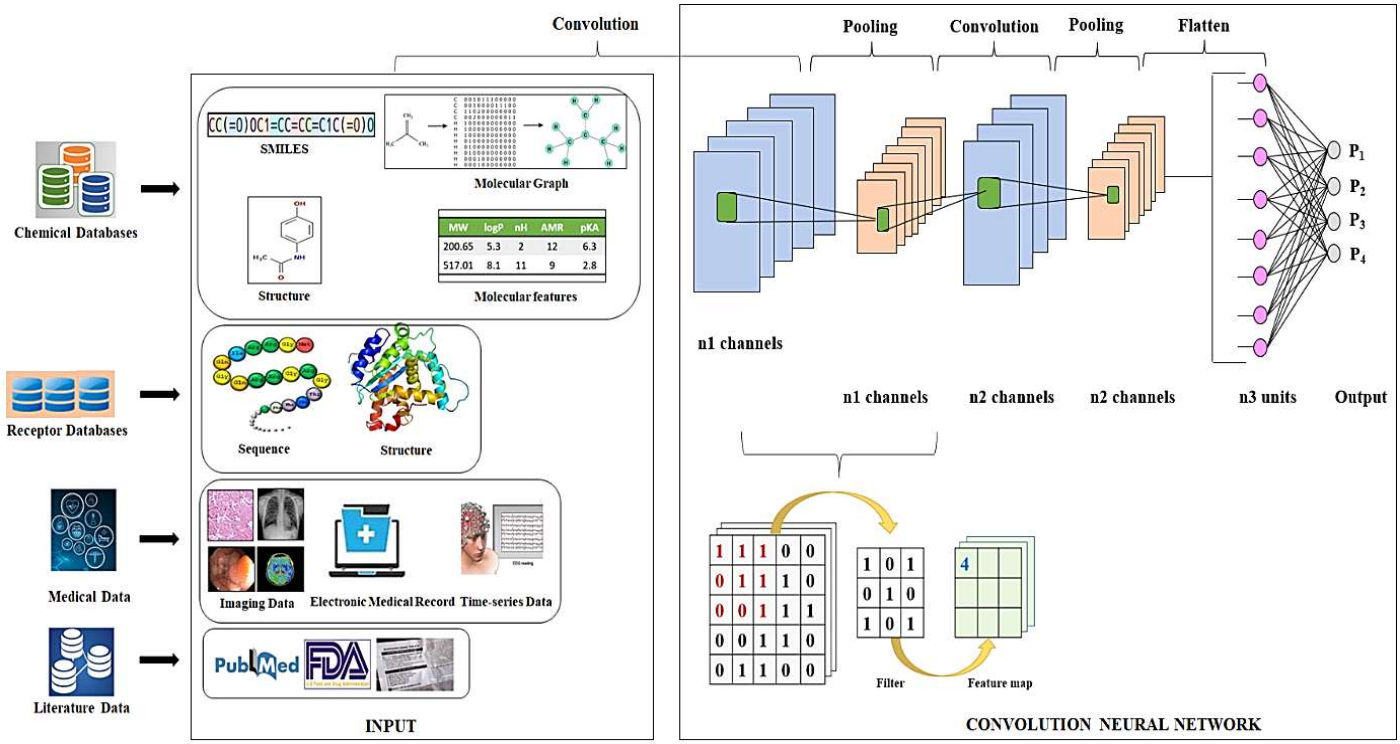

Figure 1: A simplified diagram of the applicability domain of CNN architecture.

CL can be followed by another CL, thus making the CNN's structure hierarchical, where later layers can perceive pixels from previous layers' receptive fields. Pooling layers (PL), commonly known as downsampling layers, reduce the dimensions by reducing the number of input parameters. The pooling operation sweeps a filter across the entire input, akin to the CL; however, unlike the CL, the filter does not have any weights associated with it. Instead, the filter applies an aggregation function on the values that are included inside the receptive field and returns only the most significant information from the feature map, which is passed to successive layers until the last layer.[8] The most common forms of pooling are maximum (Max) and average pooling. Max pooling, most frequently used, selects the pixel with the maximum value to send to the output array as the filter advances over the input. The average pooling method, as the name suggests, computes the average value inside the receptive field as it traverses the input and sends that information to the output array. Even though the pooling layer loses information, it improves CNN's efficiency and reduces the complexity and overfitting risk.[9] The fully connected layer (FCL) has each output layer node connecting directly to a previous layer node, in contrast to previous partially connected layers where input pixel values are not directly coupled to the output layer. The FCL gathers (flattens) all features extracted by the preceding layers and their filters to perform the classification task using softmax activation functions, i.e., to categorize inputs with a probability from 0 to 1. There are three different types of CNNs, including 1D, 2D, and 3D CNNs, depending on the input data or problem. The 1D CNN, often used with time series data or molecular features of compounds, shifts the filter in a single direction. The 2D CNNs are employed in image processing and classification tasks where the filter traverses the image in two directions. The 3D CNN utilizes 3D images from MRI or CT scans and has a kernel that moves in three directions.

The drug design and development cycle for novel drugs (small-molecule) faces several obstacles, including high cost-to-market, minimal success in clinical trials, and lengthy cycle periods.[10] Despite high expenses, the pharmaceutical industry's drug productivity continues to decline. Several factors contribute to this trend, including the complexity of obtaining approvals for novel chemical compounds, market saturation, and the tendency to spend in both developed and developing markets, among others. The potential of CNNs goes beyond computer vision, as evidenced by their successful application in a diverse range of fields, including the pharmaceutical industry.[11] Their ability to analyze complex datasets and make predictions on a range of molecular properties has led to their widespread use in the pharmaceutical industry over

Figure 2. A diagrammatic illustration of CNN applicability in a pharmaceutical domain.

the past decade, where they are used for everything from drug design and discovery to medication safety reviews (Fig 2). This review article explores various applications of CNNs in pharmaceutical sciences, including drug discovery and design, pharmacokinetics and pharmacodynamics, drug formulation and delivery, pharmaceutical manufacturing, diagnosis of disease, and classification of its progression.

Drug discovery and design

Drug design is a multifaceted process that involves designing molecules, identifying and optimizing lead compounds that are capable of interacting with specific targets in the human body or possess desirable drug-like properties. Drug development relies heavily on protein-ligand scoring because it allows researchers to reduce the vast chemical space to a manageable number of molecules for further study. CNNs have been applied in various stages of drug design and discovery, including virtual screening, de novo drug design, and structure-activity relationship (SAR) analysis as well as in generating novel compounds with desired properties. The input to the CNN is usually a SMILES notation or 3D representation of the drug molecule or target receptor, and the output is a predicted affinity score or novel compounds with the desired structure or property. Once a CNN model is trained on a large dataset of known drug-target interactions or compounds, it can be used to screen a large database of compounds to discover potential drug candidates that are likely to bind to the target or similar to the training set compounds. This can greatly speed up the process by plummeting the number of compounds that need to be experimentally tested. Several prediction models have been reported, each capable of designing or generating molecules based on the training set data. Segler et al. used SMILES notations of more than two lakh drug-like molecules from the ZINC database to generate novel molecules that were similar to the training set. The generated molecules were successfully evaluated based on their similarity to the training set and their drug-like properties.[12] In a similar study, a 3D chemical structure was used to train cycle-consistent adversarial networks (CycleGAN) for successfully generating novel compounds with similar attributes to those of the training set and predicting their drug-likeness properties.[13] CycelGAN is an image-to-image translation model that comprises two generators and two discriminators. The generator is a neural network that learns to create new data samples similar to a training dataset from noise data, while the discriminator is another neural network that learns to distinguish between real and generated data. Together, they are trained in an adversarial process to produce high-quality synthetic data. A prototype-driven diversity network, also called a generative chemistry architecture, was proposed by Harel et al. that incorporates an encoder (responsible for mapping real data samples to a lower-dimensional space), CNN, and recurrent neural network (RNN; a feedback-connected network that processes sequential data by maintaining state information from prior inputs) components to build diverse molecules with molecular template-like features without explicit prior chemical knowledge.14 Maziarka et al. introduced a novel approach called Mol-CycleGAN for molecular optimization in drug discovery. The authors proposed the use of a CycleGAN to learn the mapping between molecular structures and their corresponding properties. By training the Mol-CycleGAN on a dataset of molecules with known properties, the model generated new molecules with desired properties, thereby showcasing its potential as a powerful tool for molecular optimization in drug design and discovery.

There has been extensive usage of CNN algorithms in virtual screening to automatically extract features from the two- or three-dimensional structure of a receptor (protein or gene) to predict its binding affinities to a ligand and identify potential drug candidates.15 AtomNet, the first structure-based deep CNN application to predict binding affinity, was trained with ChEMBL dataset (comprising 78,000 actives and 2,000,000 decoys, spanning 290 targets)

using a 3D grid technique to encrypt the binding site surroundings of individual atoms into voxelized feature vectors.[16] The voxelized feature vector is a 3D grid of voxels, each of which represents an object's color, texture, material, and other characteristics. Another structure-based virtual screening study employed densely connected CNNs and a transfer learning approach (use of a pre-trained model to start a new task and allows the model to leverage knowledge learned from one domain to another) to create protein family-specific models that outperformed the machine learning (ML) benchmark models.[17] An ensemble model (combination of multiple models) based on CNN was reported by Paul et al. to improve structure-based drug design using a dataset of 22.5 million ligand poses docked into various binding locations across the Protein Data Bank (CrossDocked2020 dataset). The ensemble model classified binding positions and selected poses appropriately.[18] Shayakhmetov et al. constructed a CNN model for gene-specific virtual screening using the SMILES strings of 3,000 chemicals from the ChEMBL database to identify chemicals that activate specific genes in a transcriptome with an accuracy of 80%.[19] Another CNN model reported by Ragoza et al. used a 3D grid point of a protein-ligand complex, where the atom densities are stored at each grid point,[20] to classify correct and incorrect protein-ligand binding poses and scores for binding and nonbinding pairs. The multichannel topological neural network (TopologyNet) by Cang et al. uses a topological approach to represent the 3D biomolecular geometry[21] while maintaining important biological information to predict binding affinities. Other published models to forecast drug-receptor binding affinity based on drug SMILES, drug maximum common substructure, amino acid sequence, information about protein domains and motifs, and more are DeepDTA,[22] WideDTA,[23] DeepAffinity,[24] and PADME[25].

The potential use of CNN in drug development and material chemistry was made possible by DeepChem, a Python program. It has the potential to streamline many steps in the drug development process, including virtual screening, molecular property prediction, and molecule production. The platform is intuitive and flexible, allowing researchers to fine-tune their models for usage with a wide variety of datasets and queries.[26] Several parallel CNN architectures were trained to identify chemicals based on their toxicity and to predict the activity of certain drugs utilizing molecular descriptors, images, and genomic data. Some models reported attaining accuracies as high as 99%, albeit this ranged widely depending on the dataset and the model employed. Overall, CNNs have shown great promise in drug design and discovery and have the potential to revolutionize the field by enabling the design of new drugs more quickly and cost-effectively than traditional methods.

Pharmacokinetics and Pharmacodynamics

Pharmacokinetics (PK) and pharmacodynamics (PD) are important aspects of drug development that involve the study of the absorption, distribution, metabolism, and excretion (ADMET) of drugs, as well as their effects on the body. CNNs have been used to predict the PK and PD properties of drugs such as solubility, toxicity, and bioactivity, based on their chemical structure and physicochemical properties. Chemception, a deep CNN model, predicts periodicity, molecular descriptors and fingerprints, toxicity, activity, and solvation characteristics using 2D molecular images.[27] Several groups have applied CNNs using molecular properties to predict various properties, such as aqueous solubility,[28] blood-brain permeability,[29] etc. Recently, graph convolutional neural networks (GCNN),[30] a variant of CNN has been successfully applied for predicting molecular properties.[31] GCNN first turns the topology of a molecule into a graph, with bonds as the edges and atoms as the nodes. It then creates hierarchical representations of molecules based on how far apart the bonds are around the atomic centers. This encoding gives "neural fingerprints'' that are rich, multiscale vectorial representations of molecules that are fed into additional layers of the neural network to classify activity.[32]

Additionally, CNNs have been applied in the development of personalized dosing regimens based on patient-specific factors. Deep-dose, a novel CNN-based method for estimating the distribution of personalized internal radiation dosage, leverages information from medical imaging scans to construct a personalized 3D model of a patient's anatomy. It effectively predicted the radiation dose distribution with an average error of less than 10%.[33] Similarly, another study reported the development of a CNN model using computed tomography (CT) images and contour masks as input to predict the dose distribution for nasopharyngeal carcinoma patients (NPC) treated with tomotherapy with high precision. The model provides a therapeutically viable result

and a training strategy for a dose prediction model experimentally.[34] Another study explored the application of the CNN model (Tox_R) to predict toxicity from DNA-specific fluorescent probe (DAPI)-stained cell images using several drugs with different toxicity mechanisms. Tox_R was able to automatically generate feature maps that categorized drugs by mechanism of action and further extrapolated the results to categorize nuclei and predict per-cell toxicity from raw screening images using fully automated region-based CNNs (RCNN).[35] Future studies may incorporate genomic and proteome data into the CNNs framework to improve PK and PD prediction and enable personalized treatment.

Disease Diagnosis and progression

CNNs have performed exceptionally well in diagnosing diseases using data from various imaging techniques such as radiology, magnetic resonance (MR), CT, X-ray, and microscope imaging.[36] CNN training traditionally began with image pre-processing, including scaling and augmentation. Data augmentation randomly selects a few images to flip vertically and horizontally, adjust height and breadth, and zoom up to a certain percentage. CNN models have demonstrated promising applications in the diagnosis of diabetic retinopathy,[37] skin lesion classification,[38] identification of lymph node metastasis,[39] lung nodule classification,[40-42] gastrointestinal disease classification,[43-45] tuberculosis,[46] and cancers such as breast cancer,[47] brain tumors,[48] lung cancer,[49,50] etc. Several researchers have reported the successful implementation of CNN algorithms, with high accuracy, to diagnose infections from chest X-rays, which could aid in the early identification and care of COVID-19 patients.[51-54] One such system is COVID-Net, an open-source diagnosis system, based on a deep CNN for detecting COVID-19 patients using X-ray images.[55] Mahmud et al. designed a deep CNN architecture, CovXNet, that makes use of depthwise convolution with different dilation rates to extract various features from chest X-rays.[56] Another intelligent diagnostic system using switch-controllable nanocatcher and CNN has been developed by Feng et al. to analyze pathological images for Cryptococcus infections (a common cause of illness and death in HIV/AIDS patients).[57]

In addition to classifying medical images, CNNs have also been used to identify or annotate the regions of abnormality within the images, for example, segmentation of tumor region in the uterus,[58,59] polyps in the colon,[60] liver,[61] etc. For this, a probability map of the organ or anatomical structure is first constructed using CNN and image patches, and then the segmentation is refined utilizing both the probability map and the overall context of the images. Unlike MR, ultrasound, and CT, microscope imaging has complex characteristics. Stains, background clutter, inhomogeneous intensity, contacting or overlapping nuclei/cells, etc., make it difficult to manually interpret pathology images. Due to their ability to learn complex features and patterns, CNNs have been successfully implemented to identify the cell nucleus, cell count, cell area, and mitosis in the microscopic images of different cancers (brain, lung, cervical, and brain tumors) without prior knowledge.[62-70] In a nutshell, various studies have underlined the promising role of CNN-based intelligent diagnostic systems for early and fast diagnosis of diseases with high sensitivity and specificity.

Drug-Drug Interactions

Interactions between drugs are referred to as drug-drug interactions (DDIs) which are classified as synergistic, antagonistic, or neutral. DDIs are crucial to drug development and disease detection, yet they require substantial investments of time, money, and resources.[71,72] The earlier DDI prediction models focused on the biological interactions between drugs only rather than the intricate atomic interactions. In contrast, the CNN-based DDI prediction models built are more robust and account for atomic interactions. The CNN model described by Liu et al. was the first to apply the CNN algorithm for DDI prediction based on position embeddings and word embeddings, which capture the relative distances between words and semantic information of words for two drugs of interest.[73] Quan et al. resolved the three major issues associated with extracting biological relations from medical records (namely vocabulary gap, incorporation of semantic information, and manual feature selection) by integrating the CNN framework with multichannel word embedding (MCCNN) to predict DDI.[74] MCNN consists of three components: improving word representation by increasing vocabulary and decreasing unfamiliar words, application of attention mechanism, and using DL models for prediction. DeepCNN, a 10-layer CNN architecture, showed improvement in extracting DDI information by building a high-quality learning representation of long input sequences using multi-channel word embedding.[75]

CNN-DDI, a semi-supervised CNN system (comprising of five convolutional, two fully connected, and a CNN-based softmax layer) by Zhang et. al. predicts DDI and associated events using input vectors comprising feature interactions from pharmacological categories, targets, pathways, and enzymes.[76] To forecast DDI incidents, Yang et al. suggested the CNN-Siam algorithm, which makes use of the drug multimodal information and a Siamese neural network (SNN) architecture. SNNs have two or more identical subnetworks with the same configuration, specifications, and weights. All subnetworks undergo identical parameter updates, and their output feature vectors are compared to identify input-output pairs with shared characteristics. CNN-Siam learns a representation for an individual drug by feeding its chemical substructure, target, and enzyme information into two CNNs with shared parameters; the resulting drug pair representations are then fused and further sent to a multilayer perceptron for classification.[77] Another end-to-end model, an attention-convolutional neural network (ACNN), for predicting DDI from solely drug sequence information (feature matrix) was given by Wang et al. By assigning a different attention vector to each atom in the drug feature matrix, ACNN was able to simulate the intricate interaction between the drug atoms.[78] A unique neural technique to extract DDIs from texts by utilizing drug molecular structure was proposed by Asada et al. where textual drug pairs are encoded using CNN, while their molecular pairs are encoded using graph convolutional networks (GCNs). The outputs of these two networks are then combined for final prediction.[79] CNN-based models have proven their usefulness in extracting and learning different drug representations to predict DDIs, allowing for more accurate prediction of drug efficacy and safety.

Drug Safety and Adverse Event Prediction:

Drug safety is a critical aspect of drug development and involves the identification and mitigation of potential adverse drug reactions (ADRs). While ML algorithms have been widely utilized for ADR prediction, only a small number of CNN-based models currently exist; nonetheless, research into this area has shown that CNNs can be useful for predicting and detecting ADRs, which can lead to their early discovery and elimination. Current CNN models predict the safety profile of drugs based on their chemical structure and physicochemical properties. Chen et al. presented a novel CNN model to predict drug ADRs with 88% accuracy from chemical structures by using bioactivity data of FDA-approved drugs.80 Yao et al. successfully trained a CNN model with semantic embedding to predict the likelihood of ADRs for a given drug using a dataset of drug labels and their associated ADRS from the US Food and Drug Administration (FDA).[81] In another study Dey et al. used SIDER database drug-ADR information, to develop a neural fingerprint technique that investigates all possible substructures present in drug molecules up to a certain radius, where the radius is defined as half of the maximum path length between any two atoms of that substructure, and trained a CNN framework with an attention mechanism to identify which drug-molecule substructures linked to a given ADR and also determines if the substructures could be used to anticipate ADRs in novel drugs. This study is helpful for drug developers to discover problematic substructures and may improve pipeline drug safety reviews.[82]

Additionally, CNNs could be applied to predict adverse events based on patient-specific factors, such as genetic information, electronic medical/health records (EMR/HER), and medical history.

Pharmaceutical Manufacturing:

Pharmaceutical manufacturing involves the production of drugs in large quantities while ensuring consistent quality and purity. CNNs have been used to optimize various aspects of pharmaceutical manufacturing, such as the optimization of production processes and the detection of defects in drug products. Additionally, CNNs have been applied in the development of automated systems for quality control and assurance. Optical coherence tomography (OCT) is a real-time and contactless process analytical technology (PAT) for solid dosage form coating operations in the pharmaceutical industry. Researchers have successfully employed CNNs to evaluate OCT images for pharmaceutical solid dosage forms using image data from both in- and at-line OCT implementations and for monitoring film-coated tablets and single- and multi-layer pellets.[83] Researchers have also used CNNs to detect defects in tablet production and to monitor the quality of drug products during the manufacturing process.[84]

Drug Formulation and Delivery:

Drug formulation and delivery is a crucial aspect of drug development that involves the development of

safe and effective drug delivery systems. CNNs have been used to optimize drug formulations by predicting the stability, solubility, and bioavailability of drugs based on their physicochemical properties. Additionally, CNNs have been applied in the development of drug delivery systems, such as liposomes and nanoparticles, to improve drug targeting and efficacy. For example, researchers have used CNNs to predict the optimal size and shape of nanoparticles for drug delivery.[85] Recently, using the position of the drug injection and the geometry of the blood vessel as inputs, CNN has been successfully used to develop a data-driven reduced-order model (ROM) for real-time prediction of the spatial-temporal drug trajectory and concentration field in trans-arterial chemoembolization therapy.[86]

Personalized Medicine:

To capture the unique characteristics of each patient, personalized prediction models draw data from cohorts of patients with comparable characteristics. CNNs have demonstrated remarkable performance in tasks such as tumor detection, segmentation, and classification, aiding in personalized medicine by providing precise and individualized insights.[87] These networks can capture subtle visual cues and intricate spatial relationships in medical images, enabling the identification of biomarkers, early disease detection, and assisting in treatment planning. The article highlights the potential of CNNs in revolutionizing radiology and emphasizes their contribution to personalized medicine through the accurate and efficient analysis of medical images. Suo et al. introduced a novel time fusion CNN framework that learns patient representations and measures pairwise similarity, considering the temporal relationships and contributions from different time intervals.[88] The framework aims to accurately identify similar patients and utilizes the similarity scores for personalized disease predictions, evaluating the effects of different vector representations and similarity learning metrics.

Other studies

The CNN application is not just limited to drug research, but it has also proven to be useful in other studies. Beck et al. utilized a pre-trained hybrid CNN and RNN model called molecule transformer-drug target interaction (MT-DTI) to predict whether any commercial antiviral medicines would function in SARS-CoV-2. Remdesivir, ritonavir, atazanavir, efavirenz, and dolutegravir were computationally identified for SARS-CoV-2 treatment.[89] DeeplyTough, a 3D CNN model, quantifies pocket similarity by analysing binding sites of proteins without alignment. It encodes 3D protein pockets into descriptor vectors to compute pairwise Euclidean distances using a positive and negative selection of proteins that bind chemically similar and dissimilar ligands.[90] CNN has also exhibited success predicting protein structures in the absence of a template structure.[91] Tong et al. employed a CNN model for analyzing protein structures to forecast how mutations may alter protein structure, and study amino acid microenvironments without any prior information or feature assumptions. The method was reported to double the accuracy of predictions compared to models that necessitated the selection of features by hand.[92] Images of cells pre-treated with a variety of drugs have been used by CNNs to make predictions about their toxicity. The model was able to effectively predict a broad variety of toxicity pathways from different medicines, nuclear stains, and cell lines.[93] Finally, to extract biomedical relationships from PubMed literature, Nourani et al. created a hybrid transfer learning framework (Deep-GDAE) using attrition-based BiLSTM and a CNN based on textual feature extracted using BERT (Bidirectional Encoder Representations from Transformers, a pre-trained natural language processing model) and BioBERT base models.[94]

Future prospects and challenges

CNNs have demonstrated exponential development across all fields as they excel at finding patterns and features in various types of data (textual, image, audio, video, time-series) with minimal pre-processing, handling enormous volumes of data with end-to-end training, attaining excellent accuracy while being robust to scaling, rotation, and translation invariance. It correctly performs the task (classification, segmentation) by reducing the image down to its fundamental features via convolutions and pooling mechanisms. Compared to conventional neural networks, they are easier to train owing to relatively few initial parameters and the capacity of convolutions to manage all hidden layer discoveries. CNNs have also shown great promise in various applications of pharmaceutical science, such as drug discovery, molecular property prediction, and image-based-disease diagnosis. CNNs could speed up drug discovery by evaluating enormous databases of

chemical compounds and identifying promising drug candidates or molecular targets (receptors) based on their molecular features or structure, thereby reducing the time and expense of drug development. CNNs could be trained to design drug formulation strategies, predict solubility (a critical factor in drug formulation), and identify manufacturing defects. Another area where CNNs can be employed directly with pharmaceutical applications is analysing microscopic images of cells and tissues to identify disease states or drug effects. CNNs can also be used in personalized medicine by analysing genetic data to predict drug responses or identify potential adverse reactions. This could lead to more effective and safer treatment options for patients. Overall, the future of CNNs in pharmaceutical science is very promising. As technology advances, we can expect to see even more applications in drug discovery, personalized medicine, and other areas of pharmaceutical research.

Despite the promising results obtained and bright future, there are some challenges associated with CNNs that need to be addressed. First, training a CNN model is computationally taxing and requires considerable memory, necessitating the use of graphics processing units (GPUs). Second, the lack of large, diverse, and well-labeled datasets or appropriate regularization limits CNN performance, and they are susceptible to overfitting. Third, as the CNN's depth increases, more and more input-related data may be lost or "washed out" before it reaches the output layer.2, 3,[95] Short pathways between layers and feed-forward networks, in which each layer is connected to all other layers, have been offered as potential solutions to these and related issues.[96] The application of CNNs in pharmaceuticals is still in its early stages, and more research is required to fully explore its potential. Despite the challenges, CNNs offer a promising approach to improving drug development and patient outcomes.

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Abbreviations

ACNN	Attention Neural Network
ADMET	Absorption, Distribution, Metabolism, Excretion, And Toxicity
ADR	Adverse Drug Reaction
ANN	Artificial Neural Network
BERT	Bidirectional Encoder Representation
CL	Convolutional Layer
CNN	Convolutional Neural Network
CYCLEGAN	Cycle-Consistent Adversarial Network
DAPI	DNA-Specific Fluorescent Probe
DDI	Drug-Drug Interactions
DL	Deep Learning
EMR	Electronic Medical Record
FCL	Fully Connected Layer
FDA	Food And Drug Administration
GCNN	Graph Convolutional Neural Network
GPU	General Processing Unit
HER	Health Electronic Record
LSTM	Long Short-Term Model
MCCNN	Multichannel Word Embedding CNN
MGT-DTI	Molecule Transformer-Drug Target Interaction
ML	Machine Learning
MR	Magnetic Resonance
NPC	Nasopharyngeal Carcinoma
OCT	Optical Coherence Tomography
PAT	Process Analytical Technology
PD	Pharmacodynamics
PK	Pharmacokinetics
PL	Pooling Layer
RCNN	Region-Based Convolutional Neural Network
RELU	Rectified Linear Unit
RGB	Red, Green, Blue
RNN	Recurrent Neural Network
ROM	Reduced Order Model
SAR	Structure-Activity Relationship
SNN	Siamese Neural Network

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Exploring Foldamers for the Inhibition of Amyloid-β (Aβ) Aggregation: Current Trends and Future Perspective

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Alzheimer's disease is becoming more prevalent, affecting individuals of various age groups; with individuals above the age of 85 being the most susceptible. Despite significant treatment efforts, the disease remains incurable. Over the years, variety of small molecules and peptide derivatives have been designed and developed to inhibit Aß-oligomerization, but the development of highly efficient inhibitors has still eluded researchers. Recently, bioinspired peptidomimetics, particularly foldamers, have emerged as a new alternative for inhibiting Aß-aggregation due to their structural tunability, high proteolytic stability, and versatile functionality. Foldamers have shown to effectively modulate Aß-aggregation with minimal cytotoxicity, making them intriguing candidates for the next generation of anti-amyloid therapeutic agents. In this mini-review, we will discuss a few of the most important studies regarding the potential use of foldamers to inhibit Aß-aggregation.

Introduction

Alzheimer's Disease (AD) is one of the most prevalent neurodegenerative diseases accounting for majority of Dementia cases.[1] As a result of chromosomal mutation, the sequential cleavage of transmembrane Amyloid Precursor Protein (APP) by ß-secretase and γ-secretase generates 37-43 amino acids containing isoforms of Aß-peptide amongst which 42-isoform is the most toxic one.[2] Further, these sticky monomers aggregate to form plaques of cross-amyloid-ß fibrils. These not only hinder the synaptic signaling, but also trigger immune responses and cause inflammation, ultimately damaging the surrounding neurons. A secondary pathogenic event often thought to be triggered by this plaque deposition is the hyperphosphorylation of τ-proteins resulting in its decreased tendency to support the microtubules and further disintegration of the entire axonal membrane.[3]

Although the pathway for Aß aggregation is not of complete clarity yet, it follows a typical sigmoidal pattern. The transient native form of Aß monomer is a 3_{10}-helix before it transforms to a ß-folded structure. They further associate to form dimers and multimers, all of which fall into the Initial lag phase. Rapid polymerization leads to protofibril and cross-ß fibril formation ultimately reaching the Plateau phase.[4,5,6,7] Such a mechanistic process provides ample opportunities for intervention, like either inhibiting the formation of the misfolded protein, or if formed, interfere with its polymerization by stabilizing the monomers.[8,9]

Decades of research went into designing small molecules for inhibition of Aß aggregation as well as the respective proteases involved- ß-secretase and γ-secretase.[10] However, the small molecule amyloid precursors do not form uniquely defined three-dimensional structures which makes structure-based drug design very complex and molecules so developed lack selectivity and have poor affinity. In contrast, peptides exhibit a notable binding affinity to proteins due to formation of multiple hydrogen bonds and the presence of various hot-spot binding residues.[11] Furthermore, they exhibit minimal cellular toxicity as the metabolites generated are simple amino acids. Several peptides have been developed to inhibit amyloid protein aggregation by assessing

Keywords: Alzheimer's Disease, amyloid ß, self-aggregation, ß-peptides, γ-peptides, oligoarylamide foldamers

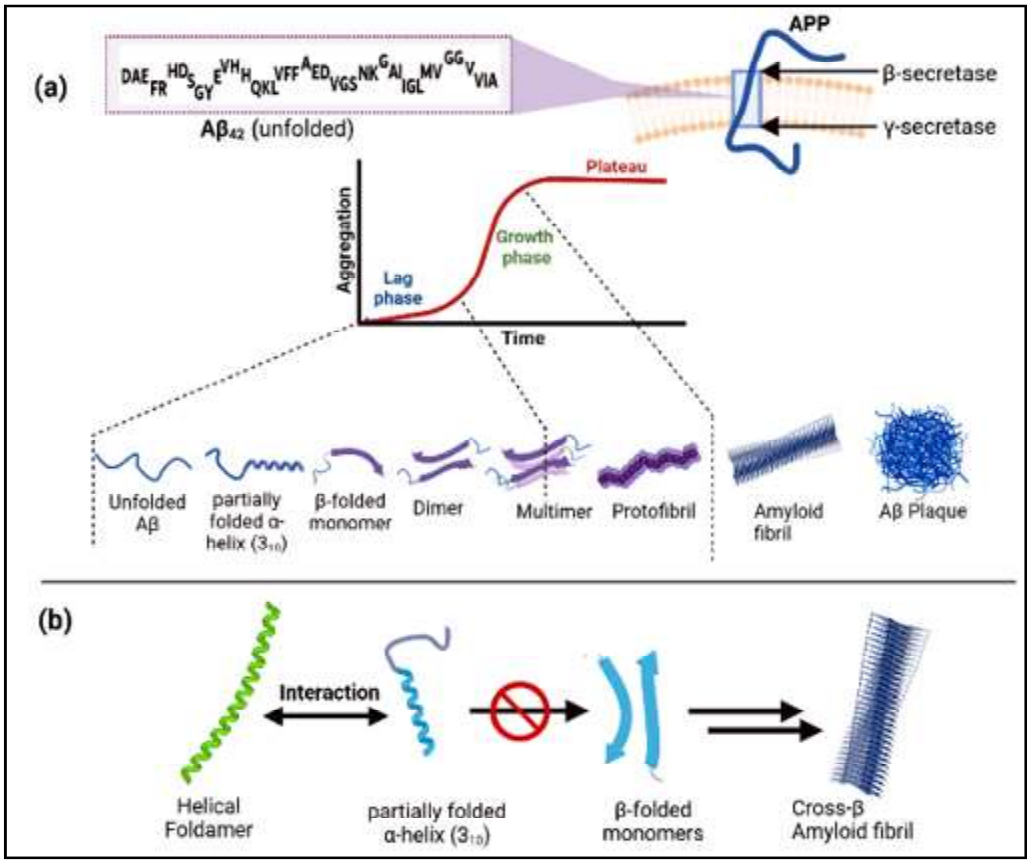

Fig. 1. (a) The mechanism of action of ß-secretase and γ-secretase on Amyloid Precursor Protein (APP) generates an unfolded Aß monomer which further undergoes misfolding and aggregation to ultimately generate the Aß-plaque. The sigmoidal curve shows kinetics of Aß aggregation with distinct Lag, Growth/Elongation and Plateau phases. (b) Inhibition of ß-structure mediated Aß oligomerization through foldamers.

native amyloid sequences, along with proline substitution, and other approaches.[12] However, such peptides made of natural amino acids are an easy target of proteases thus, have a low serum half-life. Hence, there has been a growing interest for unnatural amino acid-based peptides and foldamers which are not easily recognizable by native proteases and have a much longer half- life as compared to the native ones. Foldamers are synthetic oligomers which inherently fold into definite conformation through a variety of non-covalent interactions such as hydrogen bonding, van der Waals interactions, π-π stacking, electrostatic forces and hydrophobic interactions.[13] They possess a variety of functional groups which enhances their affinity towards biomacromolecules.[14] They lack a canonical backbone while having a tendency to adopt a conformationally ordered state in solution even with fewer monomeric units. In addition, they show structural tunability and have better pharmacokinetic properties than small molecule or protein-like drug candidates.[15] Their semi-rigid nature, proteolytic stability, along with their ability to mimic the natural peptide conformation makes them attractive therapeutic candidates for amyloid inhibition.

In this article, we present an overview of some recent advances in the use of foldamers derived from ß -amino acids, γ-amino acids and oligoarylamides for the inhibition of Aß fibrilization.

ß-amino acid based foldamers

ß-Amino acids result from mono homologation of α-amino acids. This elongated backbone enhances their flexibility as well as helical propensity. ß-peptide can form wide range of helical conformation including 14-, 10-, 8-, 10/12-helices depending on the stoichiometry and substitution patterns of amino acids in the backbone.[15] Among these various helical conformations, the 14-helix has been the most extensively studied. This helical conformation is stabilized by fourteen atoms containing H-bonded pseudo-rings between the N-H (i) and C=O (i+2) atoms having approximately three residues per turn. The morphological similarity of ß-peptide foldamers with the biomolecules could be used as an advantage and be utilized for targeting protein-protein interactions. Another significant advantage is that the incorporation ß-amino acid into a native peptide sequence increases its serum half-life by several fold due to protease resistance.[16] ß-Peptide foldamers earlier have proved useful as antibacterial,[17] for cell penetration,[18] and therapy for HIV-AIDS.[19] In addition, ß-peptides can also prove to be a novel and potent therapeutic against Alzheimer's Disease. Such unnatural amino acid oligomers that take up specific helical conformations can mimic the transmembrane domain of Amyloid Precursor Protein (APP), and thus interfere with γ-secretase functioning.

In one interesting example, Imamura and his colleagues designed a series of ß-peptides comprised of sterically-constrained, enantiomeric ß-amino acids: *(S,S)-trans*-2-aminocyclopentane carboxylic acid (ACPC) and *(R,R)*-ACPC (Fig. 2a).[20] The

designed peptides (P1-P6) are intended to form 12-helical conformation that mimics the APP transmembrane domain. Furthermore, due to hydrophobic nature of the foldamers they could be good candidates for binding to γ-secretase, which generally prefers to target hydrophobic substrates. The secondary structures of the oligomers were confirmed through CD spectroscopy; oligomers composed of *(R, R)-trans*-2-aminocyclopentane carboxylic acid (ACPC) (P1-P3) showed a low minimum at ~222 nm and a maximum at 204-205 nm, which are in good agreement with previously studied left-handed 12-helical ß-peptides (Fig. 2b). In contrast, the oligomers of *(S, S)*-ACPC (P4-P6) showed exactly opposite CD spectra suggesting right-handed 12-helical conformation (Fig. 2b). Interestingly, the intensity of signal increased proportionally with increase the length of oligomers (6→9→12). This enhanced intensity pointed towards the occurrence of a higher proportion of 12-helices upon increasing the chain length. Subsequent *in vitro* and cell-based studies demonstrated that the shortest oligomers exhibited either no or moderate inhibitory activity, whereas the dodecamers exhibited the highest inhibitory activity (in the nanomolar range) (Fig. 2c). This shows that, along with hydrophobicity, helicity is also a key factor for their inhibitory potential. Strikingly, it was observed that ß-peptides comprised of *(S,S)*-ACPC residues were far more effective inhibitors than their *(R,R)*-ACPC counterparts. The dodecapeptide of *(S,S)*-ACPC (P6) exhibited strong APP specificity while exhibiting no activity towards other hydrophobic-substrate cleaving proteases (such as α-secretase) and no action on intra-membrane cleaving proteases. Further, *in vivo* inhibitory potency of the dodecapeptide of *(S, S)*-ACPC was assessed using N2a cells confirming it to be a strong and direct inhibitor of γ-secretase (Fig. 2d, 2e). Moreover, reported inhibitory activity of helical ß-peptide is comparable to other established γ-secretase Inhibitors (GSIs) and could serve as a good lead for developing newer highly specific GSIs.

γ-Amino acid based foldamers

γ-peptides are bio-inspired synthetic oligomers whose backbone is double homologated as compared to α-peptides. The additional methylene units in their backbone limit the potential H-bonding interactions instead, make them more flexible and enhance the helical propensity. γ-peptides have exhibited their ability to mimic diverse secondary structures observed in natural proteins, such as helices, sheets and turns. In their seminal work, Seebach[21] and Hanessian[22] reported the formation of stable 14- and 9-helical conformations of γ-peptides in solution, which was further supported by Hoffman's computational methods.[23] In addition to their ability to form stable helical structures, proteolytically stable γ-peptides have been explored for the development of effective and less cytotoxic antimicrobial agents,[24] prospective cellular-uptake agents,[25] binders of somatostatin receptor,[26] and transmembrane ion and water channels.[27] Very recently, helical γ-peptides have been shown to function as inhibitors of Aß aggregation. Foldamers

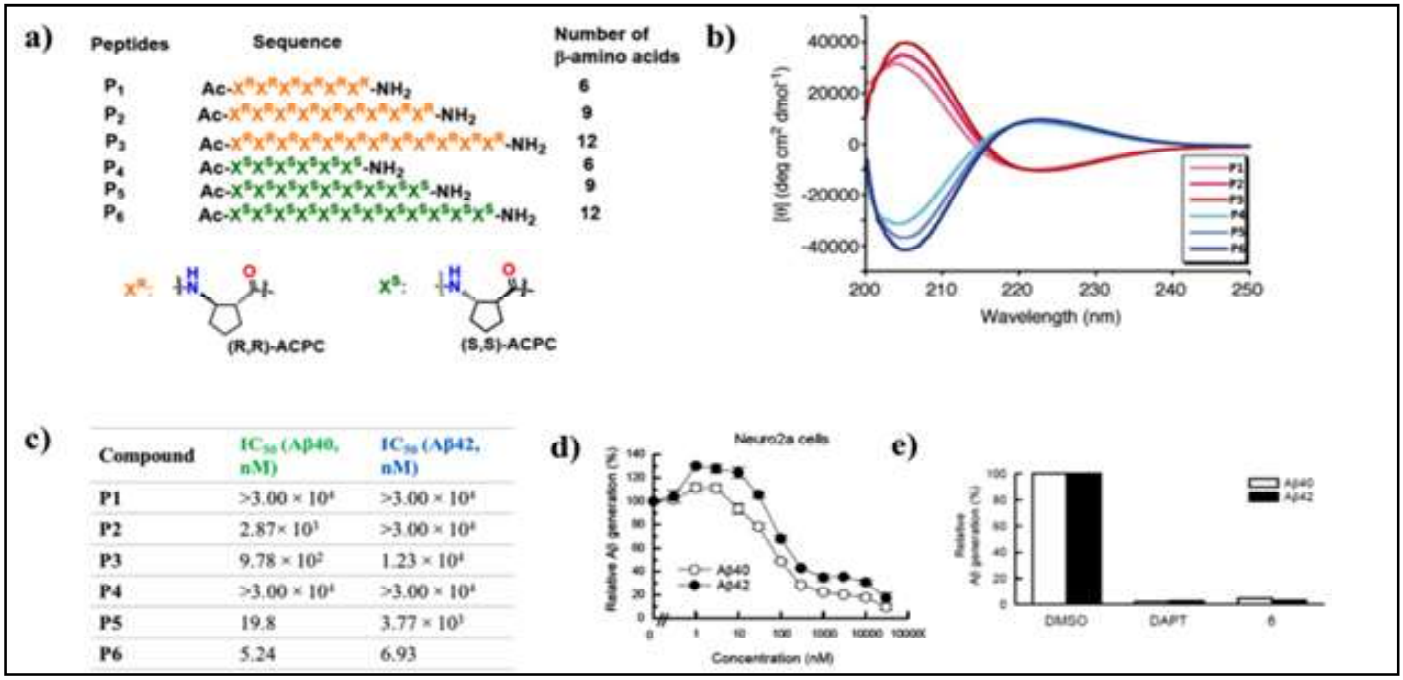

Fig. 2. (a) Sequence of the synthesized ß-peptide foldamers. (b) CD spectra of the foldamers P1-P6. (c) Inhibitory effect of ß-Peptides P1-P6 against γ-Secretase. (d), (e) Inhibitory effect of foldamer P6 on cell based γ-secretase activity. Reproduced from ref. 20. Copyright 2009 American Chemical Society.

which are reminiscent of native 3_{10}-helix can be designed such that they can interact with the transient 3_{10}-helical conformation of the Aß-peptide so that its transformation to a ß-sheet structure followed by oligomerization can be prevented.

Ongeri and colleagues have demonstrated the efficacy of γ-peptide foldamers in taking control of the Aß fibrillization process. Their aim was to target the Aß-peptides in their monomeric form using sterically constrained γ-amino acid based foldamers.[28] They designed and synthesized a series of helical γ-peptide foldamers comprised of 4-amino(methyl)-1,3-thiazole-5-carboxylic acid (ATC) (Fig. 3a). Six different γ-peptides (Fig. 3b, 3c) were synthesized with various side chains to enhance their interaction with the 3_{10}-helix (P7-P12). Keeping in mind that the KLVFF segment of Aß is mainly involved in ß-sheet formation, they designed the first series of γ^4-dipeptide, tetrapeptide and hexapeptide containing benzylic substitution that could recognize the phenylalanine residues in the amyloidogenic sequences (P7-P9) (Fig. 3b). Additionally, amino group at the 2nd carbon atom of thiazole enhanced the solubility and promoted H-bond formation with amyloid. In the second series, the aim was to utilize cationic groups to antagonize the amyloidogenesis. Here, instead of hydrophobic side chains, the γ-position was substituted with cationic aminobutyl group with the attempt of establishing electrostatic interaction with the negatively charged Asp and Glu residues of Aß (P10-P12) (Fig. 3c). 2D NMR spectroscopy and circular dichroism studies revealed that the designed γ-peptides adopted a 9-helical conformation reminiscent of native 3_{10}-helical structure. Further, *in vitro* Thioflavin-T assay was conducted to investigate the dose-dependence of ATC foldamers on plaque inhibition. Intriguingly, the inhibitory activity increased in the second series of γ-peptide foldamers with increasing length from di to hexamers. Most significant activity was shown by the cationic hexapeptide (P12). Complete absence of fluorescence was observed with ratio of 10:1 for P12/$Aß_{1-42}$. An inhibitory action was observed even with a minimal ratio of 0.1:1 (Fig. 3c). On the contrary, benzylic side chain containing 6-ATC due to its high hydrophobicity underwent self-aggregation and thus, failed to interact with the monomers. In subsequent TEM images, use of cationic P12/$Aß_{1-42}$= 10:1 showed a significant decrease in the fibrillar material as compared to the $Aß_{1-42}$ control (Fig. 3e, 3f and 3g). Furthermore, mass spectroscopy (MS) and capillary electrophoresis (CE) studies revealed that most active foldamer P12 predominantly interacts with the monomer species and monomeric form $Aß_{1-42}$ endures for prolonged period in the presence of foldamer P12. The result demonstrated the utility of thiazole γ-peptide

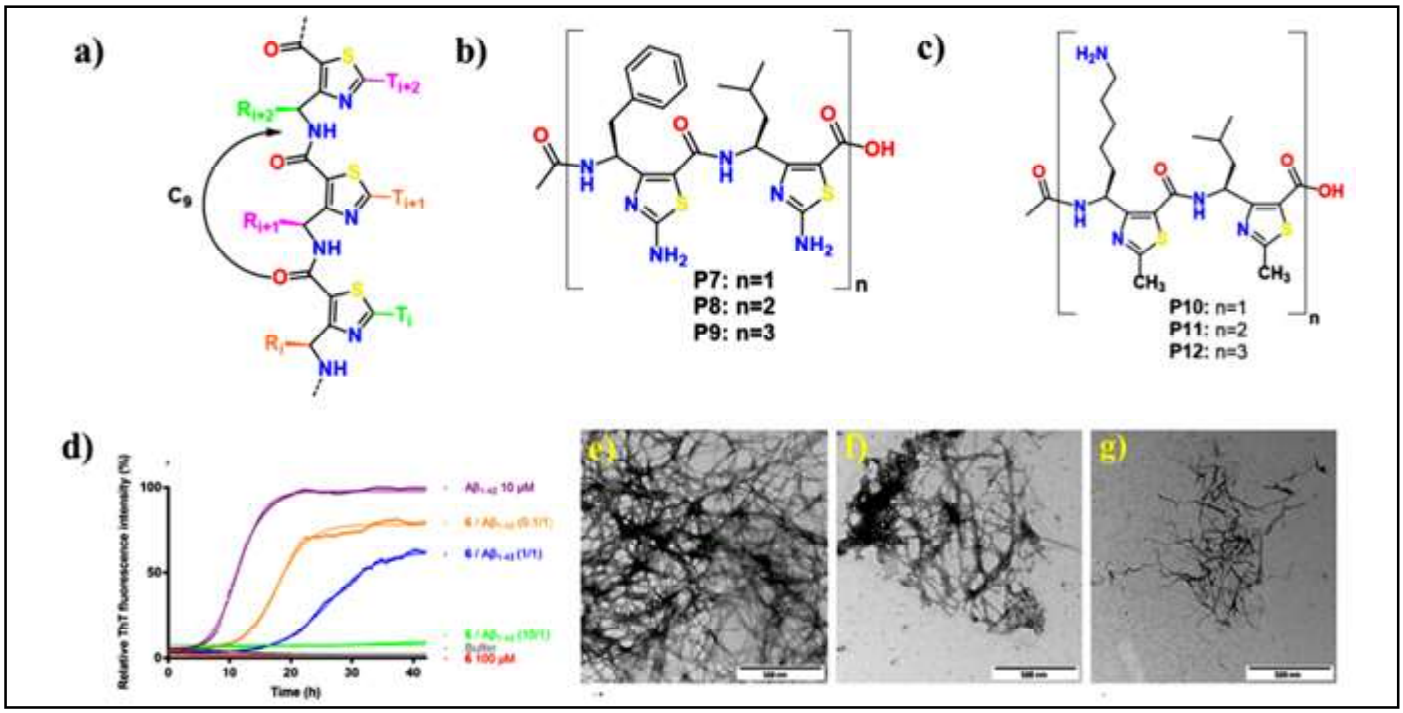

Fig. 3. (a) C_9 helical conformation of 4-amino(methyl)-1,3-thiazole-5-carboxylic acid(ATC) oligomers, T and R represents the thiazole and γ substituent. (b), (c) Chemical structure of divergent ATC foldamers. (d) ThT fluorescence curve in presence different ratio of conc. $Aß_{1-42}$ and foldamer P12. (e), (f), (g) Effect of fibril formation of $Aß_{1-42}$ peptide in the presence of foldamer P12 at various time intervals, as depicted in Fig. g, indicates that fibrils are significantly less dense after 42 hours. Reproduced with permission from ref. 28 . Copyright 2021 Wiley-VCH.

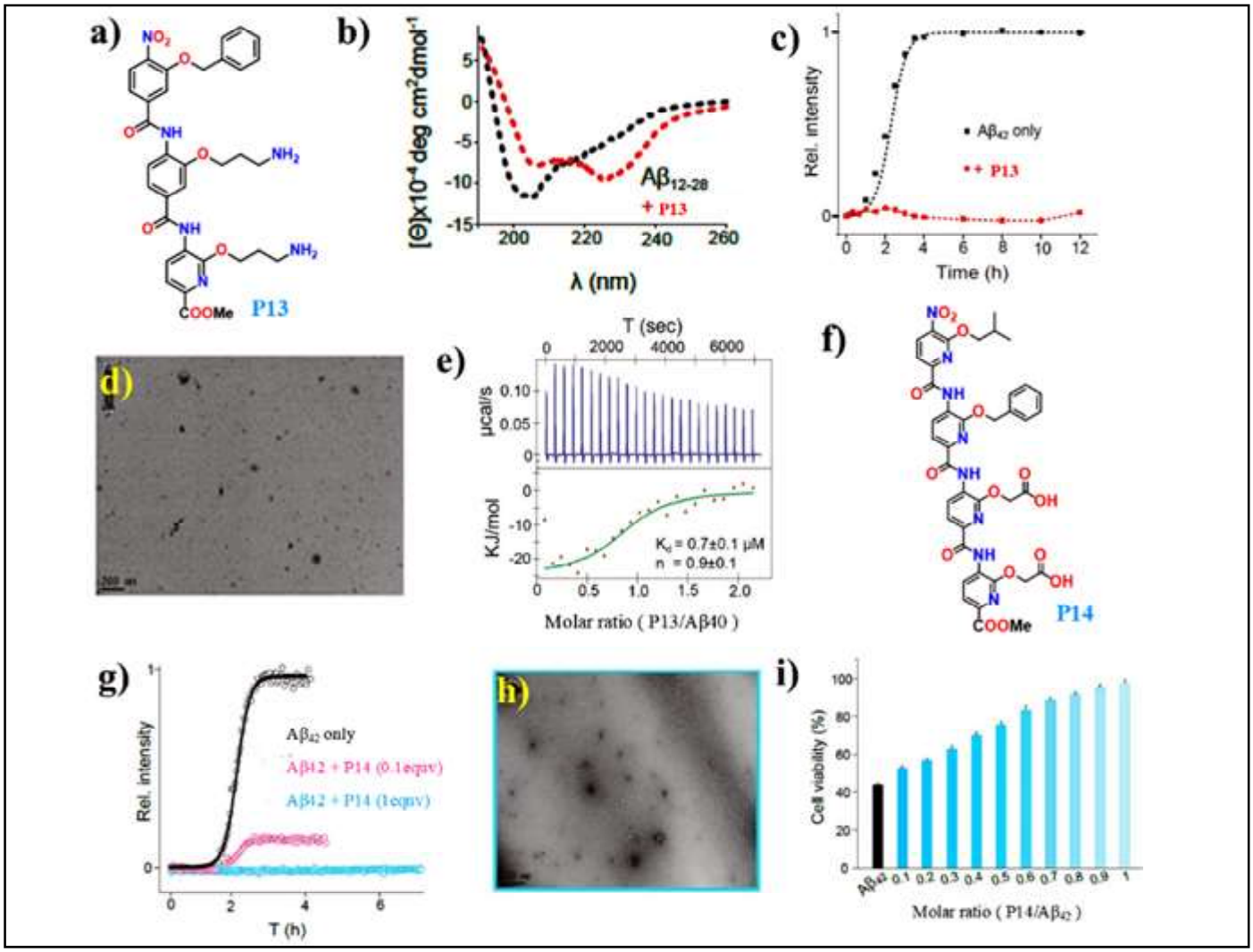

Fig. 4. (a) Chemical structure of helical peptidomimetic P13. (b) CD spectra of 1: 1 $A\beta_{12-28}$ and P13. (c) Kinetic study of Aß fibrilization via ThT fluorescence assay in the presence and absence of P13. (d) TEM image of Aß in the presence of 0.1 equivalent of P13 after hours. (e) Binding affinity of P13 and Aß1-40 measured through ITC. (f) Chemical structure of helical peptidomimetic P14. (g) Kinetic study of Aß fibrilization in the presence and absence of P14 in different ratio. (h) TEM image of Aß in the presence of equimolar ratio of P14 after 6 hours. (i) Cytotoxicity of N2a cells treated with 5 µM $A\beta_{42}$ in the absence or presence of P14 after 72 hours. Reproduced from ref. 34 and 35. Copyright 2018 American Chemical Society.

foldamers in intervening the amyloidogenesis during its initial stages and how the side chain substitutions on the γ-amino acids can be modified synthetically to modulate their interactions with the amyloid proteins.

Aromatic oligoamide based foldamers

Aromatic oligoamides are amide sequences with aryl/heteroaryl moieties that can form intramolecular H-bonds between ring components and adjacent amide-NH, thereby adopting rigid conformations.[29] Aromatic oligoamides can mimic diverse secondary structures of protein, including helices, ß-sheets, and linear strands, depending on the monomer employed and the formation of inter-amide hydrogen bonds. In their pioneering work, Huc and Gong have shown the applicability of oligoarylamides as biomodulators.[30,31] Numerous quinoline-, pyridine-, and pyrrole-based monomers have been employed to construct oligoarylamide foldamers. They exhibit an impressive mix of structural predictability, tunability, stability, and synthetic ease. More importantly, their length can be easily altered and the backbones and side chains can be readily functionalized. As a result, this foldamer family has been extensively used for protein-protein interaction[32] and molecular recognition.[33] In addition, these foldamers are recently employed as modulators of Aß aggregation.

In this context, Hamilton and his colleagues have developed a series of α-helical mimetic oligopyridylamide sequences that can target the helical domain of Aß-peptide.[34] Among the various oligopyridylamide sequences, the most potent is P13; a tripyridylamide with one benzyloxy group and two primary ammonium residues (Fig. 4a). The detailed

HSQC NMR studies revealed that, the aromatic benzyl moiety interacts with the hydrophobic (Leu^{17}-Phe^{20}) region and positively charged ammonium with anionic residues of Aß (Glu^{22}-Asp^{23}). Circular dichroism studies demonstrated that the helical peptidomimetic induces a-helical conformations in the $Aß_{42}$ peptide and prohibits its transformation into the amyloid ß-sheet structure (Fig. 4b). The inhibition was further supported by TEM and Thioflavin T assay (Fig. 4c, 4d) Further ITC studies reflected that P13 formed stable 1:1 complex with $Aß_{40}$ and inhibit the aggregation (Fig. 4e). In continuation of their previous work, the same group screened another series of anionic tetrapyridylamide scaffolds and identified that compound P14 was the most effective.[35] The side chains of foldamers; isobutyl and phenyl groups, collectively interacted with the hydrophobic residues of Aß, while two carboxylate groups interacted with (His^{13}-Lys^{16}) to stabilize Aß in a helical state. The efficacy of inhibiting this aberrant self-assembly has been supported with an array of biophysical techniques for instance, TEM (Fig. 4h), AFM, Thioflavin T assay (Fig. 4g), ELISA, CD spectroscopy, fluorescence titration and so on. More intriguingly, it also inhibited the Aß42 mediated toxicity in N2a cells at a sub-stoichiometric concentration (Fig. 4i). As evident from this study, the surface functionalities on the oligopyridylamides mainly influence their binding to Aß, making them the primary deciding factor for generation of a stable Aß helix. Any alteration in the substitutions resulted in compounds with reduced antagonistic activity.

Conclusion and Future perspectives

In conclusion, we have summarized recent progress regarding the potential use of foldamers in the treatment of Alzheimer's disease. The reported foldamers could inhibit the pathogenesis either at the initial stage of Aß monomer synthesis or the formation of the Aß oligomers. Notably, despite their ability to mimic the APP recognition surface, these foldamers exhibit no amyloidogenic potential. They also have a longer serum half-life due to their proteolytic resistance. However, certain important criteria such as the ability of foldamers to cross the Blood Brain Barrier under therapeutically relevant condition need to be addressed for their future use as drug candidates for the treatment of Alzheimer's disease. Nevertheless, the non-natural oligomers have been and will continue to be important platforms for advancing our understanding of protein folding and how these folded structures may be used to modulate the Aß aggregation and other rare diseases. The quest to find a highly specific and efficient treatment for neurodegenerative diseases continues and such inhibitors would be important tools for identifying newer drug prototypes.

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Review Article

Efficacy of Pharmacological & Non Pharmacological Treatments for Fibromyalgia: A Systematic Literature Review and Meta-analysis

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Background: The management of fibromyalgia (FM) poses a complex challenge due to its multifaceted and often severely debilitating symptoms. This study delves into the effectiveness of pharmacological and non-pharmacological interventions in tackling the intricate nature of FM symptoms.

Methodology: We conducted a comprehensive search using e-databases (PubMed and Embase), from inception to 16th March 2023. Randomized controlled trials (RCTs) that evaluated the efficacy of pharmacological and non-pharmacological treatments for FM with primary outcomes impact of fibromyalgia on a person's daily life, Pain, Depression and health related Quality of Life. For data synthesis, we calculated the standardized mean differences (SMD) accompanied by 95% confidence intervals (CI) were included. To combine the findings from the included studies, we performed a meta-analysis using the Der-Simonian and Laird method.

Results: This study comprised 27 RCTs encompassing 2390 participants, allocated to three interventions categories. A meta-analysis utilizing the Visual analogue scale (VAS) score, Fibromyalgia impact questionnaire (FIQ) scores, Tender point count (TPC) indicated a non-significant effect size, therapy over treatment as usual (TAU), with a SMD of 0.02 (95% CI: -0.57 to 0.54), -0.31 (CI: -0.64 to 0.02), -0.17 (95% CI: -0.55 to 0.21) respectively. The SF-36 score demonstrated that the intervention group had a higher score than the TAU group, with an SMD of -0.15 (95% CI: -0.18 to 0.48). The Beck's Depression Inventory (BDI) score meta-analysis showed an SMD of 0.79 (95% CI: -1.14 to 2.72) in the intervention group compared to the TAU group.

Conclusion: The approach that combines both pharmacological and non-pharmacological interventions shows potential for achieving the most favourable outcomes.

Introduction

Fibromyalgia (FM) is a chronic condition, which is characterized by widespread and multifocal pain, exhibiting fluctuations in both its spatial distribution and intensity across the course of the illness. Patients suffering from FM exhibit augmented sensitivity towards various stimuli, including thermal and mechanical pressure, as well as ischemic pressure. Such stimuli evoke pain responses in patients even when applied at levels of intensity that are non-painful for healthy individuals.[1] Ranking as the third most common chronic condition[2,3,] FM's prevalence varies from 0.5% to 5% in the general population and up to 15.7% in clinical settings.[4] While more common among older adults, its precise origins remain elusive. Hypotheses suggest a blend of genetic predisposition, stressful life events, and both peripheral (inflammatory) and central (cognitive-emotional) mechanisms contributing to pain perception development, referred to as "nociplastic pain." In recent years, research has linked FM pathogenesis to factors like inflammation, immunity, endocrine function, genetics, and psychosocial

Keywords: FM Interventions, Efficacy, Treatment, Meta-analysis

elements. Diagnosis by rheumatologists usually involves considering a patient's history of body-wide pain lasting at least three months and the elicitation of pain through digital pressure in at least 11 out of 18 specific tender points.[5] The Fibromyalgia Impact Questionnaire (FIQ) is a validated assessment tool designed to measure the overall impact of fibromyalgia on a patient's life, encompassing aspects like pain, functioning, fatigue, and psychological well-being.[6] The Visual Analog Scale (VAS) is a self-reporting tool that employs a 10-point scale to quantify the intensity of pain experienced by individuals, aiding in tracking pain fluctuations over time.[7] The Tender Point Count (TPC) involves the manual palpation of specific tender points on the body, aiding in the diagnosis of fibromyalgia by identifying localized pain and tenderness.[8] These tools are crucial in clinical settings to comprehensively evaluate fibromyalgia symptoms, monitor progress, and tailor treatment strategies.

The management of FM is currently very challenging due to its multiple etiological factors and the lack of a straightforward cure. The primary focus of treatment is symptoms relief and enhancing affected individuals' quality of life, given the substantial impact these symptoms can have on overall well-being.[9] Regrettably, the efficacy of interventions has been limited, as only a minority of patients report substantial clinical improvements from these therapies. Commonly recommended medications, such as antidepressants, anticonvulsants, and opioids, exhibit restricted efficacy, leading to a mere 10% to 25% reduction in pain intensity and a 50% reduction in specific symptoms[10] Conversely, non-pharmacological approaches like cognitive-behavioural therapy, exercise, and relaxation techniques show promise in mitigating FM symptoms. These strategies are viewed as safe, non-invasive, and cost-effective alternatives to pharmacological treatments. However, concerns persist regarding their effectiveness, safety, and potential adverse effects associated with prolonged usage.[11]

While both non-pharmacological and pharmacological interventions have been explored for FM treatment, their comparative effectiveness remains a point of contention. Furthermore, consensus on the optimal treatment approach is lacking.[12] The objective of this meta-analysis review is to offer a comprehensive and updated overview of the existing evidence regarding the effectiveness and safety of these interventions. Such insights can significantly inform clinical decision-making and provide guidance for future research endeavours.

Methods

Search Strategy and Study Selection

This systematic review (SR) and meta-analysis (MA) was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines.[13] The study participants followed the American College of Rheumatology preliminary diagnostic criteria for fibromyalgia classification and definition of FM and measurement of symptom severity.[14] The study employed the PICOS framework (Population, Intervention, Comparator, Outcome, Study Design)[15] as a criterion for including relevant studies. It integrated Randomized Clinical Trials (RCTs) that involved patients aged 18 years or older with FM. The inclusion encompassed studies where patients were designated to receive either pharmacological or non-pharmacological interventions, which were compared with placebo, alternative therapy, treatment as usual (TAU), or no intervention. No restrictions were placed on publication dates, Studies published in English language only were included. The study excluded the trials with a crossover design, surgical treatments, non-randomized RCTs, reviews, editorials, case reports, conference abstracts and studies that did not meet the intended outcomes. The protocol was pre-registered in Prospective Register of Systematic Reviews (PROSPERO) registration no - CRD42022378125.

In order to identify pertinent studies, a systematic preliminary search was conducted on PubMed and EMBASE, commencing from inception till 16th March 2023. In addition, a manual search was conducted on Google Scholar and the reference lists of the articles that met the pre-determined criteria for inclusion and exclusion, with the objective of discovering additional studies that may be of relevance. The key search terms were as follows, "FM" and "non-pharmacological" and "pharmacological" treatments, such as cognitive behaviour therapy, manual therapy, manipulation therapy, exercise, Pregabalin, duloxetine, milnacipran, amitriptyline, and gabapentin. The search terms were adjusted to align with the glossary of each database searched. Duplicate records were identified and eliminated using the Endnote software. A comprehensive search strategy has been provided in tables S3.

Data extraction, and Quality Assessment

The two authors (S.K.B and K.C) conducted an autonomous data screening and extraction process. Any discrepancies encountered were resolved through discussion with a third author, DB. The

screening process began by evaluating the titles and abstracts of the retrieved citations based on pre-determined eligibility criteria. Subsequently, they meticulously scrutinized the full text of the potentially relevant citations for suitability. A standardized excel sheet were utilised to extract all the necessary study data, including variables such as publication year, country, participant demographics, baseline characteristics, intervention details, reported outcomes, and any other relevant information mentioned in the included studies.

The methodological quality of each included RCT was critically appraised using the revised Cochrane Risk-of-Bias-2 (ROB-2) tool.[16] Two independent reviewers (S.K.B and K.C) performed the assessment and resolved any disagreements through discussion. The ROB2 tool assesses five domains of bias: randomization process, deviations from intended interventions, missing outcome data, measurement of outcomes, and selection of reported results. Each domain has been assigned a score of low, moderate to high.

Primary and Secondary Outcomes

The management of FM focuses on alleviating the prominent symptoms associated with the condition, such as chronic widespread pain, fatigue, insomnia, and cognitive impairment. Effective treatment approaches are tailored to suit the specific needs of each patient and incorporate a combination of nonpharmacological and pharmacological interventions. The primary outcomes of interest were FM Impact Questionnaire (FIQ), Visual Analog Scale (VAS), and the Tender Point Count (TDC). As secondary outcomes, we analysed additional objective and subjective measures of efficacy like Beck's Depression Inventory (BDI) score and Quality of Life using the 36-Item Short Form Survey (SF-36). For outcomes that are reported at multiple time intervals, the longest time point available is taken. Standardized mean difference (SMD), 95% confidence intervals (CI), and P-values were calculated for outcome analysis.

Statistical analysis

To account for the anticipated heterogeneity in methodology and clinical features across the encompassed studies and to achieve the highest level of generalizability in the meta-analytic assessments, a random-effects model was employed. Since all efficacy outcomes represent continuous data, the standardized mean difference (SMD) or Cohen's d was used to determine the effect size, along with 95% confidence intervals (CI). Data analysis was done by using the "meta" and "dmetar" package of RStudio (R Foundation). Standardised mean difference (Cohen's d) was used to measure the effect size (ES) as the data were continuous. All analyses were based on the random-effects model using the Der-Simonian and Laird method. The I2 statistic was utilized to evaluate the heterogeneity present among the studies. An I2 value of 0% to 40% suggested that the heterogeneity may not have significant implications, whereas an I2 value of 30% to 60% indicated moderate heterogeneity. A value of 50% to 90% signified substantial heterogeneity, and an I2 value of 75% to 100% represented considerable heterogeneity.[17] The effects of the various types of intervention like pharmacological, non-pharmacological and multicomponent therapy (MCT) were pooled and analysed. Since the population in the included studies is predominantly female, and the data is relatively homogeneous, coupled with a low sample size, it was not imperative to conduct a subgroup analysis.

Results

Search Results and Study Characteristics

A total of 791 articles were identified through the searches conducted, three additional articles were discovered by scrutinizing the references of the papers that surfaced during the screening process. After removing 385 duplicates, 406 articles were subjected for screening based on title/abstract. Out of these, 357 articles were excluded as they did not meet the inclusion exclusion criteria, finally 27 articles were included in our study according to inclusion criteria 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 (Fig: S1). The analysis includes a total of 2390 patients, randomly assigned across three categories of intervention: Five pharmacological interventions (6 studies), 19 non-pharmacological interventions (20 studies), and MCT (4 studies). The non-pharmacological interventions employed in the study included strengthening exercises, aerobic exercises, Stanger bath therapy and spa therapy, Whole body vibration, Mud Bath therapy, Aquatic Respiratory therapy, Basic Body Awareness Therapy, Exercise Therapy, Psychological Support, and Nature Exposure and Motivational Interviewing. Pharmacological interventions, such as Pyridostigmine, creatinine, Growth hormone (Nutropin), Pregabalin, Opioid and Paroxetine were utilized. MCT incorporated both pharmacological and non-pharmacological treatments. The studies were conducted across various countries, including Spain (6 studies), Brazil (4 studies), Denmark (1 studies), USA (6 studies), Turkey (6 studies), Italy (2) and

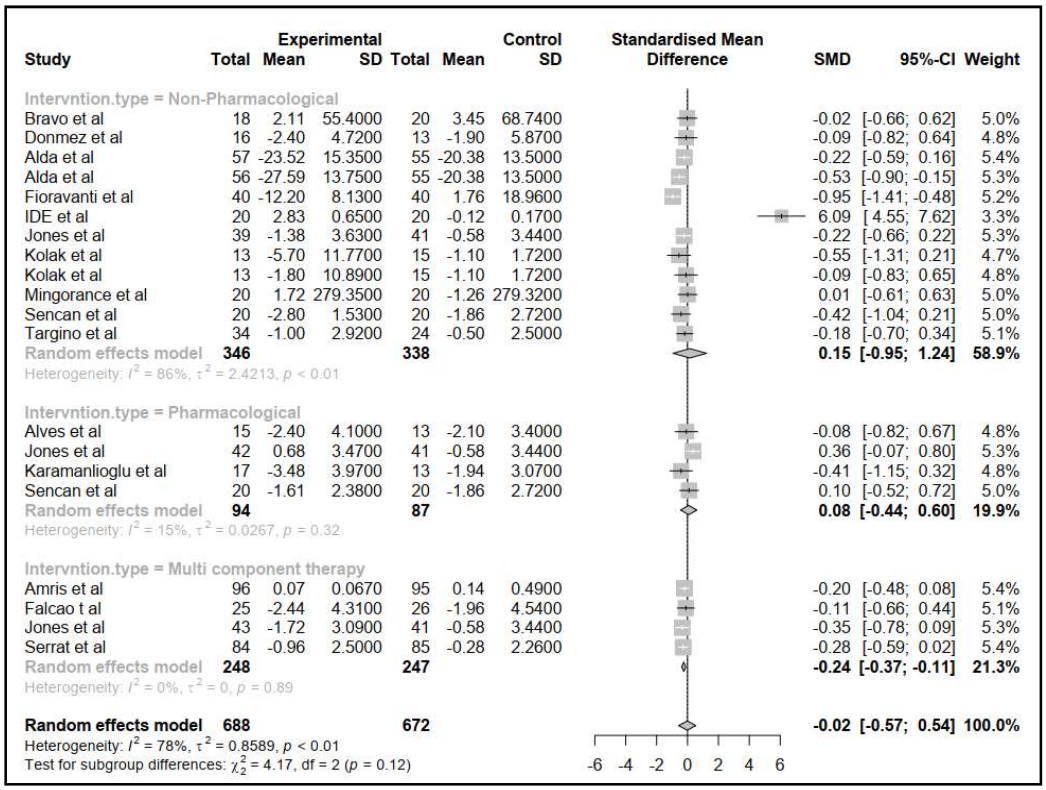

Figure 4: Forest plots for of VAS outcome

Sweden (1 studies), between 1998 and 2022. Among these, 25 studies were single centric while 2 were multicentric. The study included a predominantly female population, with 2097 participants (96.6%) and a mean age of 47.03 (SD = 8.8) years (Fig: S3). A baseline study characteristic of included RCTs were provided in Tables S1.

Risk of Bias

Overall, the risk of bias was deemed low for 12 studies, moderate for 11 studies, and high for four study. The majority of the studies had low risk of bias in the domains of randomization process, Deviations from the intended interventions, Missing outcome data, measurement of outcomes, and selection of reported results. However, two studies were judged to have high risk of bias in the domain of Measurement of the outcome, as measurement were made at a number of time points or using multiple scales. Overall, the ROB2 assessment suggests that the included studies have a generally low to moderate risk of bias (Fig S2).

Study Outcome

Efficacy Analysis

The efficacy analysis comprised of twenty-seven studies. Among the participants, 233 patients received pharmacological intervention while 1637 patients were given non-pharmacological interventions and MCT provided to 520 patients. The control group comprised of Placebo or patient receiving treatment as usual (TAU).

Visual Analogue Scale

The meta-analysis conducted on VAS comprised 18 RCTs [18, 19, 20, 22, 24, 25, 28, 29, 30, 32, 38, 39, 40, 41, 44,] wherein 16 interventions were categorized into three groups, namely non-pharmacological (nine interventions), pharmacological (four interventions) and MCT (four interventions). The collective sample size for the outcome assessment was 1360 participants, with the non-pharmacological group accounting for the majority of interventions (n=9) and the pharmacological group having 181 participants. The MCT group comprised four interventions, and a total of 495 participants were included in this category. Overall, the meta-analysis showed a small and non-significant effect size favouring pharmacotherapy over TAU with an SMD of 0.02 (95% CI: -0.57 to 0.54). The forest plot suggests that most of the individual studies are consistent with this overall finding, as the confidence intervals for each study's effect size overlap with the summary effect size. However, the meta-analysis also revealed significant heterogeneity among the studies, with an I2 value of 78%.

The subgroup analysis revealed that the non-pharmacological intervention was associated with a small effect size (SMD = 0.15; 95% CI: -0.95 to 1.24), indicating no significant difference compared to treatment as usual. The Pharmacological intervention was associated with a small effect size (SMD = 0.08; 95% CI: -0.44 to 0.60), indicating no significant difference compared to treatment as usual. However, the MCT had a moderate effect size (SMD = -0.24; 95% CI: -0.37 to -0.11), indicating a statistically significant improvement compared to treatment as usual.

FM Impact Questionnaire

The meta-analysis performed on FIQ consisted of 15 RCTs [18, 21, 23, 24, 26, 31, 32, 33, 35, 36, 37, 38, 42, 43, 44]

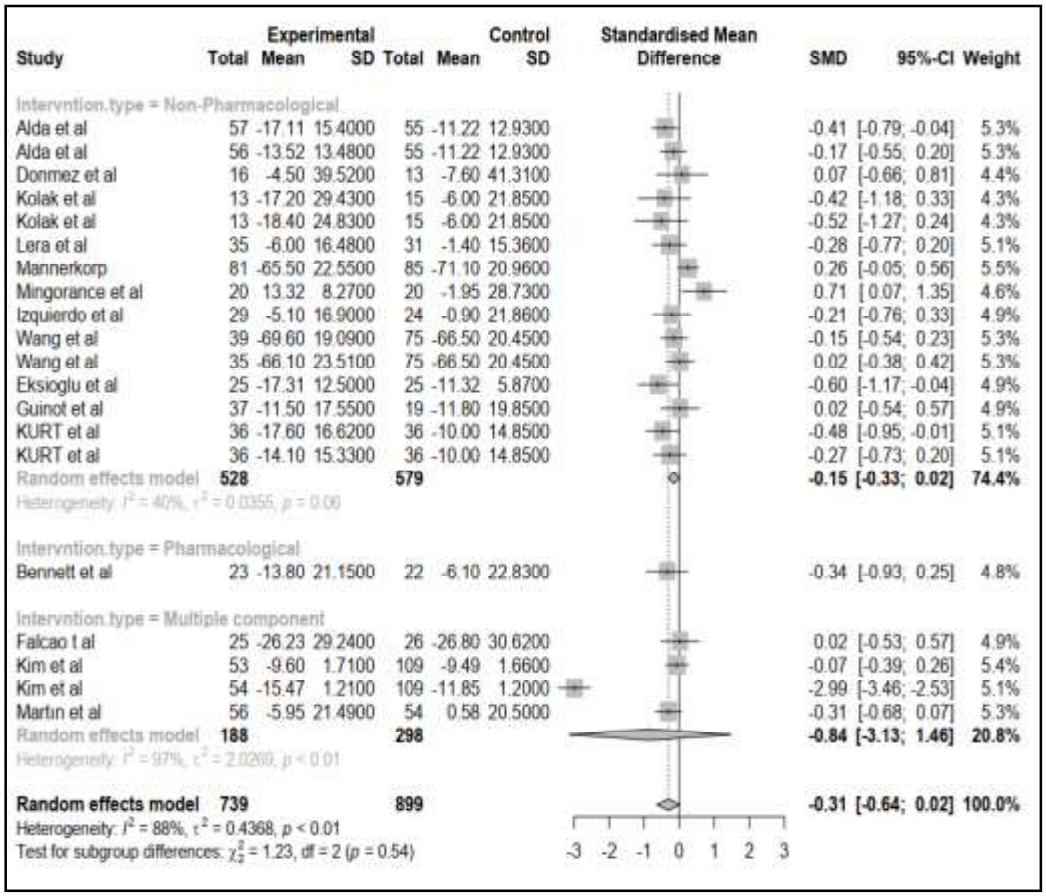

Figure 5: Forest plots for FIQ score outcome

involving 1638 participants. Out of the 15 studies, 11 evaluated non-pharmacological treatments, comprising 1107 participants. Only one study assessed pharmacological treatment, involving 45 participants, and three studies with four interventions assessed the FIQ score for MCT.

The forest plot displays the meta-analysis findings of the FIQ score, comparing the intervention group (Treatment) to the comparator group (Treatment as usual). The overall result of the meta-analysis revealed that SMD = -0.31 (CI: -0.64 to 0.02), indicating a statistically significant enhancement in the FIQ score for the intervention group compared to the treatment as usual group. Furthermore, the forest plot also demonstrates the findings of subgroup analyses based on the type of intervention. The Non-Pharmacological intervention subgroup revealed a statistically non-significant effect on the FIQ score SMD = -0.15 (CI: -0.33 to 0.02), while the Pharmacological intervention subgroup showed a statistically significant improvement in the FIQ score SMD = -0.34 (CI: -0.93 to 0.25). The MCT subgroup exhibited a large but statistically non-significant effect on the FIQ score SMD = -0.84 (CI: -3.13 to 1.46). (Figure 5).

Tender Point Count

The meta-analysis performed on TPC consisted of 7 RCTs [21, 23, 27, 29, 35, 42, 44] involving 535 participants, of which 6 studies evaluated non-pharmacological treatments, comprising 323 participants and two study assessed pharmacological treatment, involving 212 participants. The overall pooled estimate found to be, SMD = -0.17 (95% CI: -0.55 to 0.21). The subgroup analysis of the TPC score based on the type of intervention showed that the non-pharmacological group had a pooled effect size of SMD = -0.11 (95% CI: -0.69 to 0.47). On the other hand, the pharmacological group had a pooled effect size of SMD = -0.28 (95% CI: -1.37 to 0.82). The forest plot also shows that there is moderate heterogeneity among the studies, with an I2 = 64%, and the p-value for heterogeneity is less than 0.01, indicating that the heterogeneity is significant (Figure 6).

Secondary outcome

The health status and depression of patients with FM was evaluated as a secondary outcome using the Short Form-36 [27, 32, 36, 40, 41, 43] and BDI score [22, 24, 26, 32, 34, 43, 44]. A total of 607 and 463 patients were included in the meta-analysis for each outcome, respectively, across six studies. The pharmacological intervention was compared to the usual treatment. The meta-analysis indicates that the SF36 score in the intervention group was SMD= -0.15 (95% CI: -0.18 to 0.48) higher than in the treatment as usual group, with an I^2 value of 48%. Overall, the findings suggest a non-significant difference in SF36 score between the intervention and treatment as usual groups (Figure 7).

While for the other outcome meta-analysis showed that the intervention group had a BDI score of SMD = 0.79 (95% CI -1.14 to 2.72) compared to the treatment as usual group. The subgroup analysis based on intervention type revealed that MCT had an effect size of SMD = 0.01 (95% CI -0.54 to

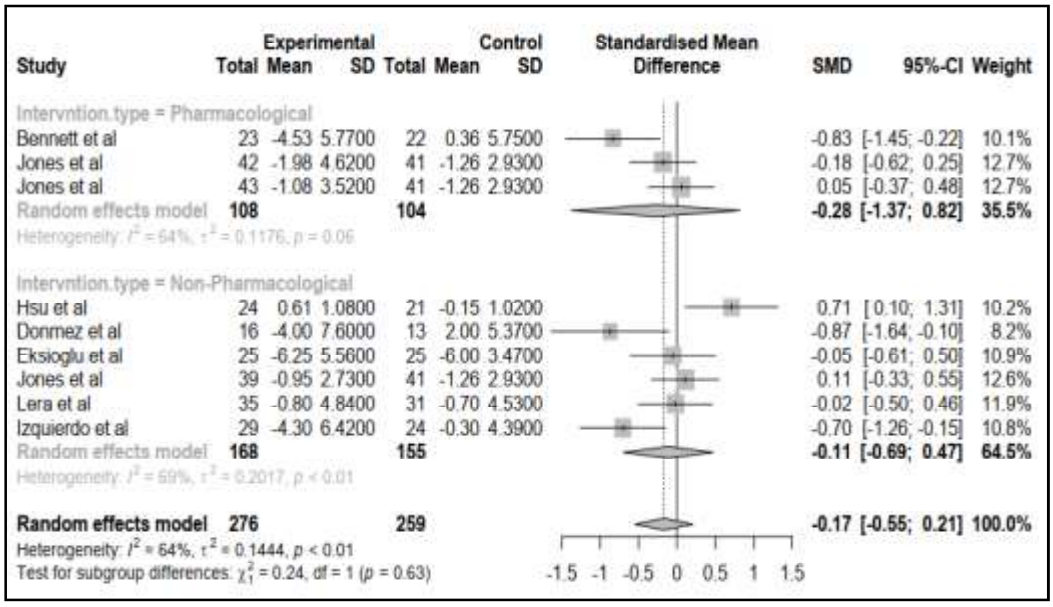

Figure 6: Forest plots for TPC outcome

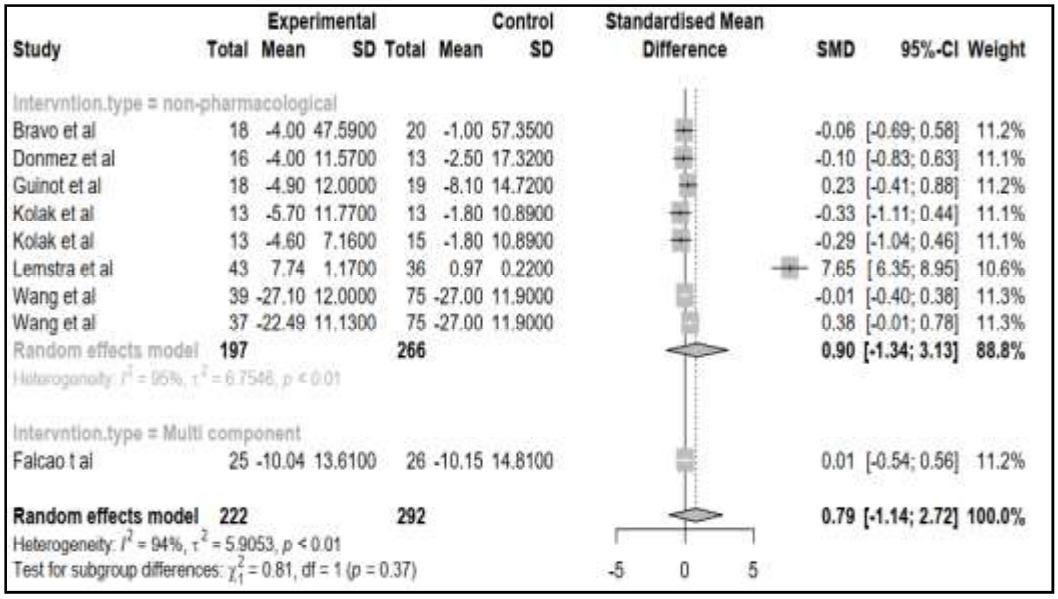

Figure 7: Forest plots for meta-analysis of SF-36

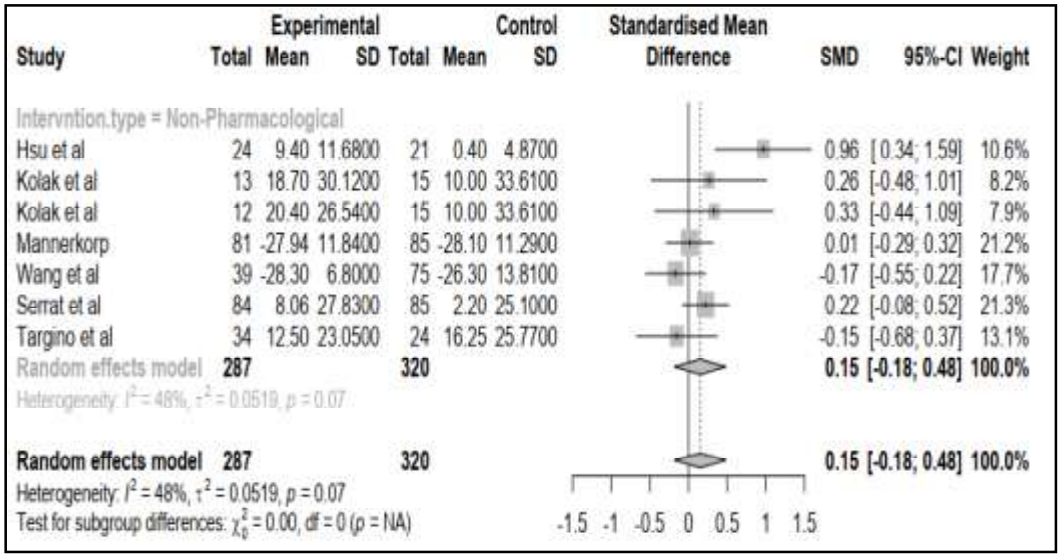

Figure 8: Forest plots for meta-analysis of BDI score

0.56), while pharmacological therapy had an effect size of SMD = -0.90 (95% CI, -1.34 to 3.13), both compared to treatment as usual (Figure 8).

Figure 8: Forest plots for meta-analysis of BDI score

Discussion

The current study evaluates both pharmacological and nonpharmacological interventions for FMS. The analysis was based on a pooled effect of 27 studies that involved 2390 patients which mainly address the symptomatic aspects of FMS reported by the patient. Only those trails were included who have assessed the effectiveness of pharmacotherapy or non-pharmacotherapy compared with TAU or Placebo. The prevalence of FMS shows a remarkable gender disparity, with women having a higher incidence than men. Thus, the current study focuses on evaluating the SMD of pain score VAS, FIQ score, and TPC, in addition assessing depression and quality of life. In a study conducted by Muhammad et al., it was observed that the ratio of female to male patients suffering from FMS was 9:1.[46] This finding is corroborated by a review conducted by Heidari et al., which estimated the total prevalence of FMS in

women to be 3.98%, while in men, it was found to be only 0.01%.[47] A similar result was observed in the present study, where 96.6% of the participants with FMS were female. The reasons for the higher prevalence of FMS in women remain uncertain. Additionally, women tend to exhibit more severe and unpredictable symptom progression compared to men. FMS predominantly occurs in adults aged between 40-50 years.[48] The study conducted herein has demonstrated that the mean age of individuals diagnosed with FMS is 47.03 (SD 8.8) years. The present study's results align with those of Walitt et al. investigation, which derived analogous findings via an interview-based survey. Our investigation discovered that the incidence of FM syndrome (FMS) was least prevalent among individuals aged 18-29, at a rate of 0.76% (0.05, 1.46), and rose to 2.41% (1.49, 3.33) among those aged 50-59 years. Moreover, there was no significant difference in the prevalence of FMS compared with older age groups. These results provide important insights into the age distribution of FMS and could inform the development of targeted interventions for this population.[49] Despite being recognized for several decades, the diagnosis of FMS remains difficult due to the absence of a definitive pathophysiological mechanism. The diagnostic and therapeutic procedures for FMS patients are protracted and intricate, encompassing multiple consultations with healthcare providers and a prolonged waiting period averaging two years prior to diagnosis.[50]

Current research has examined the effectiveness of various agents in the treatment of FMS. However, the use of pharmacological interventions alone is insufficient in treatment this condition. Despite the potential benefits of pharmacological interventions, non-pharmacological interventions have been shown to be equally effective in managing FMS symptoms. The results showed a non-significant improvement in the FIQ score with a SMD of 3.61 (CI: -0.79-8.01), p-value of 0.1, while the VAS score showed a significant improvement with a SMD of 1.41 (CI: 0.08-2.73), p-value of 0.003, in favour of the non-pharmacological intervention group. Among non-pharmacological interventions, MCT followed by aerobic exercise Low to moderate intensity endurance and strength training are strongly recommended. Chiropractic, laser therapy, magnetic field therapy, massage and transcranial magnetic stimulation are not recommended and CBT was most promising for reducing pain and improving quality of life. Of the non-pharmacological interventions, only exercise was evaluated in one large trial.[51] Pharmacological treatments show limited clinical evidence, and non-pharmacological interventions also lack substantial support. However, healthcare professionals concur that enhancing daily function and quality of life relies on crucial self-management strategies. Despite the scarcity of scientific evidence endorsing their effectiveness, self-management strategies can incorporate complementary and alternative medicine interventions.[52]

The present meta-analysis is subjected to several limitations. Firstly, the only few studies [53, 54, 55, 56] included trials that directly compared the efficacy of pharmacological therapy to non-pharmacological therapy, which limits the conclusions that can be drawn about the relative effectiveness of these treatments. Secondly, the majority of studies included in the analysis had small sample sizes, which may have reduced the quality of the pooled results. Finally, the predominantly female sample may limit the generalizability of the findings to male populations, as FM is a syndrome that affects women more frequently than men.

The main reason for the disparity in recommendations for pharmacological treatment of FMS is the lack of sufficient high-quality randomized control trials in the field. As a result, the guidelines have to rely on evidence of lower quality and expert consensus.[57] The use MCT (combination of aerobic exercise with at least one psychological therapy) with a duration of at least 24 h is strongly recommended for patients with severe forms of FM.[58]

Conclusion

In conclusion, the study highlights the limited evidence available about effective and clinically relevant treatments for FMS. A combination of pharmacological and non-pharmacological interventions may be most promising, but additional high-quality trials are needed to confirm the effectiveness of non-pharmacological interventions such as CBT, aerobic exercise, and MCT.

Supplementary file information

Tables S1 gives a comprehensive overview of key characteristics of included RCTs. Tables S2 outlines the exclusion of specific studies, elucidating the selection criteria with reasons. Tables S3 delineates the systematic review's search strategy, revealing the methods employed in identifying pertinent literature. Tables S4 Presents the PRISMA Checklist, which serves as a reference for assessing the adherence of the guidelines. Fig S1 shows PRISMA flow chart visually illustrates the study selection process. Fig S2 displays results from the Methodological Quality Assessment via RoB-2 and Fig S3 presents a gender-based distribution of FM in the study.

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Competing interests

Authors declare no conflict of interests

Availability of data

The data used to support the findings can be provided on relevant request.

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CRIPS Digest

Phase-separating peptides for direct cytosolic delivery for macromolecular therapeutics

Biotechnologically derived products such as peptides, recombinant proteins and mRNAs possess high target specificity, efficacy and lower risk profile; however they have poor aqueous solubility, stability and cellular permeability. To counter these pitfalls, various formulation techniques have been exploited. One of these novel strategies employ smart peptides that respond to physiological stimuli like pH, redox reactions, temperature, etc. The researchers used short Histidine-rich break peptide (HBpep) containing self-immolative moieties conjugated by disulphide bonds (HBpep-SR) in order to study the cytosolic delivery of macromolecules. HBpep was synthesized by the conventional Merrifield solid-phase peptide synthesis method, purified using HPLC and isolated by lyophilisation. The peptides so formed were modified by the addition of self-immolating moieties such as HO-SS-R and NHS-SS-R. These moieties initiated a chemical reaction between the e-amine terminal of the single Lys residue of N-terminal protected peptide and the amine-reactive species NHS-SS-R to form HBpep-K and HBpep-SR. The desired concentration of the Enhanced Green Fluorescence Protein (EGFP) was dissolved in a buffer (pH 7.5). A stock solution of peptides (pH=7.5) was mixed with EGFP-containing solution in a 9:1 ratio to form coacervates. The entrapment efficiency was quantified using a fluorescence spectrophotometer taking the supernatant buffer before and after coacervation. The mean size range of EGFP-loaded HBpep-K and HBpep-SR peptide coacervates was reported in the range of 964-982 nm with narrow size distribution. The zeta potential of peptide-coacervates was reported to be -12.4 mV. No potential cytotoxicity was reported with pristine coacervates and EGFP-loaded coacervates in the HEK293 cells. The redox-responsive behavior was screened by analyzing the EGFP release from the coacervates in the presence of glutathione (GSH) in HepG2 cells. These coacervates formed vesicular structures inside the cells. As soon as the pH was shifted from 7.5 to 6.5, the whole EGFP content was released within 24 h which confirmed the pH-responsive behavior of peptide coacervates. In the presence of a cytosolic compartment rich in GSH, the peptide moieties were reduced by GSH and the redox-responsive release of EGFP was triggered within 4 hours. To study the cell internalization mechanism, EGFP-loaded coacervates were incubated with HepG2 cells, and LysoTracker staining was performed. Confocal microscopy confirmed that the prepared coacervates did not localize into endosomes. The treatment of cell lines with endocytosis inhibitors did not reduce the internalization of coacervates. Coacervates loaded with mRNA were incubated with RNase to check if the prepared coacervates possess mRNA protective activity. The researchers reported that coacervate-loaded mRNA cargoes had a delivery efficiency of ~98% compared to 81% of free mRNA. The entrapped mRNA was protected by peptide coacervate from RNase, thus more efficient delivery was reported. Following these encouraging results, the scientists aspire to establish in vivo efficacy of the presented drug-delivery system to enhance its translational value. (Nat. Chem. 2022, 14(3): 274-283)

Mesenchymal stem cell-derived exosomes protect against liver fibrosis by delivering miR-148a to target KLF6/STAT3 pathway in macrophages

Nowadays, the Mesenchymal Stem Cells (MSCs)-based techniques are considered one of the best due to their unique characteristics and immunomodulatory effects. The applicability of MSCs as a regenerative solution for various diseases including spinal cord injury, organ fibrosis, inflammatory bowel disease and graft-versus-host disease is continuously emerging. Despite intensive research, the underlying mechanisms involved in the therapeutic potential of MSCs for liver fibrosis are still unexplored. Currently, MSCs-derived exosomes (MSC-EXOs) are widely accepted as crucial messengers for intercellular communication. In this particular study, researchers attempted to explore the therapeutic effects and mechanisms of MSC-

EXOs in liver fibrosis. Liver fibrosis was induced by carbon tetrachloride followed by intravenous injection of MSCs or MSC-EXOs to assess the therapeutic potential. The resulting histopathology, extent of fibrosis, inflammation and macrophage polarization were analyzed. The regulatory effects of MSC-EXOs on macrophage polarization were assessed by using RAW264.7 and BMDM cell lines. Then, the critical miRNA involved in the therapeutic effects of MSC-EXOs was identified by RNA sequencing and validated experimentally. Further, the target mRNA and downstream signalling pathways were elucidated by luciferase reporter assay, bioinformatics analysis and western blot. MSCs alleviated liver fibrosis by secreting exosomes which were found to be circulating into the liver after transplantation. Additionally, MSC-EXOs modulated the macrophage phenotype which then regulated the inflammatory microenvironment and repaired the liver injury. Mechanically, RNA-sequencing illustrated that MSC-EXOs enriched miR-148a targeted the Kruppel-like factor 6 (KLF6) to suppress the pro-inflammatory macrophages and promote anti-inflammatory macrophages by inhibiting the STAT3 pathway. Liver fibrosis was significantly reduced when miR-148a agomir or MSC-EXOs enriched with miR-148a were administered. Conclusively, MSC-EXOs may act as a potential therapeutic target for liver fibrosis by delivering miR-148a which normalizes intra-hepatic macrophage functions through KLF6/STAT3 signaling. (Stem Cell Res. Ther. 2022, 13(1): 330)

DNA damage assessment for precision medicine: an emerging diagnostic tool

DNA damage analysis as a diagnostic tool is a contributing factor in the development of precision medicine. The use of potential biomarkers such as phosphorylated histone 2Ax (?H2AX) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) has increased the growth in the field of DNA damage analysis. Several other novel biomarkers have been incorporated into routine patient categorization into cohorts with specific disease predispositions which allows early diagnosis of a disease. Advanced diagnostic technologies have been incorporated to evaluate a multitude of new possible biomarkers. Various approaches, features, technological advancements and prospective clinical applications of biomarkers of DNA damage in the context of precision medicine have been explored. Tumor heterogeneity is considered as a major obstacle to precision medicine. Much progress has been achieved in treating cancer patients based on the individual's diagnostic profile. A paradigm shift seems to take place where the high heterogeneity among the tumors is addressed by a more comprehensive biomarker-based identification and prediction in patients. For example, the fluorescence microscopy-assisted counting of ?H2AX foci, a marker of DNA damage, in the case of DSB assessment is getting popularity over other ?H2AX-detecting methods due to its superior sensitivity. Biomarkers of diffuse large B-cell lymphoma, one of the most aggressive forms of non-Hodgkin's lymphoma, have been diagnosed in more than 50% of lymphoma patients over the age of 65. A wide range of biomarkers would be required to enable complete and early detection of oncological illnesses. The utilization of capillary peripheral blood mononuclear cells as a potential substrate for the automated detection of "H2AX foci" is one of the most suitable diagnostic methods. There are a number of open-source image processing software programs like Cell Profiler, Icy, ImageJ/Fiji, and Find Foci, graphical user interfaces that are currently popular for counting H2AX foci. (J. Lab. Precis. Med. 2019, 4: 1-4)

Modelling metabolic diseases and drug response using stem cells and organoids

Metabolic diseases including obesity, diabetes mellitus and cardiovascular disease are the major threats to health in the modern world. Obesity is linked to non-alcoholic fatty liver disease (NAFLD) which eventually leads to non-alcoholic steatohepatitis (NASH). There is no FDA-approved therapy for NASH, but human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSCs) based technologies can generate various disease-relevant cell types. Animal models of metabolic diseases are limited due to unique human biology. Lack of proper animal models is responsible for the failure of drugs in the clinical trials. These failures are due to species specificity of the genomes and epigenomes. The goal of the research communities nowadays is to develop new models which replicate human pathophysiology and exploring the rational treatments, therapies and approaches. Organoids are 3D organ culture technologies to model human physiology and disease in biomedical research. Human adipose and liver organoid models are generated from healthy/diseased tissues and pluripotent stem cells such as hPSCs, ASCs and fully differentiated primary cells. Understanding the various cell-cell interactions through pluripotent stem cells techniques may lead to new therapeutic targets. Adipose organoids and spheroids have greater utility than classic adipose cellular models due to their greater morphological and functional resemblance to adipose tissue. LGR5+ stem cells generate organoids from other tissue stem cells. In the mouse liver, LGR5+ stem cells were

activated by carbon tetrachloride and cultured with Wnt agonist R-spondin. Human adult liver organoids were derived from EpCAM+ biliary cells and maintained genomic stability after long-term expansion. Hepatocyte organoids have high proliferative potential in-vivo. Adipose organoids can be used to model obesity and T2DM, while liver organoids can be used to model liver diseases that exhibit impaired cell-cell interaction and tissue structural alteration. Human organoid technology generates patient-specific organoid models. It can be used for drug discovery and precision medicine to understand the regulation and function of circadian clocks in humans. The use of individual-derived stem cells provides a platform for personalized medicine. High-throughput screening, precision medicine and organoid models can identify compounds promoting metabolic diseases. Advanced gene editing tools can prevent or rescue phenotypes of genetic diseases by repairing or inactivating mutations in cellular and animal models. CRISPR-Cas9 and base editing are used to revert a disease-causing mutation to wild-type in patient-derived hepatic organoids. Combining stem cell-derived organoids with gene editing and functional genomics has revolutionized the approach of finding treatments for metabolic diseases; however, it has been accompanied by many challenges and limitations such as heterogeneity and reproducibility. Ongoing development of synthetic and versatile scaffolds will reduce cost, increase reproducibility and promote the translational efficacy of organoids for future use. (Nat. Rev. Endocrinol. 2022, 18(12): 744-759)

The cGAS–cGAMP–STING pathway connects DNA damage to inflammation, senescence and cancer

Cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase (cGAS) is a DNA sensor which is activated due to presence of DNA in the cytoplasm instead of nucleus. It has been reported that cGAS acts as a danger-associated molecular pattern (DAMP) to induce type I interferons (IFNs) and other cytokines through production of a second messenger cyclic GMP-AMP (cGAMP). Further, cGAMP binds and activates the adaptor protein STING which activates TANK-binding kinase 1 (TBK1) and IFN regulatory factor 3 (IRF3) along with activation of NF-?B. However, cGAS can be activated by double-stranded DNA irrespective of the sequence. Cytoplasmic DNA is a consequence of nuclear DNA damage that is observed in the form of micronuclei. These are the by-products of chromosome damage, centromere hypomethylation and kinetochore dysfunction which occurs due to genotoxic stress. Cytoplasmic DNA may accumulate in Trex1-deficient cells to activate cGAS. MUS81 is an endonuclease which converts nuclear DNA into cytoplasmic forms. The cGAS–cGAMP–STING axis connects DNA damage to auto-inflammatory diseases. Ataxia-Telangiectasia (A-T) is a consequence of dysfunctional V(D)J, a genetic recombination phenomenon, observed in vertebrates. Dysfunctional V(D)Jcan be due to the mutation in A-T kinase enzyme. Another neuronal disease Aicardi-Goutières syndrome (AGS) caused by a recessive mutation in trex1, rnaseH2a, rnaseH2b and samhd1 is also associated withan elevation in type I IFNs. cGAS is also altered in DNA damage-induced cellular senescenceand irreversible cell cycle arrest which is induced by a variety of external and internal stress such as telomere shortening, oxidative damage and oncogenic sign through the senescence-associated secretory phenotype (SASP). SASP can mediate both positive and negative effects of senescence in cancer. Cytosolic DNA activates the cGAS–cGAMP–STING pathway in APC along with activation of Type 1 IFN mediated by nuclear MUS81 activity which is responsible for anti-tumour immunity. cGAS–STING pathway acts as an intrinsic barrier to tumorigenesis by linking DNA damage to several antitumor mechanisms like NLRP3 inflammasome activation, immune surveillance, cellular senescence and cell death. On the other hand, it also activates cGAS–cGAMP–STING pathway which further promotes inflammation-driven carcinogenesis and metastasis. cGas–cGAMP–STING pathway can be a promising target with regard to cancer therapies like chemotherapy, radiation therapy and immunotherapy. (J. Exp. Med. 2018, 21(9): 1287-1299)