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Methotrexate and curcumin: Therapeutic potential and drug delivery approaches

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Abstract
The combination of non-specific cytotoxic or immunosuppressive agents with bioactive natural substances as part of combination pharmacotherapy is an important process in modern medicine. Methotrexate (MTX) is a folic acid metabolism blocker that is used in treating various types of cancer and chronic autoimmune inflammatory diseases, like psoriasis and rheumatoid arthritis. However, it has limited efficacy due to strong off-target toxicity, rapid biological clearance, and the development of resistance to multiple drugs. Curcumin (CUR) is a non-polar polyphenol that has anticancer, anti-inflammatory, antioxidant, and chemosensitizing effects. CUR enhances the synergistic action of MTX and fights transport resistance, restoring the sensitivity of the affected cells and activating the Nrf2/ARE pathway, which helps to avoid MTX-induced liver toxicity, digestive system mucositis, and oxidative injury. Nevertheless, the clinical effectiveness of MTX and CUR combinations is limited by their disparate pharmacokinetic characteristics, low solubility in water, and rapid curcumin metabolism. Advanced drug delivery systems, such as liposomes, polymeric nanoparticles, solid lipid carriers, dendrimers and ointments containing liposomal gel were developed to tackle pharmacokinetic problems that arise due to those features. This review provides a comprehensive evaluation of the individual pharmacological properties of both MTX and CUR. It discusses the complexities of their molecular interactions, advances in drug delivery technologies including the use of liposomal and topical gel formulations, and the challenges of translating their findings into the real world.

Keywords: Methotrexate, curcumin, liposomes, liposomal gel, drug delivery systems

Introduction
India's tribunal system has repeatedly generated the same constitutional anxiety: bodies created to exercise judicial or quasi-judicial power have often remained administratively dependent on the executive departments whose decisions The treatment process of complex diseases such as cancerous tumours and autoimmune disorders increasingly relies on multi-targeted therapeutic methods. Monotherapy is often inefficient when treating chronic illnesses due to various cellular targets, compensatory mechanisms of survival signalling, and the development of cellular drug resistance [1]. In addition, increasing the dose of one effective medication can also lead to severe dose-limiting toxicity in non-target healthy tissues. Methotrexate is an antimetabolite and disease-modifying antirheumatic drug (DMARD) used in both oncology and rheumatology [2]. While very effective against rapidly proliferating cells, it is hampered by acute tissue toxicity (such as hepatotoxicity, nephrotoxicity, and enteritis) and resistance to treatment. Hence, natural phytochemicals provide a great option for an adjuvant that can enhance the treatment outcome and minimize adverse effects [3]. Despite the therapeutic potential of CUR, there are several biopharmaceutical hurdles to its clinical use. These hurdles include the extremely low solubility of CUR in water, the poor absorption of CUR when administered orally, fast metabolic reaction in the body, and quick elimination from the system [4]. In the case of using free MTX and CUR together, the difference in their pharmacokinetic profiles prevents simultaneous delivery of these compounds to target tissues at required concentrations for their synergistic effect. The techniques using nanotechnology provide the opportunity for the alignment of the pharmacokinetics of the drugs and the protection of the active ingredients from early destruction [5].

Methotrexate: Therapeutic Potential and Limitations
Therapeutic Mechanism and Clinical Applications
Methotrexate, a synthetic derivative of folic acid, acts on the molecular level by competitively and permanently blocking the enzyme dihydrofolate reductase (DHFR), which is responsible for the transformation of dihydrofolate (DHF) into tetrahydrofolate (THF). THF is an important carbon donor necessary for the biosynthesize of purine nucleotides and thymidylate. Specifically, MTX disrupts DNA synthesis, RNA transcription, and protein translation processes by consuming the stocks of reduced folates inside cells and trapping them in the S phase of the cell cycle. In addition, MTX blocks the action of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase so that adenosine accumulates outside the cell as a potent anti-inflammatory compound [6,7]. Due to its properties, MTX has become a miracle drug in the treatment of numerous cancers, from acute lymphoblastic leukaemia to breast cancer and non-small-cell lung cancer.

Biopharmaceutical and Clinical Limitations
Despite its broad clinical benefits, MTX therapy is confronted with significant problems.

- **Systemic Off-Target Toxicity:** The lack of selectivity towards the affected tissues means that MTX can also damage fast-growing healthy cells in the body. Thus, prolonged or high-dose treatment with MTX often leads to liver toxicity (high AST/ALT levels, liver steatosis, fibrosis), severe gastrointestinal injury (enteritis and

mucositis), kidney injury (due to crystalluria), as well as myelosuppression and bone marrow depression [4].

- **Short Biological Half-Life:** Unbound MTX has a short plasma half-life and is rapidly eliminated from the body, which makes it necessary to perform regular high-dose injections or take oral medications in order to maintain the desired plasma levels of the drug [8].

- **Acquired Resistance:** Extended exposure to MTX usually causes secondary resistance. The molecular mechanisms of resistance consist of amplification of the DHFR gene, mutations in the structure of the target enzyme, decreased expression levels of transporters for absorbing active substances (e.g., RFC-1) and increased drug excretion via efflux transporters like ATP-binding cassette (ABC) transporters (e.g., P-glycoprotein and multidrug resistance-associated proteins) [8].

Curcumin: Therapeutic Potential and Limitations

Therapeutic Potential and Molecular Targets

Curcumin, a type of natural polyphenol that has its origin in the rhizome of *Curcuma longa* (turmeric). CUR has multiple therapeutic characteristics such as being antiproliferative, pro-apoptotic, anti-angiogenic, anti-inflammatory, and radical-scavenging. At the cellular signalling stage, CUR connects with different regulatory systems, including: CUR interferes with NF-κB, which decreases the levels of pro-inflammatory cytokines (for example, TNF-α, IL-1β, and IL-6) and inflammatory enzymes such as cyclooxygenase-2 [9]. CUR also induces apoptosis, which leads to decreased expression of anti-apoptotic proteins (Bcl-2 and Bcl-xL) and enhanced expression of pro-apoptotic proteins (Bax, Bak). This results in an increased permeability of mitochondria and activation of Caspases-3/9. CUR also stops some survival pathways, including JAK/STAT3 and PI3K/Akt signalling pathways. Finally, CUR enables the nuclear translocation of Nrf2 [10,11].

Biopharmaceutical Limitations

Even though CUR has great therapeutic capability, it is considered to be a Class IV drug according to the Biopharmaceutics Classification System (BCS), causing serious clinical challenges:

- **Low Aqueous Solubility:** CUR has extremely low water solubility (0.1 µg/mL in physiological pH), resulting in poor absorption and slow dissolution [2].

- **Considerable First-Pass Metabolism:** After its introduction, CUR rapidly enters phase II metabolism in the liver and intestines through sulfation and glucuronidation processes and creates inactive metabolites [4].

- **Limited Systemic Bioavailability:** CUR's unformulated version has a very short half-life and low plasma level with poor accumulation in the target tissues [12].

Multi-Target Molecular Synergy and Organoprotective Crosstalk

Molecular Synergy Amplification and Signalling Networks

Administering MTX and CUR together creates a multi-level pharmacological treatment where CUR acts as a molecular synergy amplifier. Rather than targeting one enzyme, this treatment impacts whole networks of metabolism, survival, and apoptosis at the same time:

a. **Dual Blockade of Genomic and Survival Signals:** While MTX inhibits folate metabolism and blocks nucleic acid synthesis through DHFR, CUR acts downstream, blocking signals that lead to anti-apoptosis. In osteosarcoma (MG-63) cell lines, CUR co-administration leads to suppression of core Hedgehog pathway components, such as Patched-1 (PTCH1), Smoothened (SMO), and transcription factors GLI1 and GLI2. Thus, they achieve a downregulation of β-Catenin and matrix metalloproteinases (MMP-2, MMP-9), leading to the halt of cell migration processes [14,15].

b. **Potentiation of Mitochondrial Apoptosis:** This combined treatment alters the cellular redox state in neoplastic cells and increases levels of pro-apoptotic Bax while decreasing levels of anti-apoptotic Bcl-2. The increase in the Bax/Bcl-2 ratio leads to changes in mitochondrial membrane potential ($\Delta\Psi_m$) and release of cytochrome c and activation of Caspase-9 and Caspase-3 in breast cancer (MCF-7), cervical cancer (HeLa), and lung cancer (Calu-3) [12,10].

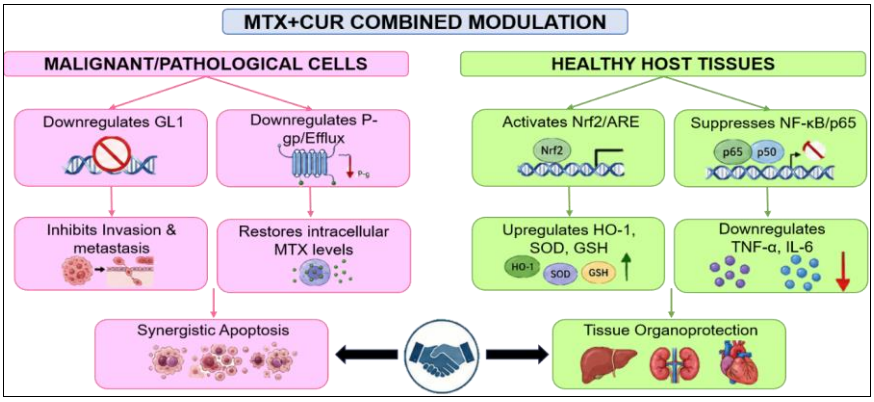

Fig 1: Schematic representation of the proposed synergistic effects of methotrexate (MTX) and curcumin (CUR) in pathological cells and healthy tissues, involving inhibition of Hedgehog/GLI and P-gp-mediated efflux, activation of Nrf2/ARE antioxidant pathways, and suppression of NF-κB-mediated inflammation, leading to enhanced apoptosis and tissue organoprotection.

Restoration of Transport-Related Multidrug Resistance

Generally, the acquisition of drug resistance to MTX is through changes in the uptake and efflux mechanisms of cells, mainly the overexpression of transport proteins that belong to the family of ATP-binding cassette (ABC) transporters, such as P-glycoprotein (P-gp/ABCB1), as well as the family of resistance proteins (MRPs/ABCC). High concentrations of P-glycoprotein enable the pumping out of MTX from the cell, and even then, cells cannot reach therapeutic MTX levels. CUR counteracts this resistance mechanism. It acts both as a competitive and non-competitive P-glycoprotein inhibitor. CUR attaches to transport areas and also inhibits P-glycoprotein expression by activating NF-κB and Akt pathways. Due to such inhibition, MTX does not experience active efflux and can reach therapeutic concentrations of this drug within cells [16,17].

Organoprotective Crosstalk: Nrf2/ARE vs. NF-κB Signaling

In addition to its combined toxic effects on affected cells, CUR shields healthy cells from damage caused by MTX. MTX causes mitochondria to function well in energy production, reduces glutathione levels, increases lipid oxidation, and creates an inflammatory response. CUR mitigates MTX-induced toxicity via interactions between Nrf2/ARE signalling and NF-κB signalling:

- **Nrf2/ARE Axis Stimulation:** CUR enhances Keap1 hyper-alkylation, resulting in Nrf2 liberation to enter into the nucleus. Inside the nucleus, Nrf2 interacts with ARE motifs to induce different antioxidant enzymes (HO-1, SOD, CAT, GPx), which clear ROS and restore the redox state of tissues [18].
- **Inhibition of NF-κB Pathway:** Nrf2 in the nucleus can displace NF-κB p65 from the CREB-binding protein (CBP) co-activator complex. The action of HO-1 also leads to direct inhibition of NF-κB gene expression. This decreases the production of pro-inflammatory factors (TNF-α, IL-1β, IL-6, COX-2), hence preserving the intestinal epithelium from enteritis and reducing liver enzyme leakage due to MTX (AST, ALT) [19].
- **Activation of the Akt/GSK-3β Pathways:** CUR enhances Akt phosphorylation at Ser473, resulting in GSK-3β inactivation via Ser9 phosphorylation. GSK-3β inactivation inhibits p53- and PARP-1-mediated apoptosis, leading to preservation of physiological liver, kidney, and gonadal cells [20,21].

Table 1: Dual Pharmacological Profiling of MTX and CUR Co-Administration across Target Tissues.

Target	MTX Pathology	CUR Mechanism	Physiological Outcome
Hepatic Tissue	GSH depletion, MDA accumulation, acute hepatocellular necrosis, AST/ALT elevation	Activates Nrf2/HO-1 axis; suppresses NF-κB p65; modulates Akt/GSK-3β	Scavenges ROS, restores SOD/CAT reserves, normalises ALT/AST levels [19,9]
Gastrointestinal Tract	Mucositis, enteritis, crypt cell atrophy, breakdown of mucosal barrier	Activates MAPK phosphatase-1; represses NF-κB and NLRP3 inflammasome	Preserves villi architecture, lowers bacterial translocation and mucosal injury [21,22]
Neoplastic Tissue	Acquired resistance, P-gp efflux, DHFR amplification, survival signalling	Downregulates Hh (PTCH1, SMO, GLI1/2), NF-κB, β-Catenin, STAT3, P-gp	Synergistic apoptosis, elevated Bax/Bcl-2 ratio, reversal of chemoresistance [23,24]
Ovarian / Gonadal Tissue	Follicular atresia, p53/PARP-1 upregulation, severe lipid peroxidation	Restores SOD/CAT activity; represses p53 and PARP-1 expression	Reduces atretic follicles, increases primordial count and AMH levels [25]

Drug Delivery Approaches for Methotrexate and Curcumin

To address the biopharmaceutical limitations of unformulated MTX and CUR—such as rapid elimination, poor aqueous solubility, and disparate pharmacokinetics—a range of advanced nanocarriers has been engineered.

Nanoparticles and nanocapsules based on polymers:

Copolymers of poly (lactic-co-glycolic acid) (PLGA) are often considered good candidates for the co-encapsulation of drugs due to their biodegradability and predictable hydrolytic degradation. PLGA nanoparticles with a size of less than 150 nm that are co-encapsulated with both MTX and CUR reach an encapsulation efficiency of around 71% for MTX and approximately 86% for CUR. These formulations are capable of releasing drugs in a biphasic kinetics pattern while achieving higher cytotoxicity against MCF-7 breast cancer cells *in vitro* and better tumour growth inhibition *in vivo* when compared to free drug combinations [26]. Alginate (ALG) biopolymeric capsules cross-linked with Ca^{2+} and stabilized by Poloxamer P407 and protect the drug payload under the simulated gastric environment while favouring drug release in a modestly acidic tumour cellular environment. Interfacial polymeric deposition also results in the formation of nanocapsules (287-325 nm) co-encapsulating both MTX and CUR, enabling the cells (Calu-3, the non-small lung cancer cell line) to trigger earlier and late apoptosis without the significant initial burst release of drugs [27,6].

Dendritic Nanosystems:

G4-PAMAM dendrimers – the fourth generation of poly(amidoamine) – have a hyper-branched polymeric structure which is monodisperse. MTX acts as a therapeutic compound alongside being a targeting ligand for FR-α when covalently conjugated with the terminal primary amines via DCC/NHS coupling technology. CUR, being hydrophobic in nature, is temporarily trapped in the internal part of the dendrimer. The formed Cur-PAMAM-MTX structure of around ~249 nm binds to FR-α-expressing HeLa cells, which results in ROS generation inside the cells and eventual Caspase-mediated apoptosis [7].

Protein Nanoparticles

BSA nanoparticles based on the Nab technology represent stable spherical carriers with a size of around ~163 nm. Albumin carriers take advantage of transport pathways

taking advantage of SPARC and the albondin (gp60) receptor, both expressed in large amounts in the tumour endothelium and inflammatory arthritic synovium. In adjuvant-induced arthritis models, BSA-MTX-CUR nanoparticles influenced the circulation time ($t_{1/2}$) and in the area under the curve (AUC), leading to greater accumulation in the structures of inflamed joints [13].

Smart Stimuli-Responsive Micelles

The micellar systems that respond to stimuli utilize the physiological gradient in pH (pH 7.4 in blood circulation versus pH 4.5-6.5 in endo/lysosomal compartments). Micelles composed of DSPE-PEG-Imine-MTX conjugates have CUR trapped within their hydrophobic lipid core. The attached MTX located on the surface of micelles induces specific cellular endocytosis due to binding to cell-surface folate receptors. Internalization into acidic environments leads to the hydrolysis of dynamic imine bonds and release of CUR along with active MTX inside the cells [1].

Liposomal Drug Delivery and Co-loading

Liposomes represent one of the most clinically established nanomedicine platforms. Composed of concentric phospholipid bilayers surrounding aqueous compartments, liposomes are uniquely structured to co-encapsulate molecules with differing physicochemical properties.

Core-Shell compartmentalization

The amphiphilic structure of liposomes resolves the solubility mismatch between MTX and CUR:

- **Hydrophobic Lipid Bilayer:** It is possible to incorporate CUR into the acyl chains of the phospholipid bilayer due to the fact that CUR is lipophilic. This prevents CUR from undergoing aggregation, hydrolytic degradation and metabolic inactivation.
- **Aqueous Core:** MTX, which is either hydrophilic or amphoteric, is incorporated in the central aqueous core of the structure.

This approach allows both drugs to be placed in a single liposome to ensure synchronization from different perspectives, such as pharmacokinetics, which enhances drug delivery to the target cells [13].

Structural Modifications and Niosomes

Standard liposomes can be modified to optimize performance:

- **PEGylated liposomes:** The application of polyethylene glycol (PEG) on the surface of a conventional liposome gives rise to a hydration layer that prevents opsonization of plasma proteins, minimizes the detection by the reticuloendothelial system (RES) and ensures a longer half-life in the bloodstream. In addition, the prolonged blood circulation allows passive accumulation of the formulation in solid tumours or inflamed joints, which is driven by the enhanced permeability and retention (EPR) effect [28,29].
- **Niosomes:** Niosomes refer to bilayer vesicular systems derived from the use of synthetic non-ionic surfactants (i.e. Span, Tween) and cholesterol. Micelles that contain MTX and CUR in one portion show better drug absorption, greater inhibition of growth and reduced cell motility in HCT-116 colorectal carcinomas in comparison with the combination of drugs [30,31].
- **Ultra-deformable vesicles (transfersomes and ethosomes):** By enriching the formulation with edge activators (surfactants like sodium cholate) and high levels of ethanol (20–40%) it is possible to produce vesicles that will be sufficiently flexible to pass through narrow intercellular junctions while preserving their structure [32,33,34].

Table 2: Comparative Physicochemical Characteristics and Biological Performances of Representative Co-Delivery Platforms

Carrier System	Structural Materials	Size & Zeta Potential	Encapsulation Efficiency (EE %)	Therapeutic Target Model	Results
PLGA NPs	PLGA Copolymers	142.3 ± 4.07 nm	MTX: 71.3% CUR: 85.6%	Breast Cancer (MCF-7)	Biphasic sustained release; enhanced cytotoxicity *in vitro* and tumor control *in vivo*[35]
ALG Nanocapsules	Alginate + Poloxamer P407	Nanoscale dimensions	High co-loading	Breast Cancer (MCF-7)	Gastric stability; pH-triggered tumor release; $CI < 1.0$ [36]
PAMAM Dendrimers	G4-PAMAM conjugated to MTX	~249 nm	CUR: ~81%	Cervical Cancer (HeLa)	FR-targeted endocytosis; ROS generation; Caspase-3 activation [7]
Niosomes	Non-ionic surfactant + Cholesterol	Nanoscale vesicles	High co-loading	Colorectal Cancer (HCT-116)	Increased cellular uptake; enhanced inhibition of cell motility and growth [12]
BSA NPs	Bovine Serum Albumin (Nab)	163.05 ± 1.71 nm	MTX: 68.2% CUR: 75.7%	Rheumatoid Arthritis	Targeted inflamed joints via SPARC; prolonged $t_{1/2}$ and AUC [13]
pH-Smart Micelles	DSPE-PEG-Imine-MTX	Self-assembled micelles	High core CUR loading	FR-Overexpressing Tumors	Dual targeting via MTX; endo/lysosomal acid-triggered drug cleavage [2]

Liposomal Gel for Various Topical Applications

Topical drug delivery is a way where topical treatments are delivered to treat skin disorders and joint problems. The stratum corneum (SC)— which is a very thick and lipid-rich layer of the epidermis— can prevent drug transport and act as a barrier for effective treatments of hydrophilic drugs or hydrophobic drugs.

Mechanistic Role of Liposomal Hydrogels

By incorporating lipid vesicles into the semi-solid hydrogel networks (e.g., Carbopol-934, poloxamer gels), an innovative dual-action system has been established:

a. **Stratum Corneum Permeation:** Flexible lipid vesicles can act as agents for penetration enhancement. Ultra-deformable transfersomes can deform to pass through the small intercellular channels under *in vivo* conditions. Ethosomes can take advantage of ethanol to enhance the stratum corneum lipid fluidity [37].

b. **Controlled Localized Retention:** The three-dimensional cross-linked structure of the hydrogel matrix results in sufficient bioadhesion. This permits the prolonged stay of the formulation on the skin and stops rapid runoff [38].

c. **Reduction of Systemic Toxicity:** Site-specific drug delivery of MTX and CUR allows for localization of the active substances in the exposed areas [39].

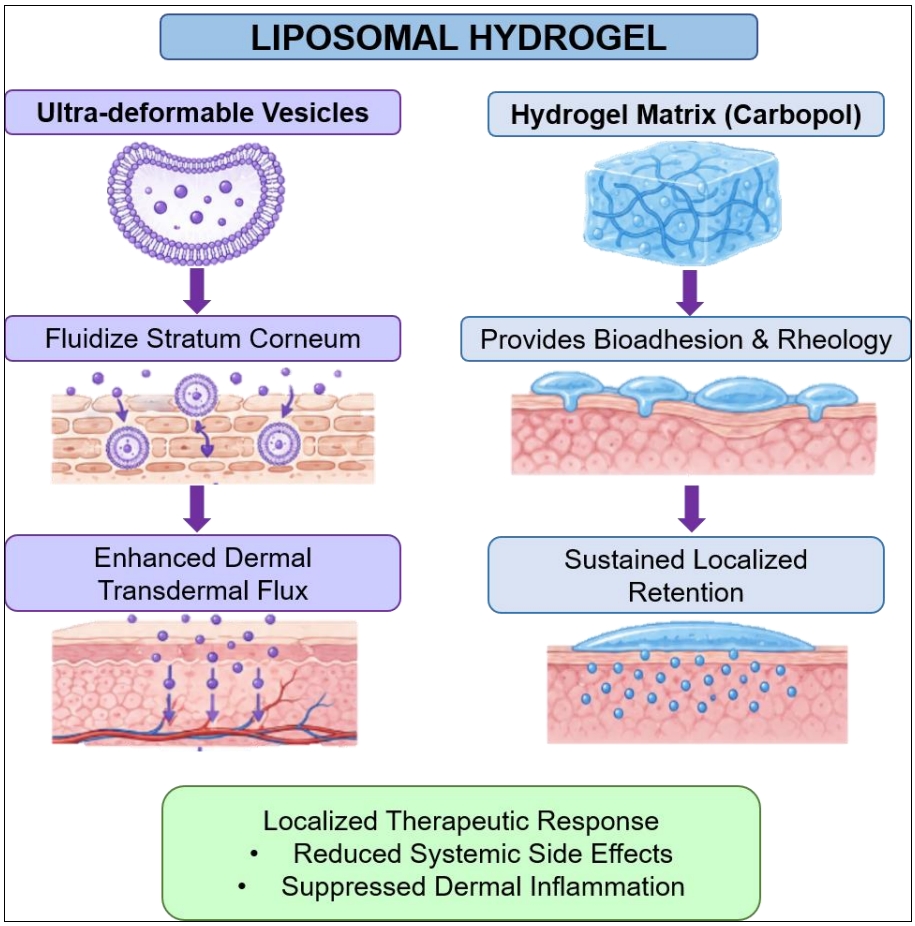

Fig 2: Schematic illustration of ultra-deformable liposomes incorporated into a Carbopol hydrogel matrix, facilitating stratum corneum fluidization, enhanced dermal/transdermal drug flux, bioadhesion, rheological stability, and sustained localized drug retention, thereby promoting a localized therapeutic response with reduced systemic side effects and dermal inflammation.

Preclinical Applications in Psoriasis and Arthritis

Management of Psoriasis: Psoriasis is characterized by epidermal hyperproliferation and inflammatory infiltration. The topical application of liposomal MTX hydrogels (LMTX gel) in association with low-level laser therapy enhances the clearance of plaques and returns normal epidermal architecture without systemic toxicity. The co-encapsulation of CUR in lipid-based gels results in improved topical bioavailability, inhibition of localized NF-κB activation, suppression of pro-inflammatory cytokines, and stopping keratinocyte hyperproliferation [40,41].

Rheumatic Arthritis: The topical application of curcumin-loaded transfersomal hydrogels (Cur-TF gel) on inflamed joints has shown better skin penetration, reduced paw swelling, and less histological inflammation in a CFA-induced arthritis model through inhibition of NF-κB signalling. The simultaneous topical application of MTX and CUR helps provide joint anti-inflammation with no GI side effects [5,42].

Challenges, Future Perspectives and Conclusion

Translational Challenges

While preclinical evaluation of MTX and CUR co-delivery systems shows strong promise, several technical and regulatory obstacles hinder clinical translation:

- The process of turning laboratory-scale methods to GMP process needs painstaking control of particle size, polydispersity, surface charge, drug loading ratio, and encapsulation efficiency. Continuous flow processing and microfluidic mixing technologies will facilitate uniform batches.
- Liposomes or multicomponent lipid-based nanoparticles experience problems with physical

stability, for instance, aggregation of vesicles, breaking of membranes, and drug leakage during storage. Thus, the aim is to create stable lyophilized compositions.

- Medicines based on nanotechnology face serious obstacles related to regulation, as authorities like the FDA or EMA consider mixed medicines complex. Hence, these medicines need a detailed assessment of every component of the product, including safety, as well as proof of their effectiveness.
- When using medicines made with polymer matrices or synthetic lipids over long periods of time, there is a need to test the effects of the medicines on metabolism and elimination from the body.

Future Perspectives

The future advancements in delivery systems of MTX-CUR are expected to include the following:

- **Smart environment-responsive platforms:** Incorporating various responses of nanocarriers to their surroundings for the release of two drugs such that these systems respond to an array of microenvironment stimuli, including hyperthermia, excessive amounts of ROS, high levels of enzyme activity (such as MMP), or acidity.

- **Active dual-targeting strategies:** Covering the surface of liposomes or nanoparticles with various targeting ligands (such as folic acid and EGFR antibody or peptide sequences) in order to increase their selectivity toward pathological cells as opposed to normal tissues.

- **Multimodal combination strategies:** Supplementing MTX-CUR carriers with PTT, PDT, or immune checkpoint therapy in order to elicit synergistic antitumor or immunomodulation effects.

Conclusion

The combination of Methotrexate and Curcumin provides an efficacious treatment option for different diseases such as cancer, rheumatoid arthritis, and chronic skin conditions like psoriasis. Curcumin not only act as a synergizer of Methotrexate's cytotoxic action and a reverser of drug resistance but also as an organo-protective agent since it activates the Nrf2/ARE pathway and inhibits NF-κB signalling, thus preventing damage to normal tissues caused by Methotrexate. Modern drug delivery systems solve the pharmaceutical limitations of drug mixtures since they use liposomal systems and topical liposomal gels that allow synchronizing pharmacokinetics regime, penetrating the skin, and allowing for minimal side effects, while continuing advancements in manufacturing, stability enhancement, and regulatory science would help to usher in a new era of using these dual drug delivery systems in clinical settings.

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