REGULAR ARTICLES

Curcumin–Piperine/Curcumin–Quercetin/Curcumin–Silibinin dual drug-loaded nanoparticulate combination therapy: A novel approach to target and treat multidrug-resistant cancers

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Abstract Curcumin is a functional food, which provides a wide range of health benefits including anti-cancer activity and considered as a suitable alternative for chemotherapeutic agents. However, cancer cells exhibit resistance to most chemotherapeutic agents including curcumin due to overexpression of adenosine triphosphate (ATP)-binding cassette transporter proteins in the cancer cell membrane, which decrease the intracellular concentration of chemotherapeutic agents. Similarly, most chemotherapeutic agents including curcumin experience lack of cancer cell targeting, lack of aqueous solubility, rapid systemic clearance, intestinal metabolism and hepatic metabolism. These limitations hinder the clinical usefulness of curcumin in the treatment of multidrug-resistant cancers. In this article, we propose curcumin–piperine, or curcumin–quercetin or curcumin–silibinin dual drug-loaded nanoparticulate combination therapy to target and treat multidrug-resistant cancers. The proposed dual drug-loaded nanoparticulate combination is expected to reverse the multidrug resistance, prevent the rapid systemic clearance, prevent the intestinal and the hepatic metabolism, increase the aqueous solubility, enhance the bioavailability, target the cancer cells, produce a synergistic anti-cancer effect and enhance the efficacy of curcumin in the treatment of multidrug-resistant cancers.

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Functional foods are those food items which provide health benefits beyond their nutritive value and are considered as a suitable alternative for potent drugs with severe systemic toxicities such as chemotherapeutic agents. Of all functional foods, polyphenols attracted many researchers as they possess a wide spectrum of health benefits including anti-cancer activity [1,2].

One such polyphenol is curcumin, which is a hydrophobic orange-yellow crystalline phytochemical isolated from various sources including *Curcuma longa* [3]. Curcumin is safe, as it is well tolerated even at a dose of 12 g day$^{-1}$ in phase-I clinical trials and has also been declared as GRAS (generally regarded as safe) by United States Food and Drug Administration [1,4–6].

Due to its diverse molecular targets, curcumin exhibits a wide range of pharmacological activities and is used in the treatment of various diseases including cataract, wound, gall stones, allergy, pancreatitis, gastric ulcer, inflammatory bowel disease, fever, acquired immune deficiency syndrome, psoriasis, Alzheimer’s disease, scleroderma, hypothyroidism, cystic fibrosis, atherosclerosis, myocardial infarction, osteoporosis, lung diseases, malaria, arthritis, leishmaniasis, diabetes mellitus, multiple sclerosis, epilepsy, Parkinson’s disease and cancer [1–4].

Curcumin exhibits its therapeutic potential (Fig. 1) against a wide spectrum of cancers including breast cancer, oral cancer, oesophageal cancer, lymphoma, gastric cancer, cervical cancer, intestinal cancer, multiple myeloma, hepatic cancer, pancreatic cancer, leukaemia, colorectal cancer, bladder cancer, melanoma, kidney cancer, ovarian cancer, prostate cancer, sarcoma, thymic cancer, uterine cancer, skin cancer, neurological cancer, bone cancer, brain cancer and head and neck squamous cell carcinoma [7–10].

However, most cancer cells show resistance towards most conventional chemotherapeutic agents including curcumin due to overexpression of drug efflux ATP-binding cassette transporter proteins including P-glycoprotein (P-gp), multidrug resistance protein 1 (MDR1), multidrug resistance protein 2 (MDR2) and breast cancer resistance protein (BCRP), which drive the drug out of cancer cells and decrease intracellular drug concentration. Similarly, most conventional chemotherapeutic agents including curcumin experience lack of cancer cell targeting, lack of aqueous solubility, rapid clearance from the systemic circulation, intestinal metabolism and hepatic metabolism [1,11]. These limitations hinder the clinical usefulness of curcumin in the treatment of multidrug-resistant cancers.

In recent years, various approaches have been tried to overcome these limitations, which are tabulated in Table 1 [12–24]. However, in this article, we propose a dual drug-loaded nanoparticulate combination therapy containing curcumin and a bioenhancer such as piperine, quercetin or silibinin, which can significantly overcome the multidrug resistance and other limitations including lack of cancer cell targeting, lack of aqueous solubility, rapid systemic clearance, intestinal metabolism and hepatic metabolism and is expected to enhance the efficacy of curcumin in the treatment of multidrug-resistant cancers.

**Hypotheses**

We propose curcumin–piperine, or curcumin–quercetin or curcumin–silibinin dual drug-loaded nanoparticulate combination...
therapy containing poloxamer 188 as a polymer and beta cyclodextrin as a stabiliser.

We hypothesise the following:

1. Bio-enhancer and poloxamer in the proposed dual drug-loaded nanoparticulate combination therapy can significantly modulate the ATP-binding cassette transporter proteins and thereby reverse multidrug resistance.

2. Nanosizing of the drugs in the proposed dual drug-loaded nanoparticulate combination therapy is expected to increase the aqueous solubility and thereby increase the bioavailability of both curcumin and bio-enhancer.

3. Bio-enhancer in the proposed dual drug-loaded nanoparticulate combination therapy is expected to minimise/prevent intestinal and hepatic metabolism by a competitive mechanism and thereby increase the bioavailability of curcumin.

4. Poloxamer in the proposed dual drug-loaded nanoparticulate combination therapy is expected to adsorb onto the surface of curcumin and bio-enhancer and prevent the rapid systemic clearance and thereby increase the bioavailability of both curcumin and bio-enhancer.

5. Nanosizing of the drugs in the proposed dual drug-loaded nanoparticulate combination therapy is expected to enhance the reactivity of both curcumin and bio-enhancer to specific molecular targets and produces required pharmacological actions at much reduced dose levels.

6. Bio-enhancer in the proposed dual drug-loaded nanoparticulate combination therapy also exhibits a wide spectrum of pharmacological activities including anti-cancer activity and is expected to produce a synergistic anti-cancer effect with curcumin.

7. Nanosizing of the drugs in the proposed dual drug-loaded nanoparticulate combination therapy is expected to target cancer cells by a passive mechanism through an enhanced permeability and retention effect and thereby enhance the anti-cancer activity of curcumin and bio-enhancer.

8. Beta cyclodextrin in the proposed dual drug-loaded nanoparticulate combination therapy is expected to increase the aqueous solubility, prevent photodegradation, provide stability during storage and also prevent drug–drug/drug–excipient interactions.

### Evaluation of hypotheses

The proposed hypotheses can be tested by the following studies:

1. We propose to compare the anti-cancer potential of pure curcumin, a mixture of curcumin with bio-enhancer and the proposed dual drug-loaded nanoparticulate combination on multidrug-resistant cancer cell lines using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, or the sulfhorhodamine B assay or the clonogenic assay. This study is required to confirm the enhancement of anti-cancer activity of the proposed dual drug-loaded nanoparticulate combination by modulating the ATP-binding cassette transporter proteins in comparison with pure curcumin and a mixture of curcumin with bio-enhancer.

2. We propose to compare the solubility of pure curcumin and the proposed dual drug-loaded nanoparticulate combination. This study is required to confirm the enhancement of the water solubility of the proposed combination in comparison with pure curcumin.

3. We propose to administer the pure curcumin, a mixture of pure curcumin with bio-enhancer and proposed dual drug-loaded nanoparticulate combination at the same dose level to three different animal groups and collect the blood samples at fixed intervals and analyse the blood samples for the presence of curcumin and bio-enhancer using a validated high-performance liquid chromatography (HPLC) method and calculate the bioavailability of curcumin and bio-enhancer. This study is required to confirm the enhancement of the bioavailability of the proposed combination in comparison with pure curcumin and pure curcumin with bio-enhancer.
(4) We propose to perform an accelerated stability study for pure curcumin, mixture of pure curcumin with bio-enhancer and the proposed dual drug-loaded nanoparticulate combination after maintaining the samples at 40 ± 2 °C/75 ± 5% RH (relative humidity) for 6 months. This study is required to confirm the stability enhancement of the proposed combination in comparison with pure curcumin and a mixture of curcumin with bio-enhancer.

(5) We propose to compare the anti-cancer potential of pure curcumin, mixture of curcumin with bio-enhancer and the proposed dual drug-loaded nanoparticulate combination on various cancer cell lines at various lower dose levels using the MTT assay, or the sulforhodamine B assay or a clonogenic assay. This study is required to confirm the enhancement of anti-cancer activity of the proposed combination at reduced dose levels in comparison with pure curcumin and a mixture of curcumin with bio-enhancer.

(6) We propose to compare the anti-cancer potential of pure curcumin, mixture of curcumin with bio-enhancer and the proposed dual drug-loaded nanoparticulate combination in living female athymic NCr-nu/nu mice bearing subcutaneous multidrug-resistant breast cancer (MDA-MB-435S MDR) tumour xenografts. This study is required to confirm the enhancement of anti-cancer activity of the proposed combination by reversing the multidrug resistance in comparison with pure curcumin and mixture of curcumin with bio-enhancer.

Discussion

A novel approach to target and treat multidrug-resistant cancers using dual drug-loaded nanoparticulate combination has been proposed in this article.

According to Noyes–Whitney’s equation, size reduction to the nanometre range can significantly increase the interfacial surface area, thereby increasing the rate of dissolution and aqueous solubility, which in turn leads to enhancement of drug bioavailability. However, increase in interfacial surface area also increases the reactivity of a drug to specific molecular targets and enhances its pharmacological action [25,26]. Most chemotherapeutic agents including curcumin and bio-enhancers including piperine, quercetin and silibinin are hydrophobic in nature, which significantly contributes to low bioavailability. Hence, based on the above facts we propose to use a nanoparticulate drug delivery system, which not only improves the bioavailability but also enhances the reactivity of both curcumin and bio-enhancer to specific molecular targets.

Natural bio-enhancers such as piperine, quercetin and silibinin can significantly suppress the drug-metabolising enzyme cytochrome P450 3A, hepatic glucuronidation and intestinal glucuronidation and thereby increase the bioavailability of a drug. These bio-enhancers also reverse multidrug resistance by modulating ATP-binding cassette transporter proteins such as P-gp, MDRP1, MDRP2 and BCRP. Moreover, these natural bio-enhancers also exhibit a wide spectrum of pharmacological activities including anti-cancer activity [27–30]. Hence, based on the above facts we propose to use these bio-enhancers to reverse multidrug resistance, to increase the bioavailability of curcumin by preventing hepatic and intestinal metabolism and to produce a synergistic anti-cancer effect with curcumin.

Opsonin is a blood serum protein that binds to hydrophobic drug particles and gets easily recognised by the macrophages of the mononuclear phagocytic system, which in turn leads to the removal of the hydrophobic drug particles from the circulatory system. However, adsorption or grafting of polyethylene glycol or a polyethylene glycol-containing copolymer such as poloxamer to the surface of a hydrophobic drug provides a hydrophilic coat, which in turn repels the opsonin proteins via steric repulsion, thereby blocking the first step in the opsonisation process and increasing the bioavailability of the drug. However, poloxamer also possesses an ability to modulate the ATP-binding cassette transporter proteins such as P-gp, MDRP1, MDRP2 and BCRP, thereby reversing the multidrug resistance [11,31]. Hence, based on the above facts we propose to use poloxamer 188 as a polymer to reverse multidrug resistance and to prevent the rapid systemic clearance of curcumin and bio-enhancer.

In contrast to normal tissues, tumour tissues are highly permeable with a gap ranging between 200 and 600 nm in diameter and they also lack a lymphatic system, which is responsible for the drainage of macromolecules. This defective architecture of tumour tissues allows the long-circulating nanoparticles with size < 200 nm to accumulate in tumour tissues via enhanced permeability and retention effect [11,32]. Hence, based on the above facts we propose to design a nanoparticulate drug delivery system for the combination of curcumin and bio-enhancer.

Cyclodextrins are cyclic oligosaccharides with a lipophilic central cavity and a hydrophilic outer surface. They are mainly used as complexing agents to increase the aqueous solubility of hydrophobic drugs, thereby increasing drug bioavailability. However, complexation with cyclodextrin also reduces gastrointestinal drug irritation, increases drug stability and prevents drug-drug/drug–excipient interactions [33]. Hence, based on the above facts we propose to use beta cyclodextrin to prevent drug-drug/drug–excipient interactions, increase the drug stability, increase the aqueous solubility and thereby increase the bioavailability of curcumin and bio-enhancer.

Conclusion

In this article, we propose a novel approach to target and treat multidrug-resistant cancers using a dual drug-loaded nanoparticulate combination. The proposed nanoparticulate combination is expected to reverse the multidrug resistance, increase the water solubility, prevent the rapid systemic clearance, prevent the intestinal and hepatic metabolism, enhance the bioavailability and enhance the cancer cell targeting. The proposed dual drug-loaded nanoparticulate combination seems to have potential in treating multidrug-resistant cancers.
The authors state that they do not have any conflict of interest.

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