

# Berberine hydrochloride: anticancer activity and nanoparticulate delivery system

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**Background:** Berberine hydrochloride is a conventional component in Chinese medicine, and is characterized by a diversity of pharmacological effects. However, due to its hydrophobic properties, along with poor stability and bioavailability, the application of berberine hydrochloride was hampered for a long time. In recent years, the pharmaceutical preparation of berberine hydrochloride has improved to achieve good prospects for clinical application, especially for novel nanoparticulate delivery systems. Moreover, anticancer activity and novel mechanisms have been explored, the chance of regulating glucose and lipid metabolism in cancer cells showing more potential than ever. Therefore, it is expected that appropriate pharmaceutical procedures could be applied to the enormous potential for anticancer efficacy, to give some new insights into anticancer drug preparation in Chinese medicine.

**Methods and results:** We accessed conventional databases, such as PubMed, Scope, and Web of Science, using “berberine hydrochloride”, “anti-cancer mechanism”, and “nanoparticulate delivery system” as search words, then summarized the progress in research, illustrating the need to explore reprogramming of cancer cell metabolism using nanoparticulate drug delivery systems.

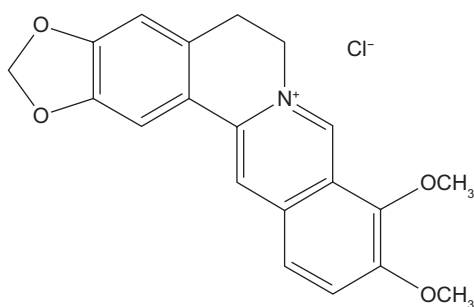
**Conclusion:** With increasing research on regulation of cancer cell metabolism by berberine hydrochloride and troubleshooting of issues concerning nanoparticulate delivery preparation, berberine hydrochloride is likely to become a natural component of the nanoparticulate delivery systems used for cancer therapy. Meanwhile, the known mechanisms of berberine hydrochloride, such as decreased multidrug resistance and enhanced sensitivity of chemotherapeutic drugs, along with improvement in patient quality of life, could also provide new insights into cancer cell metabolism and nanoparticulate delivery preparation.

**Keywords:** berberine hydrochloride, anticancer mechanisms, nanoparticulate drug progress

## Introduction

Berberine hydrochloride is an isoquinoline alkaloid (see Figure 1) isolated from a variety of Chinese herbs, including *Coptidis rhizoma*, *Phellodendron chinense schneid*, and *Phellodendron amurense*, and has diverse pharmacological actions. It has antidiabetic and antilipid peroxidation activity, as well as an anti-atherosclerotic action, and also has neuroprotective properties and improves polycystic ovary syndrome.<sup>1-5</sup> Berberine hydrochloride is widely used as an antibacterial, antifungal, and anti-inflammatory drug, and has been used as a gastrointestinal remedy for thousands of years in China.<sup>6,7</sup>

Nowadays, the antiproliferative activity and sensitivity enhancement of berberine hydrochloride in various cancer cell lines<sup>8-14</sup> have led to further research interest in



**Figure 1** Chemical structure of berberine hydrochloride.

this compound.<sup>15–17</sup> Its antineoplastic properties include induction of apoptosis and cell cycle arrest, along with inhibition of cell migration and invasion via regulation of multiple pathways.<sup>18–21</sup> The potential targets of berberine hydrochloride include reactive oxygen species generation, mitochondrial function, DNA topoisomerase inhibition, DNA or RNA binding, the estrogen receptor, matrix metalloproteinase regulation, p53 activation, and NF-kappa B signal activation.<sup>10,22–26</sup> However, it has poor water solubility caused by a quaternary amine, resulting in a low effective concentration and limited absorption in the gastrointestinal tract, which seriously limits its application and development as a pharmaceutical preparation. Furthermore, it has a risk of adverse reactions associated with intramuscular and intravenous administration, such as anaphylactic shock and drug rash, so a novel drug delivery system to improve the solubility and bioavailability of berberine hydrochloride has become a matter of urgency.

During the rapid development of nanotechnology, increasing attention has been paid to nanoparticulate drug delivery systems.<sup>27–29</sup> Modern nanoparticulate dosage forms including polymeric nanoparticles, nanocapsules, liposomes, solid lipid nanoparticles, and nanoemulsions, all of which can improve drug solubility. In general, nanoparticulate drug delivery enhances solubility and bioavailability, improving pharmacological activity and tissue macrophage distribution, while preventing physical and chemical degradation.<sup>30,31</sup> Therefore, we combined the good anticancer efficacy of berberine hydrochloride with a novel nanoparticulate drug delivery system to obtain a promising anticancer agent.

This review discusses anticancer mechanisms, with particular reference to regulation of glucose and lipid metabolism, and describes a novel drug delivery system for berberine hydrochloride, aiming to provide new insights into Chinese medicine preparations with anticancer activity.

## Anticancer mechanisms of berberine hydrochloride

The potential antitumor activity of berberine hydrochloride has always been a subject of considerable interest because of its known ability to interact with nucleic acids. Its ability to bind specifically to oligonucleotides and to stabilize DNA triplexes or G-quadruplexes via telomerase and topoisomerase inhibition accounts for its antiproliferative activity.<sup>32,33</sup> The predominant interaction between berberine hydrochloride and double-stranded or single-stranded DNA is electrostatic, and can be quantified in terms of the Hill model of cooperative interactions.<sup>34</sup> Recent novel mechanisms have a higher propensity for autophagy and autophagic regulators. Wang et al found that berberine hydrochloride induced autophagic cell death which was diminished by 3-methyladenine, a cell death inhibitor, in the human hepatic carcinoma cell lines HepG2 and MHCC97-L, through activation of beclin-1 and inhibition of the mTOR signaling pathway.<sup>35</sup> In addition, the autophagic marker, microtubule-associated protein-1 light chain 3 (LC3) was modified after administration of berberine hydrochloride in the human A549 lung cancer cell line, accompanied by shrinkage of tumor volume in a Lewis lung carcinoma model in mice, all of which indicates that autophagy might be important in cancer cell death.<sup>36</sup>

In addition to autophagy and its interaction with nucleic acid, the hypoglycemic and hypolipidemic effects of berberine hydrochloride also point to a relationship between adipose tissue/adipocytes and tumorigenesis, through upregulation of mRNA and protein levels in adipose tissue, including peroxisome proliferator-activated receptor (PPAR)  $\alpha$ ,  $\beta$ , and  $\gamma$ , CDK9, and cyclin T1.<sup>3</sup> Adipose tissue and adipocytes have a significant role in the tumor microenvironment,<sup>37</sup> and SPARC (secreted protein acidic and rich in cysteine), an adiposity inhibitor, was suggested by Nagaraju and Sharma<sup>38</sup> to be a potent anticancer molecule, and human adipose tissue-derived stem cells are known to be a source of carcinoma-associated fibroblasts in the presence of transforming growth factor  $\beta$ 1.<sup>39</sup> Further, adipose tissue-derived vascular endothelial growth factor and leptin promote adipogenesis in order to maintain the tumor microenvironment.<sup>40</sup> Hirano et al have also suggested the existence of undefined factors derived from cancer cells which promote adipogenesis, further indicating a potential relationship between adipogenesis and development of cancer.<sup>41</sup> In clinical lipofilling procedures undertaken for patients with breast cancer, there is an urgent need to clarify the issue of cancer recurrence and adipogenesis.<sup>42</sup> The adipogenesis positive regulator, PPAR $\gamma$ ,

overexpressed in ERBB2-positive breast cancer cells, enables fatty acid synthesis, mainly to support energy demands and cell survival.<sup>43</sup> Therefore, less toxic PPAR $\gamma$  agonists or antagonists, including berberine hydrochloride, are regarded as potential agents for improving adipose breast tissue and decreasing breast cancer risk, as well as suppressing proliferation and invasion of cancer cells.<sup>44</sup> By inhibiting PPAR $\gamma$  protein expression and increasing PPAR $\alpha$  mRNA levels, berberine hydrochloride has been shown to improve free fatty acid-induced insulin resistance in myotubes, and to suppress adipogenesis in white preadipocytes in humans and hepatic insulin resistance in diabetic hamsters.<sup>2,45,46</sup> Berberine hydrochloride also prevented wasting of epididymal adipose tissue and ameliorated cancer cachexia in colon 26/clone 20-transplanted mice and colon 26/clone 20 cells,<sup>47</sup> further highlighting the beneficial effect of this compound on adipose tissue in the tumor microenvironment.

Working as a potential natural compound in cancer therapy via its interaction with nucleic acid and regulation of cancer cells, as well as induction of autophagy, berberine hydrochloride augments the effects of chemotherapy/radiotherapy and has shown good prospects in cancer treatment.<sup>11</sup> After the novel mechanisms by which it interferes with the development of adipose tissue and adipocyte metabolism in the tumor microenvironment were investigated, the efficacy and potential applications of berberine hydrochloride were highlighted and emphasized. Moreover, its extensive occurrence in various plant species and low toxicity suggest that berberine hydrochloride has the potential to become an effective antitumor agent in the future.

## Nanoparticulate delivery systems

Reports on nanoparticulate delivery systems for berberine hydrochloride can be divided into three types, ie, solid lipid nanoparticles, nanoemulsions, and liposomes. Preparation, characterization, experimental design methods, and in vivo and in vitro studies are summarized here.

The first nanoparticulate delivery system uses a conventional rotary-evaporated film-ultrasonication method to make solid lipid nanoparticles of berberine hydrochloride (BH-SLN). These have good stability, a mean diameter of 60.5 nm, a zeta potential of 29.7 mV, drug loading of 8.69%, and an entrapment ratio of 97.58%.<sup>48</sup> Certain other factors have a direct impact on actual amount and quality of liposome entrapment, including preparation and manufacturing methods, and types of excipients used and particle size. Entrapment is defined as the fraction of the initial solution

remaining within the liposomes, which is the key factor in clinical application.<sup>49</sup> Wang et al established the method of coagulation centrifugation to determine the entrapment efficiency of BH-SLN.<sup>50</sup> A saturated aqueous solution of sodium chloride 0.05 mL in BH-SLN 0.5 mL was determined by high-pressure liquid chromatography, and then centrifuged at 12,000 rpm for 10 minutes to obtain the supernatant. The results show that coagulation centrifugation was rapid and accurate.

The second delivery system is berberine hydrochloride nanoemulsion, made by isopropyl myristate, EL40, and glycerin using pseudoternary phase diagrams. The nanoemulsion is a clear transparent solution with an average particle diameter of 56.8 nm.<sup>51</sup> Small spherical drops are seen under electron microscopy, with a stable content and diameter even under high humidity and temperature conditions, along with strong light, 92.5% humidity, a temperature range of 40°C–60°C, and (4500  $\pm$  500) LX.

The last system is a liposomal one, and there are several approaches used to prepare berberine hydrochloride liposomes, including the thin film evaporation method, the active loading method, and a combination of the thin film evaporation and active loading methods. The thin film evaporation process could achieve a higher encapsulation efficiency, and the optimum manufacturing processes are characterized by 60°C of hatched temperature, 30 minutes of time, and 3.3 mg/mL of cholesterol concentration.<sup>52</sup> An active loading method is better than passive loading with a higher entrapment efficiency.<sup>53</sup> There are several factors to be considered, including addition sequence, incubation time, incubation temperature, pH value of the external water phase, and the particle size of the liposome. Changing the addition sequence can also achieve different entrapment efficiencies, as can increasing incubation time and temperature and decreasing the particle size.<sup>53</sup> Chen et al used different individual variables and an orthogonal design to obtain the optimal preparation conditions for berberine hydrochloride liposomes, including incubation time and incubation temperature, proportional weights of drug and lipids, soybean phosphatidylcholine, and cholesterol.<sup>54</sup> The results indicated that the average encapsulation efficiency of the optimized liposome was 78.51%  $\pm$  2.45%, with a size range of 2.2–3.5  $\mu$ m. Based on the uniform design, berberine hydrochloride liposomes were prepared by thin film evaporation and an active loading method.<sup>55</sup> An optimal formulation was established and a high encapsulation efficiency of 79.33% was obtained with a drug loading

ratio of 30.21 and a size range of 2.2–3.8  $\mu\text{m}$ . In vivo and in vitro studies were carried out to investigate the way in which liposomal berberine hydrochloride works. Gou et al studied the effects of berberine hydrochloride liposomes on the combination of impaired glucose tolerance and hyperlipidemia, suggesting that glucose and lipid metabolism was interfered with and progression from hyperlipidemia to type 2 diabetes was also prevented.<sup>56</sup>

## Conclusion

Studies of the molecular mechanisms by which berberine hydrochloride affects lipid and glucose metabolism warrant further attention. Additionally, with the increasing research on reprogramming of cancer cell metabolism, including inhibition of glycolysis, impairment of mitochondrial function, and suppression of cell anabolism, it could be possible to reverse abnormal cancer cell metabolism in various ways, including blocking formation of the cell membrane and synthesis of macromolecules, and inhibiting growth and proliferation of cancer cells. During the process of interfering with cancer cell reprogramming, because of the alteration in the physiological properties of the cell, multidrug resistance, sensitivity of chemotherapeutic drugs, and patient quality of life could be ameliorated, which indicates the potential of berberine hydrochloride as an adjuvant drug in antineoplastic treatment. With effective troubleshooting of issues such as the hydrophobic properties, poor stability, and bioavailability of berberine hydrochloride, research on nanoparticulate delivery systems for this compound is being vigorously pursued. Furthermore, investigations in this field are likely to provide new insights into anticancer drug preparation in Chinese medicine.

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## Disclosure

The authors report no conflicts of interest in this work.

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