Combining Photothermal Therapy with A Nanohybrid-Based Drug Delivery Strategy for Slow-Released Doxorubicin: A Treatment for Hepatocellular Carcinoma

Muhammad Habiburrahman*, Afid Brilliana Putra, Muhammad Ilham Dhiya Rakasiwi
Faculty of Medicine, Universitas Indonesia - Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia

ABSTRACT

Background: Hepatocellular carcinoma (HCC) is the fifth most common malignancy in the world. Surgical intervention remains the primary treatment option for resectable liver cancer. However, the low curative resection ratio, high metastatic ratio, and risk of recurrence make this treatment less than ideal. Additionally, the choice of liver transplantation is limited by the availability of donors. This literature review aimed to discuss the combination strategy of photothermal therapy and nanohybrid-based chemotherapy delivery, which are expected to address the challenges in HCC treatment.

Methods: We conducted literature searches in Pubmed, Scopus, ProQuest, and Google Scholar using combined keywords such as “hepatocellular carcinoma”, “polyethylene glycol”, “doxorubicin”, “mesoporous silica”, “CuS”, “nanoparticle”, and “photothermal therapy”. Based on the assessment of validity and applicability aspects using modified Oxford CEBM (Center for Evidence-Based Management) and OHAT (Office of Health Assessment and Translation) checklist tools for preclinical studies, all the selected studies fulfilled the eligibility criteria.

Results: Photothermal therapy promotes necrosis and apoptosis of HCC cells by ‘heating’ the cancer cells. Meanwhile, the chemotherapy agent doxorubicin, modified with mesoporous silica nanohybrids and encapsulated copper sulfate polyethylene glycol (PEG-DOX-MSN-CuS), enhances the efficiency and duration of drug circulation in the blood, reduces drug clearance, and minimizes retention by the reticuloendothelial system. By utilizing near-infrared light induction from photothermal therapy, doxorubicin can be slowly released, leading to significantly improved effectiveness. In vitro studies have demonstrated that this combination strategy achieves over 90% HCC cell death at a chemotherapy concentration of 80 µg/mL, in conjunction with near-infrared light induction. The optimal release time for doxorubicin was recorded at a concise 20 minutes.

Conclusions: Given the numerous benefits associated with this combination of strategies, photothermal therapy using PEG-DOX-MSN-CuS holds significant expected to be a promising treatment for HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common malignancy worldwide, ranking fifth in Asia where it accounts for 72.5% of cases globally [1,2]. It is estimated that there were 905,677 cases of HCC globally in 2020, and it is projected that there will be a 55% increase between 2020 and 2040, resulting in 1.4 million new diagnoses in the next two decades (by 2040) [1,3]. Around 80% of HCC cases occur in developing countries, including Southeast Asia, South Asia, West Asia, Central Asia, and sub-Saharan Africa, with a higher prevalence among males [3,4]. Meanwhile, in Indonesia, the number of liver cancer cases was 21,392 in 2020, with an incidence rate of 13.4 per 100,000. It is the fourth most common cancer type among males. It is predicted that 20–30% of the 17.5 million Indonesians with Hepatitis B will develop cirrhosis and liver cancer in the coming decades [5]. The most common etiological factor for HCC is hepatitis B (HBV) infection. Other common risk factors...
include alcohol consumption, hepatitis C virus (HCV) infection, and non-alcoholic fatty liver disease [2]. The prevalence of HCC is widespread, resulting in a significant economic burden and compromising the quality of life for the broader community [6,7].

Surgical intervention remains the most effective approach for treating resectable liver cancer. However, its efficacy is hindered by a low curative resection ratio, a high metastatic ratio, and a risk of recurrence within five years after therapy make this treatment less than ideal [4,8,9]. Moreover, alternative managements, such as liver transplantation, are limited by the availability of donors, leading to potential delays in therapy [10].

One component of the strategy involves combining photothermal therapy (PTT) with chemotherapy. PTT is a non-invasive therapy that utilizes irradiation to “heat” cancer cells with the assistance of a photo-absorber, which converts photon energy into thermal energy [11,12]. PTT offers advantages over chemotherapy, including minimal side effects, and it can enhance the effectiveness of chemotherapy by facilitating the controlled release of anticancer drugs [13–15]. Doxorubicin (DOX) is an FDA-approved anticancer drug used in clinical practice for HCC. Its primary mechanism involves DNA intercalation and the release of reactive oxygen species (ROS), which have been demonstrated inhibitory effects on the growth of liver carcinoma cells [16].

Meanwhile, mesoporous silica nanoparticles (MSN) have gained significant attention as efficient drug carriers due to their large capacity and surface area, adjustable pore size, morphological controllability, and modifiable surface chemistry [14,15,17]. MSN nanocarriers offer controlled release capability, including those influenced by photo-irradiation [18-20]. Near-infrared (NIR, λ = 700–1100 nm) photo-irradiation has been shown to enhance deep tissue penetration [21]. Recently, copper monosulfide (CuS) nanoparticles have emerged as a new phototherapy ablation agent for cancer by facilitating the conversion of light into thermal energy upon NIR absorption [22,23]. Copper was chosen due to its lower costs compared to gold, which is commonly used, and its superior interstitial tumor penetration ability [22,24].

However, the utilization of this combination therapy for treating HCC in Indonesia remains limited. Meanwhile, the number of unresectable HCC cases continues to rise each year, highlighting the need for alternative treatment options to be explored. Therefore, the novel treatment approach that combines PTT with the slow release of drugs presents an intriguing avenue to enhance HCC curative rates. The objective of this review study was to evaluate the effectiveness of combining PTT with mesoporous silica nanohybrids and copper monosulfide to achieve controlled release of doxorubicin. These nanohybrids are encapsulated by polyethylene glycol biopolymers (abbreviated as: PTT-PEG-DOX-MSN-CuS) and hold promise as new treatment modality for HCC in Indonesia.

**METHODS**

This literature review was conducted by searching the latest sources pertaining to the potential of alternative therapies for HCC. To ensure the creation of an evidence-based review, we referenced previously published studies [25-29] and formulated the literature search process using the PIO query approach which consists of the following components: (1) Population: HCC patients or experimental animals/cell models; (2) Intervention: PTT, polyethylene glycol, doxorubicin, mesoporous silica, CuS, nanohybrid; and (3) Outcome: substance characterization, cytotoxicity, temperature effects, drug released profiles, therapeutic efficiency, and therapeutic applications.

From May to July 2022, an extensive search was performed in four journal databases, namely PubMed (pubmed.ncbi.nlm.nih.gov/), ProQuest (www.proquest.com/), Scopus (www.scopus.com/search/form.uri?display=advanced), and Google Scholar (scholar.google.com). Due to the novel nature of the study topics, we employed simple keywords during the literature search. We did not utilize Boolean operators other than AND/OR to broaden the search results. Although this approach reduced the specificity of the terms, but it enhanced the sensitivity to obtain more relevant evidence. All keywords used and a generated search flowchart are shown in Figure 1. Initially, 1,661 articles were identified, out of which 1,118 were screened based on their titles and abstracts. Following the application of inclusion and exclusion criteria, only 366 articles were reviewed, ultimately resulting in the selection of six articles [23,35–39].

The articles were selected based on inclusion criteria, which included a match with the discussed intervention, available in full text, and publication within the last fifteen years. Conversely, articles were excluded if they met certain exclusion criteria, such as having non-interventional design (should be clinical or animal/cell model), being non-English, or not discussing the application to HCC. Prior to discussing the findings, we conducted a thorough assessment of the validity and applicability of all the selected articles. A comprehensive assessment of their validity can be found in Supplementary Tables S1 and S2. To evaluate the validity of the relevant studies, we employed tools recommended by the Office of Health Assessment and Translation (OHAT) for assessing preclinical studies [30]. Additionally, adaptations of the Oxford Center for Evidence-Based Medicine (Oxford CEBM) checklist were used to assess their applicability [31]. A full assessment is available in the accompanying supplementary file.

In this review, we examined the efficacy and profiles of PTT and PEG-modified DOX, along with various nanohybrid career agents, to enhance the efficiency and effectiveness of HCC treatment. Furthermore, we
discussed the molecular mechanisms involved in the pathogenesis of HCC, the limitations of current therapies, and the pharmacological characteristics of PTT, DOX, PEG, and MSN-CuS. In addition, we conducted a comprehensive search for supporting literature published in English within the last ten years. However, some exceptions were made to include articles rarely explored data and primary theoretical forms. The methods employed in conceptualizing the review, searching for evidence, and presenting the findings align with the recommendations put forth by Ferrari et al. [32], Green et al. [33], and Gasparyan et al. [34].

RESULTS

Six studies were included in this review, encompassing experimental studies conducted both in vitro and/or in vivo. These studies have been deemed valid based on the OHAT Risk of Bias Rating Tool [30] for in-vitro and animal studies. Minor issues of clarity regarding blinding and concealment were identified, which are inherent to the experimental study design. Lin et al. [35] and Lin et al. [36] discussed the characteristic of MSN and MSN-PEG, respectively. Liu et al. [37] explored the cytotoxicity of PEG and its prolonged circulation to the organ. A study by Xiao et al. [38] and Li et al. [23] demonstrated the effectiveness of photothermal radiation and CuS nanoparticles both in vivo and in vitro. Wu et al. [39] presented the results of PEG-modified DOX loaded in MSN-CuS nanohybrid in cell culture using the HCC cancer line HepG2.

DISCUSSION

Synthesis and characterization of mesoporous silica nanohybrids and copper monosulfide containing slow-released doxorubicin encapsulated in polyethylene glycol (PEG-DOX-MSN-CuS)

The synthesis procedure of PEG-DOX-MSN-CuS is schematically shown in Figure 2 [39]. Firstly, the DOX solution, as a chemotherapy agent, is incubated with MSN. Subsequently, the negatively charged citrate-modified CuS nanoparticles are statically absorbed onto
the surface of the positively charged MSN. The presence of citrate in CuS enhances the stability of CuS in MSN. PEG biocompatibility is then modified on the surface of CuS, which significantly increases colloidal stability and improves efficiency and blood circulation time [35,36,38]. The selected area electron diffraction (SAED) pattern of CuS nanoparticles exhibits well-defined diffraction rings, indicating a good crystal structure. Transmission electron microscopy (TEM) is used to characterize the typical morphology and particle size of the modified PEG and MSN containing CuS (PEG-DOX-MSN-CuS). The MSN’s silica shell has a diameter ranging from 2 to 50 nm, providing potential for its use as a career in general medicine or specifically for doxorubicin delivery [39].

N2 adsorption techniques have been employed to characterize the pore size, revealing an average pore diameter of 2 nm for MSN. The zeta potential measurements indicate that CuS, MSN, and MSN-CuS possess values of approximately -8.73 mV, 28.7 mV, and -16.6 mV. The charged surface demonstrates the successful attachment of CuS citrate nanoparticles to the MSN surface. In the PEG-MSN-CuS system, near-infrared (NIR) rays exhibit notable absorption in the NIR-irradiated area due to the transition of d-d Cu2+ ions to CuS nanoparticles. Here, CuS nanoparticles acts as a photothermal transducer agent, absorbing NIR and generating heat and ROS upon near-infrared radiation (808 nm, 2 W/cm²). These properties result in significant anticancer effects both in vitro and in vivo. The heating effect and release of copper ions from CuS under acidic conditions constitute the primary mechanism for ROS formation, serving as a potent weapon against cancer cells [23].

**In vitro cytotoxicity of PEG-MSN-CuS**

Non-toxicity or low toxicity is a crucial requirement for any biomedical nanoparticle application [37]. Prior to evaluating the photothermal ablation capability, cytotoxicity is assessed by measuring cell viability using the methyl thiazolyl tetrazolium (MTT) assay, a calorimetric method that evaluates the metabolic activity of cancer cells. from PEG-MSN-CuS without the presence of NIR irradiation in human HCC cell models (HepG2) and normal hepatocytes. In a study by Liu et al. [37], each group consisted 5 x 10⁵ cells and was incubated in a humidified environment at a temperature of 37°C with 5% CO₂ content for 38 hours.

The results demonstrate that this nanohybrid exhibits excellent biocompatibility and is non-toxic to HepG2 cells and normal hepatocyte. Even at a concentration of 100 µg/mL and incubation periods ranging from 24 to 48 hours, the viability of the remaining cells remains above 80% [39].

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**Figure 2.** Synthesis of mesoporous silica nanohybrids and copper sulfate containing slow-release doxorubicin, which further encapsulated by polyethylene glycol (PEG-DOX-MSN-CuS) [39]. Abbreviation: CuS: copper(II) sulfide, DOX: doxorubicin, HCC: hepatocellular carcinoma, HCl: hydrogen chloride, mg: milligram, mM: millimolar, MSN: mesoporous silica nanohybrids, NaOH: sodium hydroxide, NIR: near-infrared, nm: nanometer, PEG: polyethylene glycol, rpm: rotation per minute, SH: sulfhydryl, W: Watt, µg: microgram. The copyright of this figure’s design and illustration belongs to the authors and journal.
Increased temperature and photothermal cytotoxicity of PEG-MSN-CuS

The photothermal effect of mesoporous silica nanohybrids and copper monosulfide containing the slow release doxorubicin encapsulated in polyethylene glycol (PEG-MSN-CuS) was evaluated by measuring temperature increases induced by NIR irradiation (808 nm, 2W) in a study by Wu et al [39]. They conducted a research to assess the photothermal performance by monitoring temperature changes in PEG-MSN-CuS solutions of various concentrations under NIR irradiation for 10 minutes. The initial temperature for all solutions was approximately 21°C. The temperature increase (Δt°C) after 10 minutes of NIR irradiation was measured as 21.50°C, 22.90°C, and 29.80°C for different nanoparticle concentrations (20, 40, and 80 µg/mL). The temperature reached up to 500°C when the concentrations of PEG-MSN-CuS was increased to 80 µg/mL, which was sufficient to achieve a tumor ablation effect. In contrast, the temperature of deionized water did not show a significant increase when exposed to a NIR laser irradiation for 10 minutes, serving as the control group [39].

The ability of PTT ablation with PEG-MSN-CuS in cancer cells was evaluated using a HCC cell model through confocal microscopic in the study by Wu et al [39]. The visualization of living cells was achieved using AM fluorescence dyes, which were only incorporated into living cells and not HepG2 cells. The researchers conducted a study comparing the photothermal ablation effect of HepG2 cells using four treatment groups: (1) without PEG-MSN-CuS and NIR; (2) PEG-MSN-CuS without NIR; (3) NIR without PEG-MSN-CuS; and (4) PEG-MSN-CuS with NIR. The PEG-MSN-CuS nanohybrids exhibited remarkable cell death only under NIR irradiation. Green fluorescence from AM calcein indicated the survival of HepG2 cells, while small portions of cells near the edge of the illuminated area also experienced cell death due to the heat spreading outside the irradiated zone. These experimental findings demonstrate the significant photothermal ablation capability of MSN nanohybrids containing CuS, which can act as NIR absorbers to be utilized in combination therapy for HCC [39].

Drug release mechanism from MSN nanohybrids containing PEG encapsulated CuS triggered by NIR lasers on HepG2 cells

In a study by Wu et al. [39], it was observed that the release of doxorubicin from the nanohybrids increased with longer NIR radiation time, reaching its peak after 20 minutes of irradiation. Beyond this point, no further increase in drug release was observed with longer exposure times. In a 10% Fetal Bovine Serum (FBS) solution, which has a higher solute concentration, the drug release rate was slower. Only 6.26% and 21.43% of drugs were released after 0 and 20 minutes irradiation, respectively. The slower rate of release of drug in the presence of FBS can be attributed to the absorption of FBS onto the surface of the nanohybrid, leading to pore clogging and reduced drug release [40,41]. These results indicate that the release of drugs from the nanohybrid can be controlled by regulating NIR irradiation conditions, such as adjusting the irradiation time, output power, and physiological environment [39].

The release of doxorubicin from the MSN-CuS-PEG nanohybrid, which exhibits fluorescent properties, can be easily detected using a fluorescence microscope. A
Study impact and limitation

The findings of this review provide valuable insight into the combined strategy of photothermal therapy and chemotherapy which can be an innovative approach in the field of cancer treatment. This study represents the first comprehensive review that explores the utilization of nanoparticles with chemotherapeutic agents, covering various aspects ranging from nanoparticles fabrication and toxicity testing to the release of chemotherapeutic agents upon light interaction. Additionally, this study presents a possible mechanism of action for the combination of photothermal therapy and chemotherapy.

However, it is important to acknowledge the limitations of this study. Firstly, there is a lack of research with substantial evidence, such as randomized clinical trials, which would further strengthen the findings. Secondly, the development of this combination method relies on specialized technology, and most studies have been conducted in high-tech countries such as China and the United States [23,35–39]. Conducting Phase I–III pharmacological studies will be crucial in order to assess the efficacy and clinical safety of the

Confocal beam scanning technique was applied to study the pattern of drug release triggered by NIR irradiation. HepG2 cells incubated with nanohybrids under NIR irradiation displayed clear fluorescence signals. However, no fluorescence signals were observed without NIR irradiation or in the control group. These results clearly demonstrate that the local heat generated by the irradiation of NIR rays can trigger the release of doxorubicin from the nanohybrids [39].

Therapeutic application

Based on prior in vitro research studies, it has been determined that the volume of MSN-CuS nanohybrids containing PEG-encapsulated doxorubicin was 1 mL (80 μg/mL), and a physiological solution of 500 μg/mL was added [39]. Based on in vivo studies in mice, it has been known that the possible administration routes were intravenous (IV) or intraperitoneal (IP) [42]. Subsequently, the liver region of the stomach was irradiated with near-infrared light (808 nm, 2W) for an optimal duration of 20 minutes [39]. Overall, the proposed mechanism of action of this combination therapy is summarized in Figure 3.

In vitro research dose:

1 mL of PEG-DOX-PEG-MSN@CuS nanohybrid was combined with 500 μL of a physiological solution (i.e., NaCl 0.9%) and injected intravenously

Combination of photothermal therapy with modified doxorubicin loaded into MSN nanohybrids surrounded by CuS and encapsulated in PEG

Photothermal therapy

Necrosis (T ≥50°C)

Apoptosis (T : 41°- 47° C)

Necroptosis

Based on:

In vitro and in vivo studies

Chemotherapy efficiency

High drug load capacity

Pores that filter active molecules and control the rate of drug release

Heat ↑

ROS ↑

Apoptosis/ Necrosis

Chemotherapy as a first-line chemotherapy agent for HCC

Damage to topoisomerase II → DNA unwinding → cell cycle termination

Production of ROS ↑ and H2O2 ↑

Apoptosis

MSN as a nanocarrier of doxorubicin

Prolong systemic circulation time

Efficiency of drug internalization

Uptake of the drug by the RES

PEG as a biopolymer for carrying doxorubicin

Figure 3. The proposed practical application and mechanism of action of combining phototherapy with mesoporous silica nanohybrids and copper monosulfide containing controlled-release doxorubicin with polyethylene glycol biopolymer encapsulation. The mechanisms of each component are depicted individually when applied to the human body [35–41,43]. Abbreviation: C: Celsius, CuS: copper(II) sulfide, DNA: deoxyribonucleic acid, DOX: doxorubicin, H2O2: hydrogen peroxide, HCC: hepatocellular carcinoma, mL: milliliter; MSN: mesoporous silica nanohybrids, NaCl: sodium chloride, NIR: near-infrared, nm: nanometer, PEG: polyethylene glycol, RES: reticuloendothelial system, ROS: reactive oxygen species, T: temperature, W: Watt, μL: microliter. The copyright of this figure’s design and illustration belongs to the authors and journal).
substances involved, which can include chemotherapeutic agents or herbal medicine with anticancer activities. Furthermore, randomized controlled trials are still necessary to investigate the effects of these substances in specific patient populations. It is imperative for other stakeholders, including government and pharmaceutical industry, to contribute to the research and development in this field.

CONCLUSIONS

In a nutshell, this study shed light on the efficacy of combining photothermal therapy with PEG-encapsulated MSN-CuS nanohybrids containing doxorubicin chemotherapy agents for the treatment of HCC. The study demonstrates that photothermal therapy can effectively release doxorubicin in the HCC cell model, enhancing the drug’s effectiveness. The results also show that increasing photothermal temperature and duration improves drug release and cell ablation. The PTT-PEG-DOX-MSN-CuS combination exhibits selective biocompatibility and cytotoxicity in the HCC cell model. Based on these findings, the review article recommends further evaluation of this combination therapy in orthotopic mouse models before progressing to clinical trials. This therapy shows promise as a new alternative for patients with HCC in Indonesia or other developing countries burdened with a high incidence of liver cancer.

DECLARATIONS

Ethics approval and consent to participate
Not applicable.

Competing interest
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