Preclinical evidence on the potential of silibinin in liver cancer therapy

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Abstract

Liver cancer is a public health problem worldwide, hepatocellular carcinoma (HCC) constituting 90% of cases. Although research is in continuous development, the prognosis of this type of cancer remains unfavorable, as selective treatments with low toxicity are necessary. Silibinin is a flavonoid recognized for its hepatoprotective effect, antioxidant and anti-inflammatory properties, and also for its proven effectiveness in cancer treatment when administered indiviadually or in association with conventional therapies.

Keywords: silibinin, liver cancer, synergism, cancer cell lines

INTRODUCTION

Liver cancer is a global public health problem and hepatocellular carcinoma (HCC) constitutes the majority of cases. Advances and research in the medical-pharmaceutical field are continually developing, but the prognosis for this type of cancer remains unfavourable for many patients. The mechanisms of hepatocarcinogenesis are varied and quite complex, but fibrosis is known to be the precancerous stage of HCC, thus approximately 80% of HCC develops in fibrotic or cirrhotic liver [1]. Current treatments for liver cancer depend largely on the specific stage and type of neoplasm, and include surgeries - for HCC the most effective is liver resection; radiotherapy - with poor results because the liver's tolerance to radiation is low; and chemotherapy - involving doxorubicin, 5-fluorouracil and other agents [2]. However, cancers are progressing rapidly and resistance to treatment is increasingly common, and more and more therapeutic alternatives are needed. In addition, adverse reactions and toxicity of conventional therapies are common and often unbearable for the patient. For this, natural compounds are an alternative due to their lower adverse reactions, silibinin being a compound with many biological properties that can be considered as an agent with therapeutic potential. Silibinin is extracted from the seeds of the milk thistle plant (Silybum marianum), is part of the flavonolignan class, known for its hepatoprotective, antioxidant properties and currently intensively researched for its anticancer benefits through various mechanisms of action. In several studies, silibinin has been proven effective against several types of cancers including liver, prostate, skin, breast, colon, lung and bladder cancers. The prevention and efficacy exhibited by silibinin on cancer types has been explored using either in vivo studies in rodents or in vitro experimental models such as different cell cultures, the anticancer activity of this compound being mainly related to its ability to induce cell cycle arrest and/or apoptosis [3].

Aim and objectives

This review aims to highlight the most recent advances made concerning the efficacy of silibinin in liver cancer treatment, administered both individually, and in combination with traditional chemotherapeutic drugs.

IMPACT OF SILIBININ IN PRECLINICAL EXPERIMENTAL MODELS ON LIVER CANCER

POTENTIAL OF SILIBININ IN LIVER CANCER

According to studies in the literature, silibinin is a phytocompound that has multiple actions on HCC, which derive from its ability to trigger apoptosis and cell cycle arrest, inhibit angiogenesis, reduce of cell proliferation and metastasis, and exert antioxidant and anti-inflammatory effects. The activity of silibinin on HCC has been studied in several cell lines showing an effect of interest to researchers. Niki Vakili Zahir and associates evaluated the compound in vitro on the HepG2 (Human hepatocarcinoma cell line) and HUVEC (human umbilical vein endothelial cell line) cell lines. Silibinin cytotoxicity was significant on the HepG2 cell line, while for HUVEC cell viability reached 25% even after administration of the highest concentration of 200 µg/mL. Cytotoxicity was also dose and time dependent. In the same study, the types of cell death involved were investigated, for HepG2 cells apoptosis was evidenced by microscopic fluorescence while for the HUVEC line necrosis was reported [4]. In another evaluation conducted by Varghese et. al, the impact of silibinin on cell growth, viability, apoptosis and cell cycle progression was investigated on two HCC cell lines, HepG2 and Hep3B. The results obtained reported that both cell lines were inhibited by silibinin, with

the effect being particularly strongest observed on the Hep3B cell line, associated with the induction of cell death by apoptosis. In addition, the compound of interest caused G1 arrest in HepG2 cells, and both G1 and G2-M arrest in for Hep3B cells [5]. Also other studies have noted the antiproliferative effect of silibinin on the HepG2 cell line. Therefore, at concentrations of 0-250 μg/mL, cell viability gradually decreased, with the strongest effect at the highest dose used. When silibinin was tested on normal rat hepatocytes, at concentrations of 125 μg/mL the compound induced very weak cytotoxicity, which slightly increased at higher concentrations [6]. Wei Cui and collaborators studied the impact of silibinin on nude mice bearing HuH7 xenografts. The results demonstrated that silibinin inhibits cell proliferation, cell cycle progression and PTEN/P-Akt and ERK signaling, and also can induce apoptosis-associated inhibition of survivin phosphorylation, these effects leading to a dose-dependent reduction of HCC xenograft growth [7]. John J Lah and associates demonstrated on multiple cell lines that silibinin significantly reduced the growth of human hepatoma cells (i.e., HuH7, HepG2, Hep3B and PLC/PRF/5). Cell growth reduction for HuH7 was associated with significant up-regulation of p21/CDK4 and p27/CDK4, induction of apoptosis correlated with down-regulated survivin and upregulated caspase-3 and -9 [8].

The in vitro results of silibinin in various cancer cell lines are promising and of interest to the oncology field, thus making the compound the focus of attention for many researchers and thus pointing to further studies needed for a clinical approach to the compound.

USE OF SILIBININ IN LIVER CANCER THERAPY IN COMBINATION WITH CONVENTIONAL THERAPIES

Due to the high resitance of HCC to treatments and its frequency, ranking third worldwide in terms of mortality, researchers have also turned their attention to the association between herbal agents and current conventional therapies. Li et al evaluated the combination of doxorubicin, an anthracycline chemotherapeutic extensively used in oncology, and silibin, a non-toxic phytotherapeutic agent, on orthotopic rat models of HCC. Silibin associated with doxorubicin showed a synergistic effect, inducing cell proliferation inhibition, G2-M arrest and apoptosis in HepG2 cells. In addition, simultaneous treatment with the two compounds showed an approximately 40% increase in apoptotic cell death, which was 3-fold greater than the action of the compounds individually [9]. Essential oils of Silybum marianum have been investigated in combination with 5-fluorouracil (5-FU) in HCC both in vivo and in vitro. Each preparation resulted in decreased H22 cell viability compared to controls. S. Marianum essential oils alone or in combination with 5-FU reduced cell invasion and migration, and angiogenesis-related proteins were significantly reduced both in vivo and in vitro. Furthermore, each treatment increased phospho-NF-kB (p65) and NF-kB (p65) protein levels. The results showed that this combination of S. Marianum and 5-FU prolonged survival in a mouse model of HCC compared to treatments administered individually [10]. Silibinin was in another study evaluated in combination with sorafenib, a kinase inhibitor drug approved for the treatment of advanced primary liver cancer. Results showed that silibinin combined with sorafenib induced cell death through apoptosis and potently inhibited the proliferation of HCC cell lines. It was also found that this combination inhibited the formation and the self-renewal of HCC stem cells by down-regulating the expression of stemness-related proteins such as the Homeobox NANOG protein. The study demonstrated that silibinin improves the efficacy and potency of sorafenib in therapy [11]. Considering that current treatments require improvement in all cancers, silibinin has been tested in combination with different chemotherapeutics in several cancers. Combination therapy is becoming recognised for the fact that the use of cytotoxic agents together with natural chemopreventive agents have different mechanisms of action with non-overlapping toxicity, the latter generally showing much lower toxic effects.

DISCUSSIONS

Liver cancer is currently a health challenge, with its incidence increasing worldwide. It is estimated that by 2025 more than 1 million people will be affected by this type of cancer. HCC is the most common form, with hepatitis B virus infection being the most important risk factor for the development of HCC [12]. Current therapies need improvement as resistance to treatments is increasing. In addition, the need for compounds with increased selectivity and low toxicity is imperative. Silybinin is the main compound in silymarin, and is recognised to have beneficial biological activities in several areas of health. Its beneficial effects have also been recognized in diseases such as Alzheimer's and epilepsy, as well as in cardiovascular diseases [13–15]. In recent decades, attention has also focused on the impact of silibinin in liver cancer. It is recognized and has been shown in numerous experimental studies to have antitumor effects imprinted by apoptosis induction, cell cycle arrest, angiogenesis inhibition, antioxidant and anti-inflammatory properties; basic properties in terms of the compound's mechanism and activity in cancers. Used individually, silibinin produced reduced cell proliferation on various cell lines, decreasing cell viability of tumour cells [4,5]. The exploration of the potential use of flavonoids has driven research into its use in combination with conventional therapies with known chemotherapeutic agents used in clinical practice. Sorafenib, doxorubicin, 5-fluorouracil or cisplatin are chemotherapeutic compounds with which silibinin had a synergistic effect, potentiating their activity against liver cancer [9,10,16]. Both used individually and in combination with agents recognized in currently used therapies, silibinin has proven efficacy in different forms of cancer.

CONCLUSIONS

Silibinin, already recognized in many studies for its antioxidant and anti-inflammatory properties, has demonstrated its effectiveness on liver cancers through multiple results obtained in preclinical experiments. The need for compounds with selectivity and limited toxicity, propells silibinin to future studies due to its therapeutic potential. Further research of this compound, both individually and in association with chemotherapy drugs is necessary for the complete understanding of all the mechanisms underlying the observed anti-tumor actions.

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