Coffee, Traditional Chinese Medicine and cannabinoids as potential tools for prevention and treatment of hepatocellular carcinoma

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ABSTRACT

In the last decade, the incidence of hepatocellular carcinoma (HCC) is growing in both Europe and United States. Conventional therapies such as liver resection, transplantation, ablation, chemoembolization and sorafenib are not enough to avoid a significant mortality. Many studies suggested the positive effect of caffeine for prevention of HCC. Nevertheless, the amount of therapeutic caffeine and the high-dose safety are unknown. Many authors proposed Traditional Chinese Medicine as preventive and/or curative approach. Although it reveals limits such as the uncertain safety profile and the lack of evidence about a unique product, it shows interesting results in terms of survival and quality of life if given in combination with standard loco-regional therapy. Among the future promises, cannabinoids show interesting background mechanisms of blocking cell proliferation and neoangiogenesis. It is conceivable that in the next years, some natural products may have a role in improving the standards of care of HCC.

Key words: Hepatocellular carcinoma; caffeine; Traditional Chinese Medicine; cannabinoids

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INTRODUCTION

Liver neoplasm represents the sixth most common cancer and the third cause of cancer-related mortality worldwide.[1] Hepatocellular carcinoma (HCC) is the main liver cancer, accounting for more than 90% of cases of liver tumors. In the last decades, the HCC incidence and HCC-related mortality are increasing in both United States and Northern Europe.[2] Cirrhosis due to chronic hepatitis B and C, is the major risk factor for the HCC development. However, also other

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potentially risky conditions such as alcohol intake, tobacco habit, overweight, diabetes, aflatoxin consumption and oral contraceptives use, should be considered.[3]

Barcelona-Clinic Liver Cancer (BCLC) staging system is a widely used set of criteria to guide management of patients with HCC. It takes into account tumor stage, liver functional status, physical status and cancer-related symptoms.[4] Surgical treatment of HCC is a potentially curative approach, including liver transplantation (LT) and liver resection. LT is the best treatment option for patients fitting the "Milan criteria" since it removes both neoplasm and underlying liver disease. For patients with single tumor < 2 cm, with a Child-Turcotte-Pugh class A, without clinically significant portal hypertension and with normal bilirubin, liver resection represents a feasible strategy.[4] Ablation with ethanol or acetic acid or thermal, is another potentially curative option. It is practicable in patients with single, small tumors not candidates for surgery.[4] Many HCC cases are diagnosed in stage B of BCLC algorithm, for which the standard of care is the transcatheter arterial chemoembolization (TACE).[4] Lastly, sorafenib is the unique universally approved systemic palliative drug for BCLC C patients.[4]

In the European[5] and in the North-American[6] guidelines, no natural product is mentioned neither for the prophylaxis nor for the treatment of HCC. On the contrary, in the Asian-Pacific ones,[7] natural products are cited for both primary and secondary prophylaxis.

Literature data are available regarding the coffee-derived substances as prevention tools in high-risk populations, the possible prevention or adjuvant effect of many kinds of Traditional Chinese Medicine (TCM), and possible utility of cannabinoids as antineoplastic drugs.

Hereby, we sought to review the current knowledge on the role of some natural products in the prevention and treatment of HCC. The research included published articles (peer reviewed original articles, review articles and meta-analyses). The search terms included "natural products and hepatocellular carcinoma", "natural products and liver", "hepatocellular carcinoma treatment options", "coffee and HCC", "Traditional Chinese Medicine and HCC", and "cannabinoids and HCC".

COFFEE

Many data are available about the dose-dependent protective effect of coffee respect to the development of liver disease and HCC.[8] Both *in vitro* and *in vivo* studies showed that several coffee compounds such as diterpenes, cafestol and kahweol, may act on some enzymes involved in carcinogenesis.[9,10] Diterpenes, cafestol and kahweol seem to modify the xenotoxic metabolism via induction of glutathione-S-transferase and inhibition of N-acetyltransferase.[11] Caffeine and antioxidant substances from coffee beans, may improve some liver enzymes, such as γ-glutamyltransferase and aminotransferase. Interestingly, this positive effect of caffeine is mainly relevant in heavy drinkers.[12,13] Notably, coffee consumption would be inversely related to the hazard of cirrhosis, which is the main risk factor of HCC.[14,15]

Although some authors[16,17] suggested a not statistically significant association between coffee consumption and risk of HCC, many other studies reported positive results.

In an Italian case-control study (including 250 HCC),[18] coffee intake showed a significant protective role against HCC. In all patients, ten-year coffee intake was associated with a decreased risk of HCC with a dose-effect relation (double with 3-4 cups/day respect to 1-2 cups/day).

In a further Italian study (185 HCC),[19] patients drinking ≥ 4 cups/day (no decaffeinated) had a lesser risk of HCC respect to the others.

Tanaka *et al*.[20] developed a Japanese case-control study (209 HCC) showing that coffee consumption during the last 1-2 years, was associated with a decreased risk of HCC. Another Japanese case-control study including 73 HCC, analyzed the role of coffee in patients with hepatitis C.[21] Coffee drinking ≥ 1 cup/day significantly reduced the risk of HCC compared to the abstinence. The same data were found for hepatitis B chronic carriers[22] with a risk reduction of 30-80%.

Two large Japanese prospective studies[23,24] including hepatitis B, C and sieronegative subjects, reported that drinkers of ≥ 5 cups/day had a lower dose-dependent HCC risk respect to abstinent patients.

The relationship between coffee and risk of HCC was studied also by Johnson *et al*.[25] through a large prospective study including 63,257 patients. The authors reported that subjects consuming ≥ 3 cups/day experienced a 44% of HCC risk reduction.

Hu *et al*.[26] firstly analyzed the possible association between coffee consumption, serum gamma-glutamyltransferase and HCC. The study cohorts included 60,323 patients without cancer. During a median follow-up period of 19.3 years, 128 participants developed HCC. According to the author's data, a combination of very low coffee consumption and high level of serum GGT was associated with nearly nine-fold increased risk of HCC.

In 2007, Bravi *et al*.[27] performed a meta-analysis based on 10 studies (both European and Asian) and a total of 2,260 HCC cases. Authors reported a 41% of reduction in HCC risk among coffee drinkers compared to non-drinkers. In the same year, Larsson *et al*.[28] published another meta-analysis with similar conclusions. In 2013, Bravi *et al*.[29] conducted a further meta-analysis including more recent studies. According to the authors, coffee drinkers had a decrease of 40% in the risk of HCC compared to abstinent patients. Moreover, high coffee drinkers showed more than 50% of risk drop. Notably,

the protective effect of coffee was reliable across different subgroups at increased HCC risk.

After the publication of these meta-analyses, other studies regarding the protective role of coffee in the HCC setting have been published. The first one, was a multicentre study by Bamia *et al*.[30] including 201 HCC cases. Authors demonstrated that coffee intake was associated with a decrease of 72% in HCC risk. Setiawan *et al*.[31] conducted a large population-based prospective cohort study (451 HCC) showing that drinkers of 2-3 cups/day respect to abstinent subjects, had a 38% of HCC risk reduction. In addiction, patients drinking 4 or more cups/day had a 41% of risk drop. Feld *et al*.[32] again suggested that regular ingestion of coffee in patients with chronic liver disease can make slower the progression of liver fibrosis, preventing both cirrhosis and HCC. Petrick *et al*.[33] developed the Liver Cancer Pooling Project based on North-American data and including 1,212,893 patients (with 860 HCC cases). A high caffeinated coffee consumption ($\geq$ 4 cups/day) was associated with a lower risk of HCC in comparison to a lesser intake. In a Japanese cohort-study[34] including 258 cases, an inverse association was reported between coffee and mortality associated to HCC. Interestingly, the hazard of HCC-related death for abstinent patients was two-fold higher compared to coffee drinkers, and this was true also for few consumption ($\geq$ 1 cup/day).

TCM

Many authors proposed TCM-based therapy alone or in combination with standard loco-regional therapies for prevention or treatment of HCC [Table 1]. The main TCM products include combinations of different herbal medicines or animal/insect extracts. Astragalus shows immunomodulatory properties and anti-tumor activity. It seems to reinforce Lymphokine Activated Killer cell activity restoring the T-cell function suppressed in cancer patients.[35,36] The Panax Ginseng has inhibitory effects on cell proliferation and angiogenesis[37] restraining tumor cell invasion and defeating sister chromatid interactions in human lymphocytes.[38] Toad skin secretion bufalin (Bufotoxin) could induce apoptosis in human-leukaemia cells modifying the expression of some apoptotic genes.[39] Other toad skin secretions such as 3-formyloxyresibufogenin, 19-oxobufalin, 19-oxodesacetylcinobufagin, 6-hydroxycinobufagin and 1-hydroxybufalin seem to have inhibitory properties on KB, human promyelocytic leukemia cells (HL-60) and MH-60 cancer cell lines.[40] *Mylabris phaleratais* (Mylabris) can lead to the apoptosis of tumor cells[41] while *Atractylodes* might bring apoptosis and have cytotoxic effects against tumor cells.[42] *Bupleurum falcatum* shows a significant anti-cell adhesive activity on solid tumor cells[43] and *Curcuma longa* has a relevant immunostimulatory activity.[44]

Concerning the prevention ability of herbal products, a Japanese herb called Sho-saiko-to has to be cited since it is reported in the Asian-Pacific guidelines.[7] In a randomized controlled trial (RCT),[45] Sho-saiko-to was shown to improve liver function in patients with chronic hepatitis. Also Oka *et al*.[46] reported that Sho-saiko-to may prevent the development of HCC in cirrhotic subjects. Successive studies with liver cell lines confirmed the above-cited suggestions.[47,48]

In 2013, Zhai *et al*.[49] compared in a RCT the efficacy of TCM and TACE in preventing recurrence of small HCC after resection. Authors tested TACE or TCM as adjuvant therapy for patients who underwent surgery without evidence of recidivism. One hundred and eighty-eight patients received Cinobufacini injection (extract from *Bufo bufo gargarizans* Cantor) and Jiedu Granule (a compound herbal medicine). The other patients (191 cases) were assigned to the TACE subgroup. TCM was associated with decreased HCC recurrence after resection in comparison to TACE, with similar adverse events.

Regarding the use of TCM alone as therapeutic tool, Tian *et al*.[50] demonstrated that it may be effective in subjects affected by middle/late stage HCC. In this RCT, 97 patients were treated with *Oleum fructus bruceas*, Ganji Decoction and external application of Ailitong, and 48 patients received

Table 1. The main natural products from Traditional Chinese Medicine

Product	Type	Main property/ies	Studies in humans	RCTs	Meta-analysis	Ref.
Astragalus	Herb	Restores T-cell	Yes	Yes	Yes	[35,36,53,55]
Panax Ginseng	Herb	Anti-proliferation and angiogenesis	Yes	Yes	Yes	[37,38,55]
Bufotoxin	Toad skin secretion	Induces apoptosis	Yes	Yes	Yes	[39,55]
Atractylodes	Herb	Induces apoptosis	No	No	No	[42]
Bubleurum falcatum	Herb	Anti-adhesive activity	No	No	No	[43]
Curcuma longa	Herb	Immunostimulatory activity	No	No	No	[44]
Cinobufacini	Bufo skin estract	Induces apoptosis	Yes	Yes	No	[49]
Jiedu	Herb	Unreported	Yes	Yes	No	[49]
Sho-saiko-to*	Herb	Decreases collagene type 1	Yes	Yes	No	[7,45-48]
Bruceas	Fruit estract	Unreported	Yes	Yes	No	[50]
Ganji	Herb	Unreported	Yes	Yes	No	[50]
Ailitong	Herb	Unreported	Yes	Yes	No	[50]
Kanglaite	Herb	Immunomodulation	Yes	Yes	Yes	[56]

*It comes from the Japanese Tradition

chemotherapy. The HCC progression was similar between the two groups, but the TCM approach showed less adverse reactions. Moreover, survival rate at three months was comparable, while the test group had a better half- and 1-year survival.

Man *et al*.[51] studied 94 patients with unresectable HCC. Authors compared three subgroups: (1) patients receiving TCM with non-curative antitumor treatments of Western Medicine; (2) patients receiving TCM alone; and (3) patients treated with non-curative antitumor treatments of Western Medicine or supportive treatment alone. They showed that patients treated with the combination schedule respect to patients in Western therapy alone, showed a significantly better 1- and 2-year survival (76.0% and 55.5% *vs.* 55.8% and 30.8%, respectively).

In 2005, Shu *et al*.[52] analysed 26 RCTs reporting that TCM might determine an advantage in terms of both neoplasm response and long-term patient survival. Notably, authors did not specify the kind of used natural product. McCulloch *et al*.[53] compared 34 RCTs, including 2,815 subjects, demonstrating that *Astragalus*-based TCM increased the efficacy of platinum chemotherapy. In 2009, two meta-analyses reported data concerning the possible role of TCM in association with TACE. Cho and Chen[54] analyzed 30 studies showing an improved long-term survival in patients treated with the association between TACE and TCM respect to the subjects who did not receive TCM. According to this study, TCM determined a relevant increase in white blood cell count, a substantially lower nausea and vomiting, and a significant rise in the body weight. Wu *et al*.[55] systematically reviewed and meta-analyzed a series of Chinese RCTs concerning the efficacy of TCM for the treatment of HCC. Authors reported some criticisms of the analyzed trials suggesting that the methodological issues were poor. Nevertheless, the studies with bufotoxin, *astragalus* (with or without *mylabris*) and ginseng associated to TACE, showed lower HCC recurrence and better patient survival in comparison to TACE alone. However, authors suggested that these data should be confirmed in further well-conducted Western RCTs.

Kanglaite (KLT) is a TCM coming from the seeds of a tropical Asian grass called Coix. It exhibits antitumor and immunomodulatory activity. Fu *et al*.[56] performed a meta-analysis including nine clinical trials to evaluate the efficacy of KLT injection combined with hepatic arterial intervention for the treatment of unresectable HCC. KLT injection combined with hepatic arterial intervention respect to arterial therapy alone, seemed to improve both short-term clinical efficacy and pain's control.

CANNABINOIDS

Cannabinoids are lipid mediators isolated from the hemp plant *Cannabis sativa* that can activate two G-protein-coupled receptors.[57] The active ingredients of Cannabis, as well as their synthetic analogues, are bioactive lipids that seem to block cell proliferation, reduce cell migration and inhibit angiogenesis.[58] The molecular mechanisms involved in the antineoplastic and anti-HCC action are debated. G protein-coupled receptor type 1 and 2 are typically considered the cannabinoids receptors. However, these substances may impact on other targets such as nuclear receptors peroxisome proliferator-activated receptor (PPAR)s.[59] PPARs are ligand-activated transcription factors, which belong to the nuclear receptor superfamily and mediate lipid metabolism, energy balance and anti-inflammatory cascade.[60] Several PPAR ligands have been shown to decrease HCC cell proliferation and migration through PPAR activation.[61] Moreover, utilizing a PPARg knockout mice model, it was suggested that PPAR decreases HCC carcinogenesis acting as tumor-suppressor gene in the liver.[62] Notably, the synthetic cannabinoid WIN 55,212-2 seemed to increase PPAR expression leading to apoptosis in the HCC HepG2 cell line.[63] Vara *et al*.[57] demonstrated that D9-tetrahydrocannabinol and JWH-015 (two kind of cannabinoids), might induce autophagy in HCC cells stimulating the AMP-activated protein kinase pathway. Jiang *et al*.[64] studying the PPAR-deficient mice, demonstrated the accumulation of autophagic vacuoles and up-regulation of autophagic marker LC3 protein expression. These results are in agreement with the above reported observations by Vara *et al*.[57] These authors suggested a connection between PPAR and autophagy-essential proteins in mammalian HCC. Also Vara *et al*.[65] reported the involvement of PPAR activation in the anti-cancer effect of cannabinoids. The authors showed that THC and JWH-015 might increase mRNA and protein levels of PPAR inducing PPAR activation *in vitro*. Moreover, the authors showed that, when endoplasmic reticulum stress-related protein tribbles homolog 3 (TRIB 3) is genetically inhibited, the expression of both PPAR mRNA and protein decreased. Indeed, TRIB 3 seemed to have a significant role in regulating cannabinoid-induced PPAR overexpression. Cannabinoid treatment could improve phosphorylated-eIF2a (an endoplasmic reticulum stress marker) and the endoplasmic reticulum stress-related pseudokinase TRIB 3. Notably, this latter is necessary for cannabinoid-induced cell death and the consequent anti-tumor effect.[66] Regarding the role of TRIB 3, Takahashi *et al*.[67] demonstrated that it can downregulate PPAR transcriptional activities by protein-protein interaction in 3T3-L1 adipocytes.

CONCLUSIONS AND FUTURE PERSPECTIVES

HCC represents one of the most common cancers worldwide and is the third cause of neoplasm-related death. Since chronic viral hepatitis are the main risk factors for HCC, the vaccination against hepatitis B and the treatment of both hepatitis B and C, represent the main preventive therapies. Today, the potentially curative (LT, resection, ablation) and palliative (arterial chemoembolization, sorafenib) standards of care still do not protect from a relevant rate of mortality.

Cohort studies and meta-analyses suggest that high coffee intake might prevent the HCC in subgroups of patients at increased risk. Nevertheless, the mechanisms involved and the specific components of coffee beverages that may determine this sort of protection are unknown. The available studies often report different cut-offs of coffee intake, besides not taking into account many potential confounders. Moreover, registration of coffee consumption depends mainly on the self-reporting questionarries with intrinsic relevant statistical limits. Consequently, it is difficult to establish the temporal relationship between coffee use, liver disease and HCC onset. Indeed, the open questions are the following: how much coffee is necessary and for how long time? Which is the long-term safety profile of high-dose caffeine?

Concerning TCM, many authors proposed it, alone or in association with standard therapy. Notably, the studies proposing TCM approach alone for the treatment of HCC show no strong data. The TCM treatment obtains some interesting results if administered together with TACE. In particular, RCTs and meta-analyses demonstrate an advantage in terms of both patients' survival and quality of life in comparison with the Western approach alone. However, there are many unclear aspects: which single product of the TCM large family is the best? Which is the impact of TCM on the liver function? Which is the safety profile of each TCM product?

Many basic studies suggest that cannabis could block cell proliferation, reduce cell migration and inhibit angiogenesis thus showing an anticancer attitude. Several data show a relationship between PPAR receptor and autophagy-essential proteins in HCC but the mechanisms involved in the antineoplastic action of cannabinoids are still debated. Furthermore, the lack of data on humans makes difficult to consider these substances as therapeutic choices.

It may be that the described natural products could have a future in the prevention of HCC, in the strengthening of the standard therapy and in the palliative phase. Still, further RCTs with strong results are mandatory for their effective broad application.

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