HOMOHARRINGTONINE: A NASCENT PHYTOCHEMICAL FOR CANCER TREATMENT (A REVIEW)

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ABSTRACT

Recently homoharringtonine (HHT) was approved in October 2012 by FDA for the treatment of adult patients with chronic or accelerated phase chronic myeloid leukemia. The time spend for this approval was more than 40 years, one of the historical for any drug to be approved by FDA. Looking towards potential of HHT, we aim to review its long journey. This review involves sources, chemistry, semisynthetic formulation, extraction, new methods of production, characterization, mechanism, and clinical development of homoharringtonine (HHT). The compiled data will be helpful for the researchers to focus on different aspects of HHT and its analogues. The data may prove useful for anticancer research and areas of research yet to be discovered.

KEYWORDS: homoharringtonine, omacetaxine mepesuccinate, chronic myeloid leukemia, Cephalotaxus harringtonia.

INTRODUCTION

Natural plants have been used to prevent and to treat various diseases for thousands of years. The ancient Chinese emperor, the Red Emperor, or Shen Nung, compiled the first medicinal herbal literature, \textit{Pentsao} in 2,800 BC.\textsuperscript{[1]} Phytochemicals are interesting yet mysterious group of thousands of chemicals found in natural plants. Some of them when isolated has shown promising effects to protect against cancer while many are not associated with cancer at all and undefined are yet to be discovered. There are excellent sources of bioactive components.
(phytochemicals) exerting their health beneficial effects, and very often, these sources are materials for gourmet food consumptions. Certain bioactive components (phytochemicals) from the plants have been confirmed for their anti-cancer activities. There is an estimate that approximately 50-60% of cancer patients in the United States utilize agents derived from different parts of plants or nutrients (complementary and alternative medicine), exclusively or concomitantly with traditional therapeutic regimen such as chemotherapy and/or radiation therapy.\textsuperscript{[2]} Various review articles summarized natural phytochemicals and their anti-cancer effects. In recent years, some of these reviews touched the general overview for the bioactive aspect for phytochemical compounds, or specific compounds such as Vitamin E from plant oil, boron-rich natural compound, hydroxytyrosol from virgin olive oil, resveratrol from grapes, phytoestrogens most notably from soybean or EGCG from green tea polyphenols, while the others are more specific for certain cancers, e.g., colorectal cancer, breast cancer, head and neck cancer, pancreatic cancer, prostate cancer, or protein targets and pathway mechanisms, such as Nrf2, COX-2, PLK1, angiogenesis.\textsuperscript{[3]}

Recently, homoharringtonine (HHT) an angiogenesis-inhibiting and apoptosis-inducing alkaloid which was approved in October 2012 by the FDA for the treatment of adult patients with chronic or accelerated phase chronic myeloid leukemia (CML) with resistance and/or intolerance to two or more tyrosine kinase inhibitors (TKI).\textsuperscript{[4]}

Although, homoharringtonine (HHT) has been introduced in the market under various brands after its 40 years journey from discovery to approval and its use on patients with CML, none of the article has compiled it thoroughly.

So in this review we have made an attempt to provide the comprehensive summary of Homoharringtonine (HHT) and its analogues.

**SOURCES OF HOMOHARRINGTONINE**

*Cephalotaxus harringtonia* (image-1) is the major source of homoharringtonine, it is also known as Japanese/Chinese plum yew. Because Japanese plum yew was cultivated in Europe and the United States for close to a century, *Cephalotaxus harringtonia*, was named in honor of the Earl of Harrington, one of the first to grow the plant in a European garden.\textsuperscript{[5]} The genus Cephalotaxus comprises nine species, which are mostly concentrated in China, but are also found in eastern India, Thailand, the Korean peninsula and Japan.\textsuperscript{[6]}
CHEMISTRY OF HOMOHARRINGTONINE (HHT)

In 1963, the very first time Paudler et. Al isolated harringtonine and cephalotaxine from *Cephalotaxus harringtonia*. However, in 1969, Powell et al. determined the structures of some ester alkaloids isolated from *Cephalotaxus harringtonia*. These isolated alkaloids were found to be harringtonine, isoharringtonine, deoxyharringtonine, and homoharringtonine (HHT). The difference between HHT and harringtonine structure was HHT has a methylene group inserted in the side chain. These alkaloids confirmed the antileukemic effects on mouse P-388 and L-1210 lines.

SEMISYNTHETIC FORMULATION

The natural purification of harringtonine and HHT has caused significant damage to the environment. And the development of homoharringtonine was hampered by several factors: difficult production and unreliable source supply, toxicity profile of the original dose schedules, large quantities of bulk of cephalotaxus trees required (rare, from China), the success of tyrosine kinase inhibitors in CML, and the uncertainty of the role of homoharringtonine in the context of tyrosine kinase inhibitors. So there was a vital need to look after the alternatives to produce HHT.

In 1999, Robin et al. reported, for the first time, the synthesis of semisynthetic HHT (sHHT). sHHT involves the direct esterification of cephalotaxine extracted from dry leaves of cephalotaxus, not from the bark. Only one 70th of the amount of cephalotaxus is required to extract sHHT compared with its natural counterpart, and it is also purer (99.7%). In addition, sHHT has excellent bioavailability by the subcutaneous (SC) route. sHHT is known currently as omacetaxine mepesuccinate (ceflatonin, CGX-653, Myelostat) and is being developed by ChemGenex Pharmaceuticals Ltd. (Menlo Park, CA, USA), in collaboration with Stragen.
Pharma (Geneva, Switzerland). Omacetaxine has recently been approved by FDA of the United States as an orphan drug to treat CML patients resistant to TKIs.\textsuperscript{[10]}

CHEMISTRY OF OMACETAXINE MEPESUCCINATE (SEMISYNTHETIC HHT)
It has molecular formula $C_{29}H_{39}NO_9$, Mol. Mass : 545.62 g/mol and melting point: 144-146 °C.

![Chemical structure of omacetaxine mepesuccinate (semisynthetic HHT)](image1)

**Fig1:** Chemical structure of omacetaxine mepesuccinate (semisynthetic HHT)

**Synthesis of Omacetaxine mepesuccinate (semisynthetic HHT)**

![Synthesis of Omacetaxine mepesuccinate](image2)

**Fig 2:** Synthesis of Omacetaxine mepesuccinate (semisynthetic HHT)
The oxidation of 2-methyl-1-cyclopentene-1-carbaldehyde (I) with O₃ and Ag₂O gives 2,6-dioxoheptanoic acid (II), which is esterified with cephalotaxine (III) by means of (COCl)₂, yielding the ester (IV). Reformatsky reaction of (IV) with methyl bromoacetate (V) and Zn affords the adduct (VI), which is treated with an excess of methylmagnesium iodide to provide the target homoharringtonine (as a single diastereomer), along with some starting cephalotaxine that is separated by chromatography.[11]

EXTRACTION OF HARRINGTONINE AND HOMOHARRINGTONINE

1 kg of finely ground *Cephalotaxus fortunei* Hook is extracted with 8 lt. of 90% ethanol at room temperature for 24 hrs. The solution is filtered to yield a filtrate A and filter cake. The filter cake is percolated with ethanol and filtered again to yield filtrate B. The filtrate A and B are combined and distilled under reduced pressure to recover ethanol and an aqueous residue. To this residue, 2% HCl is added to adjust the pH to 2.5. The solids are separated from the solution by filtration to yield a filtrate C. The solids are washed once with 2% HCl and filtered to yield a filtrate D. The filtrate C and D are combined and the pH adjusted to 9.5 by adding saturated sodium carbonate solution. The alkaline filtrate is extracted with chloroform and the chloroform layer separated from the aqueous layer. This extraction process is repeated five times. All the chloroform extracts are combined and distilled at reduced pressure to recover chloroform and alkaloid as a solid residue respectively. The solid alkaloid is then dissolved in 20 ml of 6% citric acid in water. The solution is divided into three equal portions. These are adjusted to pH 7, 8 and 9 by adding saturated sodium carbonate solution. The portions having pH 8 and 9 are combined and extracted with chloroform. The chloroform extracts are distilled under reduced pressure, whereby chloroform is removed and recovered and a solid residue of crude harringtonine is obtained. The crude harringtonine is dissolved in pure ethanol i.e. alkaloid: anhydrous ethanol 1:10, and crystallized. The crystals are refined by recrystallization in diethyl ether. Overall yield of Harringtonine is about 0.1% including yield from mixed HH from the subsequent process. The portion having a pH of 7 and the mother liquors from the foregoing crystallization of Harringtonine are combined and passed through a liquid chromatographic column of diameter to height ratio 1:50 packed with alumina. The column is finally flushed with chloroform and followed by chloroform-methanol of 9:1 mixture. The resulting alkaloids are mixture of HH. The mixed HH is then separated from each other by counter current distribution employing chloroform and pH 5 buffer. The first fraction of the counter current distribution is Homoharringtonine and the last
fraction of the counter current distribution is Harringtonine. Homoharringtonine is purified by crystallization in methyl alcohol.[12]

The new methods of producing homoharringtonine (HHT) are shown as follows
The good clinical results of HHT in treating cancer brought to the major problem, which is the supply of HHT both short term and long term. It is apparent that a huge amount of bark of Cephalotaxus is needed for collection, extraction and purification of HHT. It is clear that due to the slow growth of the trees of Cephalotaxus, which is a nature source of HHT, and the killing of trees by harvesting bark is not a sustainable resource for HHT production.

1. Tissue Culture (Plant Cell Culture)
Culture manipulation to promote secretion of HHT is a new way for an extracellular product HHT. The biosynthetic methods can yield more HHT through precursor of HHT feeding. The production of HHT increased significantly after the addition of the precursors and special biochemical agents. Content of precursor of HHT abounds in tree and it is very cheap. The present methods include several significant developments in technique of culture plant tissues that are
   (a) Yields of HHT selected from rapid growth, resistance to infections organisms; and
   (b) HHT can excrete into media.

2. Using Precursor of HHT
Recent research’s results have established that direct production of HHT from its precursor and advances in biosynthetic understanding for HHT metabolism. Biosynthesis or semi synthesis of HHT from major nonactivity ingredients is well established through great advances in special biochemistry reactions. Using precursor of HHT for semi synthesis and increase of production in plant cell culture are new developing methods for producing HHT.

3. Using Leaves
In this new method leaves of tree of Cephalotaxus is used and not the bark. So far, the extraction of HHT is done by using bark. The leaves are harvested from the trees of Cephalotaxus, which grow in mountains of South China. The natural source of leaves is very abundance. The new methods do not use bark. Therefore, it can avoid destroy trees. The natural source of Cephalotaxus tree is very limited and slow growing. In fact, bark of Cephalotaxus has very low yield of HHT. The yield of HHT from Cephalotaxus bark is about
50-100 mg/kg of dried bark. The present new method, therefore, has a great economic and environmental value.

4. Semi synthesis
HHT has received important chemical studies particularly in regard to structure and anticancer activity relationship and semi synthesis. A great progress in biochemistry allows semi synthesis to use precursor of HHT from leaves of Cephalotaxus and to produce HHT. The total chemical synthesis of HHT appears too long to have commercial value too. Semi synthesis method can yield a high efficient conversion of precursor to HHT. It is other better biological source for manufacturing HHT. This new method uses closing chemical analogues to convert to HHT. This analogue is produced from leaves or other organ of Cephalotaxus. The present invention disclosed that new methods and techniques of manufacturing HHT could avoid chopping down Cephalotaxus trees which governmental environmentalists are trying to have declared a threatened species.

5. Using Taxol Residual
Both taxol and HHT can be extracted from yew tree. The content of taxol is less than 0.01% in yew tree. The content of HHT in yew tree is about 0.01% -0.22%. The content of HHT is much higher than content of taxol. Taxol extracted from bark of yew is difficult and expensive. One reason is that the presences of closely related congeners are similar to taxol. A major congener is cephalomannine (CPM), which is a waste of process in manufacturing of Taxol. The chemical and physical characters are very close between taxol and cephalomannine (CPM). CPM characterized by the same ring structure as taxol and distinguishes from them only in C-13 ester structure. The present invention disclosed that CPM and related derivative are used to produce HHT.\[13\]

CHARACTERIZATION OF HHT
IR spectra
Identical IR spectra were obtained by either the KBr pellet and/or mineral oil mull preparation technique. A typical infrared spectrum (KBr) for unambiguous identification at the solid state of the crystalline homoharringtonine obtained by this process. A series of sharp absorption bands are noted at 612, 703, 771, 804, 826, 855, 879, 932, 1029, 1082, 1119, 1135, 1161, 1191, 1229, 1274, 1344, 1367, 1436, 1457, 1488, 1505, 1653, 1743, 2814, 2911, 2958, 3420, and 3552 cm\(^{-1}\)
Differential Scanning Calorimetry (DSC) and Thermogravimetry (TG)

Measurement of DSC and TG were obtained on a Mettler Toledo STAR System. Approximately 11 mg of homoharringtonine drug substance were accurately weighed (10.6251 mg) into a DSC pan. The sample was heated from 25°C to 250°C at a rate of 5°C/min. The DSC data were obtained following a standard method in the art. The DSC curve of crystalline homoharringtonine drug substance exhibits a melting endotherm at 145.6 °C.

Melting range performed by the capillary method (Bucchi Apparatus) gave 143-145°C. Literature indicated 144-146°C.[14] Crystallization medium was not published. This is the only literature reference regarding melting point of a crystalline form of HHT, X-Ray Powder Diffraction.

X-ray powder diffraction pattern was collected on an INEL microdiffractomer model

Diffractinel: Powdered homoharringtonine DS was packed in a glass capillary tube and was analysed according to a standard method in the art. The X-ray generator was operated at 45 kV and 40 mA, using the copper Kalpha line as the radiation source. The sample was rotated along the chi axis and data was collected between 0 and 120 deg 2-theta. A collection time of 1200 sec was used. The x-ray powder diffraction for this crystalline form of homoharringtonine shows a typical pattern including major reflection peaks at approximately 7.9, 9.2, 10.9, 14.9 16.0, 17.7, 19.5, 19.7, 21.78, 23.1 , 25.3, 25.4 and 25.7 deg 2-theta.

MECHANISM OF ACTION

Harringtonine and HHT inhibit protein translation by preventing the initial elongation step of protein synthesis via an interaction with the ribosomal A-site. [15,16] Recent crystallographic studies have shown that HHT blocks protein synthesis by competing with the amino acid side chains of incoming aminoacyl-tRNAs for binding to the A-site cleft in the peptidyl transferase center of the ribosome. [17] HHT leads to a general decrease in synthesis efficiency of all proteins. An important short-term effect of HHT on cells is the rapid loss of proteins with short half-lives. A number of proteins related to cell survival and proliferation with short half-lives are encoded by mRNAs that possess complex 5’ UTRs that are G/C rich and have complex 3-dimensional structures (e.g. c-Myc, Mcl-1 and Cyclin D1). HHT and omacetaxine induce the rapid loss of a number of short-lived proteins from various cell lines of hematological malignancies. These short-lived proteins clearly regulate proliferation and cell
survival and their loss is likely to be involved in the apoptosis induced by HHT and omacetaxine.

In vitro studies showed that HHT could induce apoptosis of AML (acute myeloid leukemia) and MDS cells via up regulation of pro-apoptotic bax and down regulation of the protein inhibitor surviving.\(^{[18-20]}\) Moreover, a study by Tong et al. showed that HHT might act as a broad-spectrum protein tyrosine kinase inhibitor that inhibits the phosphorylation of the signal proteins by oncogenic proteins such as JAK2V617F, Bcr-Abl, thus blocking the survival and proliferative signal pathway of primary AML cells and AML cell lines such as HEL, K562 and HL-60 cells.\(^{[21]}\)

This effect of HHT is similar to other novel protein translation inhibitors, such as silvestrol. However, the mechanisms of these protein translation inhibitors are different. Silvestrol is a cyclopenta benzofuran rocaaglate isolated from the Indonesian plant Aglaia foveolata, which has a unique dioxanyl ring-containing side chain. Silvestrol interferes with the assembly of the eIF4F translation complex by promoting an aberrant interaction between capped mRNA and eIF4A, thereby blocking protein synthesis at the initiation step. This inhibition of protein synthesis by silvestrol also results in a preferential depletion of proteins with short half-lives, such as Mcl-1, Cyclin D1 and c-Myc. Silvestrol was reported to have activity against leukemia cells in vitro and in vivo.\(^{[22,23]}\)

**HHT CLINICAL DEVELOPMENT**

The initial clinical trials of cephalotaxine esters in patients with cancer were conducted in the 1970s.\(^{[24-27]}\) Nine of 15 patients with CML treated with HHT (5 mg d\(^{-1}\) ~ 7 mg d\(^{-1}\) for 7 ~ 10 days) achieved complete hematological remission (CHR).\(^{[27]}\)

In a subsequent study, 39% (32 of 82) of CML patients treated with HHT (intravenously, 4 mg d\(^{-1}\) to 8 mg d\(^{-1}\)) achieved CHR.\(^{[28]}\) Huang et al. reported that 57.6% of 33 CML-CP patients treated with harringtonine (intravenously, 4 mg d\(^{-1}\), dose reduction and length of the course according the WBC counts) during 1991 ~ 1995 achieved CR .\(^{[29]}\) In a study carried out during 1996 ~ 2002, 76 newly-diagnosed CML-CP patients were treated with HHT (intravenously, 1.5 mg m\(^{-2}\) daily for 7 ~ 11 d every month). Among 55 patients with cytogenetic data, 38.2% achieved CyR (cytogenetic response) and 20% achieved MCR (major cytogenetic response), while only 2 of 10 patients with cytogenetic data achieved minor cytogenetic response in the group treated with hydroxyurea. The estimated 4-year
overall survival (OS) was 46.2%, which was significantly higher than that of the group treated with hydroxyurea (27%, 10/27).\textsuperscript{[30]} In 2008, Li et al. reported a low-dose and long term protocol of HHT (intravenously or intramuscularly, 1 mg d\textsuperscript{-1} for 8 weeks or 2 mg d\textsuperscript{-1} for 4 weeks, next cycle beginning after 4–5 weeks interval until 4 years), which resulted in a CHR of 66% (27 of 41) and with 5-year progression-free survival rate (PFS) 95% (39 of 41).\textsuperscript{[31]}

**SUMMARY AND CONCLUSION**

Various review articles summarized natural phytochemicals and their anti-cancer effects. In this review we have summarised the 40 years journey of Homoharringtonine (HHT) from its discovery to approval by FDA. This HHT is an angiogenesis-inhibiting and apoptosis-inducing alkaloid which was approved in October 2012 by the FDA for the treatment of adult patients with chronic or accelerated phase chronic myeloid leukemia (CML) with resistance and/or intolerance to two or more tyrosine kinase inhibitors (TKI). Its major source includes Japanese/Chinese plum yew. It was isolated from *Cephalotaxus harringtonia* as an ester alkaloid along with harringtonine, isoharringtonine and deoxyharringtonine. Semisynthetic HHT (sHHT) involves the direct esterification of cephalotaxine extracted from dry leaves of cephalotaxus. sHHT is also known as omacetaxine mepesuccinate. The extraction and isolation of harringtonine and homoharringtonine is done from *Cephalotaxus fortunei* while ethanol used solvent. Certain new methods are reported and proved to produce HHT, these methods are having great economic and environmental value. Different analytical data of HHT has been reported to confirm its identity. Various in vivo and in vitro mechanism of actions are proposed for HHT to be effective AML (acute myeloid leukemia) and chronic myeloid leukemia (CML). The clinical development/trials of HHT showed its use for cancer patients. HHT has witnessed a long time from isolation to its final use on cancer patients.

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