Section A-Research paper
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Role of Heat Shock Proteins as an Emerging Target to Ameliorate Chronic Liver Disease: A Critical Study

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Abstract

Every year, two million people worldwide die due to chronic liver disease, with 1 million of those fatalities coming from cirrhosis-related complications, and 1 million from hepatic cancer and viral hepatitis. Hepatocellular carcinoma is currently the 16th most prevalent cause of mortality worldwide, while cirrhosis is currently the 11th; together, they account for 3.5% of all mortality worldwide. The complex process of liver regeneration or treatment restores functional tissues after injury/resection and hepatic parenchymal cells undergo momentary ATP depletion and undergo metabolic stress. When cells are under stress, heat shock protein acts as a chaperone and triggers the release of various inflammatory cytokines which have been recognized as important participants in the initial phases of liver regeneration. Heat shock proteins were thought to be an emerging therapeutic target for chronic hepatic disease due to their participation in the development of a number of liver ailments. In this study, we concentrate on how various liver-related disorders are treated by natural, synthetic, and semi-synthetic medications that regulate heat shock proteins.

Keywords: Chronic liver disease, heat shock proteins, hepatocellular carcinoma, natural and synthetic drugs

1. Introduction

Globally, chronic liver disease or injury is a severe health issue. Numerous hepatic diseases, such as fibrosis, fatty liver, hepatocellular cancer, hepatitis, and cirrhosis, are included in chronic liver disease. Current synthetic medications are not very effective to ameliorate chronic liver disease, and they also have unfavourable side effects. In order to treat chronic liver illnesses, a wide variety of medicinal plants and phytochemicals have been researched as alternative and complementary therapies. Chronic liver disease, which is still among the most significant health problems globally, may impact greater than 10% of the global population. Chronic liver disorders are conditions that gradually degrade and repair the liver parenchyma in a clinical environment. Cirrhosis and hepatocellular cancer develop as a result of these disease processes if they are not treated. The most prevalent types of chronic liver disorders are cirrhosis, hepatocellular carcinoma (HCC), non-alcoholic or alcoholic fatty liver disease, viral hepatitis, and autoimmune hepatitis.[1] Hepatic cancer is a prevalent liver cancer with a high fatality rate that occurs all over the world. According to figures from 2018, HCC is thought to be the 6th most known cancer to be diagnosed and the 4th foremost cause of death globally.[2] The development of HCC is affected by numerous risk factors, such as hepatitis B & C, alcoholic cirrhosis, exposure to chemical carcinogens, non-alcoholic steatohepatitis, and intake of food contaminated with aflatoxin.[3] Under pathological circumstances, heat shock proteins

(HSPs) start the folding, restoration, folding back of misfolded peptides, and probable degradation of damaged proteins, among other cytoprotective actions. Few HSPs occur constitutively, but the majority of heat shock proteins are molecule-level chaperones that are frequently overexpressed by cells in response to trigger signals that may result in protein degradation. It is well recognized that excessive apoptosis plays a function in the pathogenesis and development of a number of human inflammatory disorders (HIDs) and cancer. This is due to the elevated cellular amount of reactive oxygen species and the ensuing amplified responses of inflammation. By inhibiting pro-inflammatory factors, HSPs are believed to influence the efficacy of inflammatory cascades that cause endogenous oxidative species production and intrinsic apoptosis and consequently play a key role in the pathogenesis of cancer and inflammatory injuries.[4,5] The mechanism by which various environmental and pathological conditions affect the heat shock proteins regulation is shown in Figure 1. Heat, nutrient shortage, chronic or acute inflammatory illnesses, gravity, viral infection, oxidative stress, ischemia, exercise, bacterial infection, and heavy metals are some of the stressors.[5-8] Heat shock proteins (HSPs) are vast members of molecular chaperones that have undergone evolutionary conservation and play a crucial function in the progression and survival of cells. In general, HSPs can be divided into two subgroups depending on alike molecular weight.[9] In both prokaryotic and eukaryotic animals, heat shock proteins are broadly conserved and distributed protein families that preserve cellular proteostasis and shield cells from stressors. According to their molecular weights, HSP protein families are divided into large HSPs, HSP100 HSP90, HSP70, HSP60, HSP47, and tiny HSPs. As an integrated network, they serve as molecular chaperons in cells and are involved in the folding of freshly produced polypeptides, the refolding of metastable proteins, the building of protein complexes, the dissociation of protein aggregates, and the destruction of misfolded proteins. They are crucial for the control of cell signaling transmission, the cell cycle, and apoptosis in addition to their chaperone duties. HSP dysfunction is thereby linked to an array of diseases, including cancer, neurodegeneration, and other diseases.[10,11] These families' roles are engaged in the entire metabolic process of proteins. As holdase, sequestrase, foldase, disaggregase, aggregase, or, they act as a framework that sustains the proteostasis in cells and serves as a powerful first class of defence in instances of stress.[12,13]

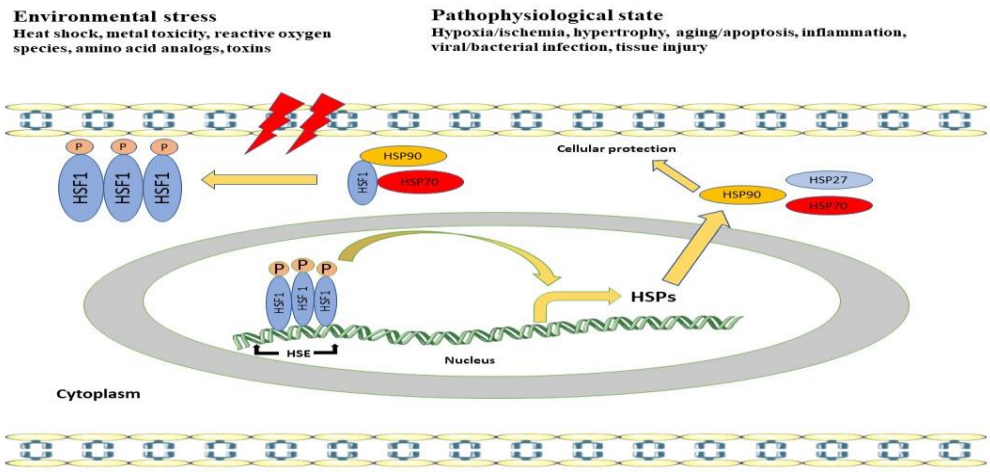


Figure: 1 The heat shock transcription factor 1 (HSF1) controls HSP expression. In non-stressful circumstances, HSF-1 gets pushed away in the cytoplasm by HSPs (HSP90, HSP70), which attaches to HSF1 and prevents its transcriptional activity. In consequence of

stress, such as pathophysiological circumstances like, apoptosis, hypertrophy, aging, inflammation, hypoxia or ischemia, viral or bacterial infection, and other tissue harm, HSPs dissociate from the complex that activates HSF1. High temperatures, reactive oxygen species, metal toxicity, toxins, and analogs of amino acids, are a few examples of environmental stressors. In order to activate the transcription of HSP genes and assist cellular survival by enhancing cellular protection, after nuclear translocation, HSF1 attaches to certain heat shock element sequences that are promotors of the upstream heat shock gene.

2. Role of heat shock protein as an emerging target for liver disease

Heat shock proteins function as chaperones by helping in the gathering and folding of freshly produced proteins, preventing the adhesion of proteins, and restoring cell homeostasis.[14] HSP70 is up-regulated in cells in response to an array of stimuli, involving heat, chemical injury, and oxidative damage, to assist in preventing protein aggregation. A unique therapeutic approach for fibrosis involves procedures that attempt to restore normal HSP70 expression because HSP70 deficiency causes fibrosis.[15,16] The expression of HSPs, which are stress proteins, is strictly regulated by gradually elevating temperatures, making them inactive under normal circumstances. HSPs are quickly activated and up-regulated, however, when living organisms are subjected to an array of physiological and environmental factors, involving heavy metals, heat shock, ultraviolet radiation, membrane perturbations, and oxidative stress. This can either help cells survive or foster the death of an untreatably damaged cell. Heat shock proteins (HSPs) play a crucial function in a variety of fibrosis, including liver, idiopathic pulmonary fibrosis, and lung by controlling inflammatory response and cytokine generation[17,18] The potential role of HSPs in different liver-associated inflammatory diseases via regulating inflammatory pathways is shown in Figure 2. The ubiquitous and highly maintained proteins known as stress-induced HSPs are triggered by an array of environmental and physiological stressors, including heat, noxious chemicals, alcohol, and hydrogen peroxide. These proteins were initially discovered for their cytoprotective properties, but it has since been discovered that they also play a significant causal function in chronic diseases like atherosclerosis, cancer, diabetes, and neurodegeneration. HSP90 is also known to be a promising therapeutic target in hepatocellular carcinoma, plays a substantial role in fibrosis, and promotes hepatitis C virus replication when exposed to alcohol. Chronic alcohol use activates hsp90 in the liver, just like other stress signals do.[19] Whenever there is cellular stress, the HSP70 also serves as a chaperone and triggers the release of a number of inflammatory cytokines that were previously discovered as important participants in the initial stages of liver regeneration. Additionally, it was proposed that Hsp70 would be necessary for the start of regeneration.[20] The role of various HSPs and their localization is shown in Table 1.

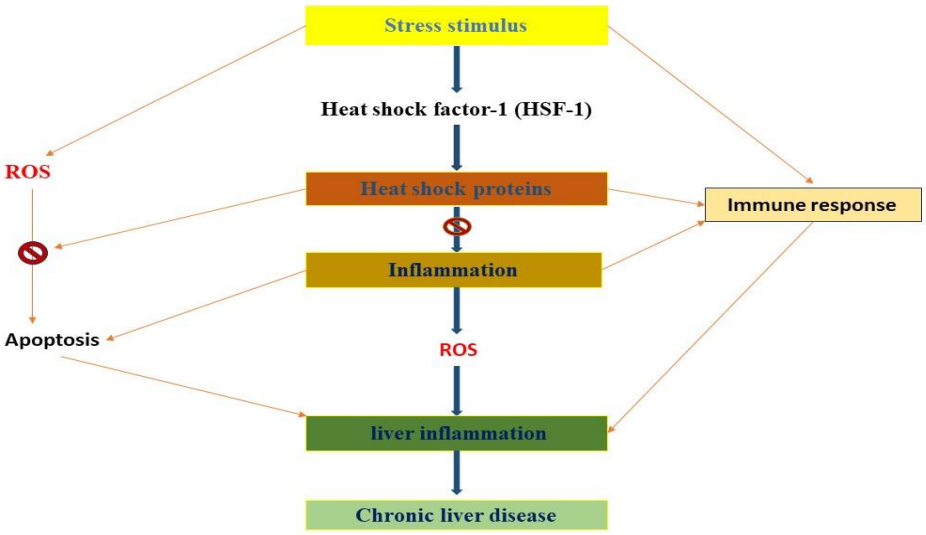


Figure: 2 Potential roles of HSPs in chronic liver disease via regulating different inflammatory pathways.

Name	Localization	Mol. weight (kDa)	Function	References
Small HSPs	Cytoplasm/nucleus	12-45	Tumor growth, apoptosis, tumorigenesis, metastasis, chemo-resistance.	21
HSP40	Cytoplasm/nucleus	40	Binds non-native proteins and regulates the activity of HSP70.	22
HSP60	Mitochondria/cytosol	57-69	facilitating folding proteins in collaboration with HSP10 and also playing a role in the immune system and apoptotic control.	23
HSP70	Cytosol, nucleus, mitochondria, endoplasmic reticulum	70	several physiological processes, such as protein folding, protein complex disintegration, and the transfer of proteins across the membrane.	24,25
HSP90	Cytosol, endoplasmic reticulum, mitochondria	90	HSP90 proteins participate in signaling events, apoptosis, folding of proteins, cell viability, and deterioration in regulatory pathways that control cellular functions.	25

HSP110	Cytosol/nucleus	110	Maintain cellular protein proteostasis, and act as cochaperones for HSP70s and as cochaperones, the main nucleotide-exchange factors (NEFs) for cytosolic Hsp70s are Hsp110s, which make it easier for Hsp70s to exchange ADP for ATP.	26

Table 1: Different Functions and Localization of heat shock proteins

3. Treatment of Chronic Liver Disease by Targeting Heat Shock Proteins

A tragically significant percentage of people worldwide die each year as a result of liver disease, which has now become a serious condition. Numerous treatments have been proposed; these putative medicinal substances are frequently described as herbal or conventional medicines. They can regulate heat shock proteins either positively or negatively. Among various treatments, natural and synthetic remedies are the most commonly reported which are shown below.

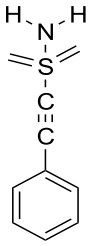
3.1 RDPEER Supplementation

It was discovered that adding RDPEER, an antioxidant oligopeptide from watermelon seeds, prevented heat stress-related liver damage in rats and HepG2 cells. By boosting enzymes having an antioxidant activity (GSH-Px, CAT, and T-SOD), reducing MDA (Malondialdehyde) level, and decreasing reactive oxygen species overproduction, it was found that RDPEER supplement lessen heat stress-induced hepatocyte damage. In addition, RDPEER decreased inflammatory factor transcription (NF-κB) and HSPs expression (HSP90/72/27), antagonize HPA axis hyperactivity, serum ALT, AST, and level of inflammatory factor (TNF-α, IL-6, and INF-γ), triggered the endogenous antioxidant pathway (Keap-1/ Nrf2), and activated the endogenous antioxidant pathway to protect against liver damage from heat stress. HSP72 and HSP90 were significantly upregulated by heat stress at the mRNA level when compared to the control group ($p<0.05$). The western blot technique revealed that heat stress raised several HSPs expressions ($p<0.05$). However, RDPEER supplementation prevented the rise in HSPs (27/72/90) mRNA expression brought on by heat stress.[27]

3.2 2-phenylethynesulfonamide

By specifically targeting the NHE1-HSP70 complex in mice, it has been shown that 2-phenylethynesulfonamide (PES) inhibits the production of pro-inflammatory factors and reduces hepatic damage brought on by LPS. After intraperitoneal injection of PES (antagonist of HSP70 substrate binding activity), pro-inflammatory variables were reduced in C57/BL6 mice. It was discovered that PES inhibits LPS-induced changes in ALT and AST activity, inflammatory cell infiltration, and liver cell death. PES decreased the levels of serum nitric oxide (NO), interleukin-6, inducible nitric oxide synthase, and tumor necrosis factor-α protein in LPS-stimulated animals. It was determined that blocking the NHE1-Hsp70 association, which in turn lessens the activation of the I-κB-NF-κB pathway in the liver, inhibits Hsp70 substrate binding activity in vivo and

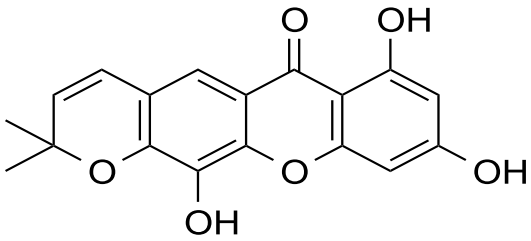
decreases the stimulation of pro-inflammatory factors and protects LPS-induced liver injury.[28]



2-phenylethynesulfonamide

3.3 1,3,5-trihydroxy-13,13-dimethyl-2H-pyran [7,6-b] xanthone (TDP)

It has been proven that TDP extracted from the herb *Garcinia oblongifolia* utilized in traditional Chinese medicine causes apoptosis in HCC by antagonizing the expression of Hsp27. In HCC, *garcinia oblongifolia* substantially suppresses the proliferation of cells and induces caspase-dependent mitochondrial death. To identify the targets of TDP in Hepatocarcinoma cells, a mass spectrometry-based comparative proteomics, and two-dimensional gel electrophoresis were carried out. There were found to be 18 proteins that were differentially expressed, with the Hsp27 protein being one of them that was considerably down-regulated by TDP. It was proposed that TDP induces apoptosis by resolutely inhibiting the expression of the protein Hsp27, which is directly linked to the caspase-dependent pathway's mitochondrial death.[29]

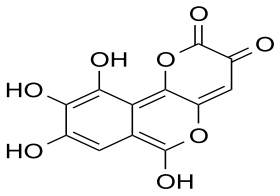


1,3,5-trihydroxy-13,13-dimethyl-2H-pyran [7,6-b] xanthone

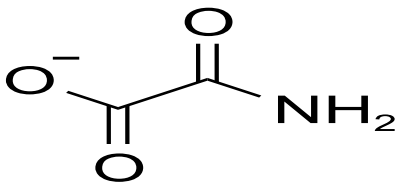
3.4 Galloflavin & Oxamate

The ATPase activity of two of the investigated HSPs (Hsp72, Hsp90) is inhibited by the polyphenol galloflavin, a derivative of gallic acid, according to a prior study. This inhibited HSR results in much-reduced levels of alpha-fetoprotein and induces senescence. Heat shock protein (HSP) overexpression has been frequently documented in human cancers and has been linked to tumor development. Among the conditions where HSR activation has been found to have the most clinical significance is hepatocellular cancer. The transcription factor HSF1, which is very active in cells of cancer, is the main inducer of HSP expression. HSF-1 has also been discovered to control glucose metabolism and boost lactate dehydrogenase (LDH-A) expression in its A isoform. These results demonstrated a link between the HSR and Warburg effect. The Warburg effect, which is a well-known characteristic of cancerous cells, is characterized by an increased

transformation of pyruvate to lactate even when there is enough oxygen present. The increased expression of LDH-A is directly related to this metabolic alteration associated with cancer. Galloflavin (GF), an enzyme inhibitor, was used for investigation into the interaction between LDH and HSR. The standard LDH inhibitor, oxamate (OXA), particularly antagonizes the enzyme by outcompeting its natural substrate. Like other polyphenols, GF inhibits LDH activity but also has a variety of metabolic attributes. In addition to mimicking OXA effects, it was discovered that GF was a more effective inducer of cell senescence and inhibited the ATPase activity of HSP90 and HSP72.[30,31]



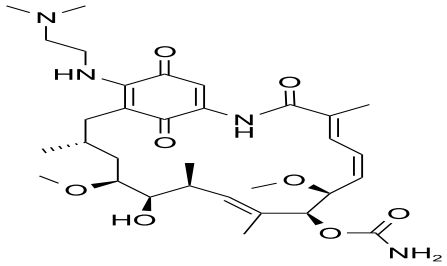
Galloflavin



Oxamate

3.5 17-dimethylamino-ethylamino-17-demethoxygeldanamycin (17-DMAG)

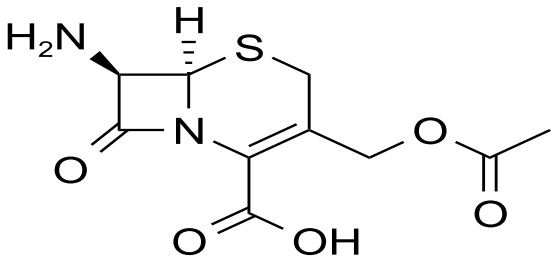
Inhibitors of HSP90 were discovered to provide protection for numerous organs. In the rat bile duct ligation model, the HSP90 inhibitor (17-DMAG) was demonstrated to have a protective effect. The effectiveness of 17-DMAG at treating the liver damage brought on by cholestasis was tested after it was supplied intraperitoneally at a dosage of 0.002g/kg. HSP90 expression was elevated in relation to cholestatic liver damage. By minimizing the expression of interleukin-1 and interleukin-18 in in-vitro sinusoidal endothelial cells of the liver, DMAG also protected hepatocytes from cholestatic damage.[32]



17-dimethylamino-ethylamino-17-demethoxygeldanamycin

3.6 7-aminocephalosporanic acid (7 ACA)

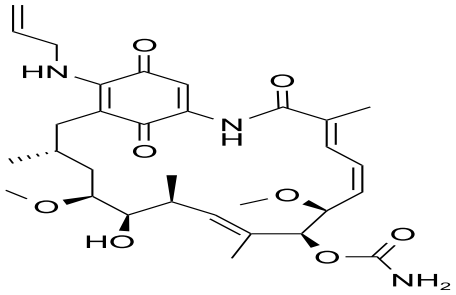
It was shown that HFD-induced hepatic steatosis may be prevented by 7 ACA, a strong HSP90 inhibitor. An antibiotic's 7-aminocephalosporanic acid has a KD value of 6.201μM between it and the non-N-terminal domain of HSP90. Additionally, it was anticipated that 7ACA would engage with an intermediary domain of HSP90. It was discovered that 7 ACA decreased sterol regulatory element-binding protein (SREBPs), resulting in reduced cellular total cholesterol and triglycerides in HepG2 cells. The treatment with 7ACA (0.005, 0.01, and 0.025 g/kg daily for 12 weeks) in HFD-fed animals reduced serum TC and TG in a dose-dependent manner and was critical in preventing lipid build-up in the adipose tissue and hepatocytes.[33]



7-aminocephalosporanic acid

3.7 17-N-allylamino-17-demethoxygeldanamycin (17-AAG)

It was thought that 17-AAG (an HSP90 antagonist) was successful in treating mice with thioacetamide-induced liver fibrosis. Swiss albino male mice of the CD-1 strain received a dose of 17-AAG (0.025, 0.05g/kg) intraperitoneally to reduce thioacetamide-induced liver fibrosis. The expression level α-SMA, Malondialdehyde, Col1A1, HSP90, and TIMP-1 were substantially decreased ($p< 0.05$) by the 17-AAG higher dose as compared to the thioacetamide group after receiving a 50mg/kg intraperitoneal dose of 17-AAG. The group that was treated with 17-AAG (0.05g/kg) had substantially ($p<0.001$) increased GSH level, caspase-3, and Fas than the other groups. By balancing oxidative stress and increasing apoptosis in cells, the Hsp90 antagonist 17-AAG diminished thioacetamide-induced hepatotoxicity.[34]

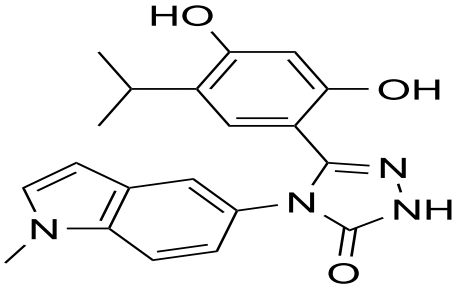


17-N-allylamino-17-demethoxygeldanamycin

3.8 STA9090 (Ganetespib)

According to prior work, the HSP90 inhibitor STA9090 reduces mRNA transcription and DNA-PKcs protein stability, making HCC more susceptible to DNA damage brought on by hyperthermia. The DNA-dependent protein kinase catalytic subunit (DNA-PKcs), which somewhat is imperative for DNA double-strand break repair, is mandated for the non-homologous end-joining mechanism. To survive in the hostile tumor microenvironment and numerous anticancer medicines, tumor cells have greater amounts of DNA-PKcs. In hepatocellular carcinoma (HCC), enhanced levels of HSP90, and PKcs-DNA were found to be associated with poor overall survival. It was also discovered that the C-terminal domain and HSP90 terminal domain have differing impacts on mRNA and DNA-PKcs protein levels. The nucleotide-binding region at the N-terminal of Hsp90 was positively correlated with DNA-PKcs stability. HSP90-SP1 interaction, SP1 level, and

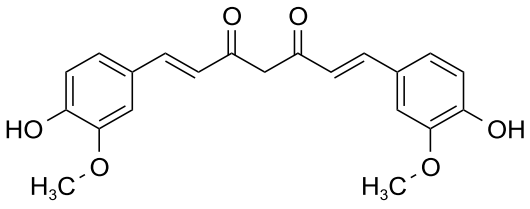
HSP90/SP1 communicating with the proximal promoter area of PRKDC were all reduced when the Hsp90 N-terminal was inhibited. STA9090, an HSP90 inhibitor, was shown to reduce DSB repair pathways by decreasing the stability of the mRNA transcription and DNA-PKcs protein. We explained the mechanism by which HSP90 N and C-terminal activity on PKcs-DNA varied and reiterated that HSP90 in HepG2 cells controlled PRKDC transcription via SP1.[35]



STA9090 (Ganetespib)

3.9 Curcumin

According to research, curcumin prevents hepatic cancer by preventing the DAMP molecule HSP70 and TLR4 activation. A polyphenol called curcumin, which was extracted from the rhizome of traditional Chinese turmeric, has an array of anti-tumor, anti-inflammatory, and anti-oxidant actions. HepG2 cells had TLR4 activated on their surface, HSP70 was excreted from HepG2 cells, and heat stress boosted HSP70 secretion into the HepG2 cell culture medium. Heat stress can produce HSP70 from cells and dramatically raise eHSP70, as shown by the fact that the levels of intracellular HSP70 were lower in heat-stressed cells than they were in non-stressed HepG2 cells. When curcumin was co-cultured with HepG2 cells, eHSP70 drastically decreased; yet, after curcumin's influence was removed, eHSP70 increased once more. The enhanced expression of TLR4 in HepG2 cells was shown to be linked to eHSP70, as evidenced by the observation that the cellular expression of TLR4 showed a concentration-dependent connection. It was established that curcumin may block the HSP70-mediated activation of TLR4 signaling, and this inhibition was linked to curcumin's anticancer activity.[36]

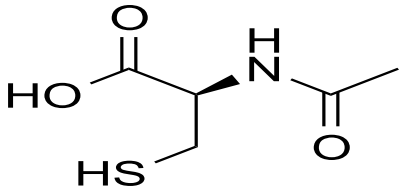


Curcumin

3.10 N-acetylcysteine (NAC)

Aspartate transaminase, hepatic hydroxyproline, total bilirubin, superoxide dismutase, albumin content, malondialdehyde, and HSP47 protein expression activity were all significantly decreased by NAC treatment. It also substantially decreases inflammation in

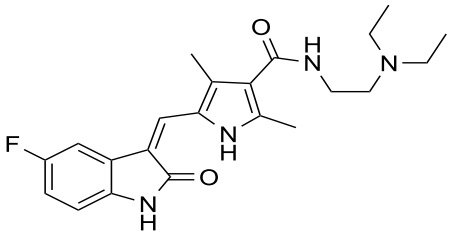
the liver and collagen deposition. When NAC was given to MG132-treated cells, Hsp70 levels increased. The degree of fibrosis decreased in the cirrhotic group that received NAC treatment. When contrasted with the group of cirrhosis having no treatment, this group has also displayed less cell membrane degradation, reduced decline in glutathione peroxidase amounts, and a decreased expression of iNOS.[37-39]



N-acetylcysteine

3.11 Sunitinib

In clinical trials, sunitinib showed strong activity against angiogenesis and cancer in a range of cancer types. In liver fibrosis models, sunitinib was previously shown to lessen inflammatory infiltration and the production of fibrotic biomarkers such as HSP47. In cirrhotic rats, sunitinib was similarly reported to lower portal vein pressure and the proportion of ICAM-1 and VCAM-1 hepatic positive vasculature.[40] As a result, in cirrhotic rats, sunitinib significantly reduces inflammatory infiltrate, hepatic vascular density, the amount of α-SMA, portal pressure, collagen expression, and LX-2 viability, which in turn lowers portal pressure and fibrosis as well as inflammatory infiltration.[41]



Sunitinib

3.12 SB203580

Stress kinase inhibitor SB203580 has a structure of 4-(4-fluorophenyl)-2-(4-methyl sulfinyl phenyl)-5-(4-pyridyl)-imidazole. Through the suppression of MAPKAPK-2 activation and HSP phosphorylation, it inhibits p38 MAPK. Stress kinase inhibitor SB203580 reduced hypoxia-induced expression of collagen XVIII, VEGF, and CBP2/Hsp47. Additionally, it was shown that SB203580 lessened the severity of hepatic fibrosis.[42-44]

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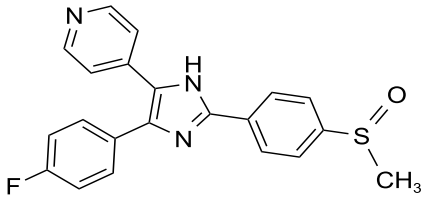
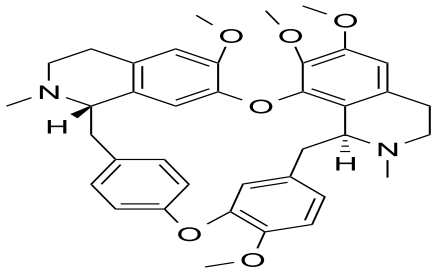


4-(4-fluorophenyl)-2-(4-methyl sulfinyl phenyl)-5-(4-pyridyl)-imidazole

3.13 Tetrandrine

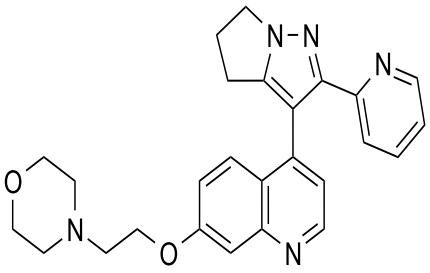
The measurement of Pcol1A1, collagen 1, HSP47, and α-SMA in precisely cut hepatic slices from rat's fibrotic livers after the application of tetrandrine were significantly reduced in the in vivo comparison experiments on BDL rats. Tetrandrine was reported to significantly reduce the abundance of liver collagen in rats with dimethylnitrosamine-induced fibrosis. The count of α-SMA and NF-kB positive cells in the hepatic fibrosis was decreased by tetrandrine application. Tetrandrine therapy reduced plasma transaminase activity (ALT & AST) levels as well as intercellular adhesion molecule 1, TGF-1 mRNA, and α-SMA expression. Tetrandrine was also discovered to promote the apoptosis of activated HSCs.[45-48]



Tetrandrine

3.14 LY2109761

A small chemical inhibitor called LY2109761 has the potential to suppress TGF-β, which plays a crucial role in the angiogenesis and spreading of cancer cells. Precision-cut rat liver slices exposed to LY2109761 exhibit an antagonistic effect on the HSP47 expression, which is exacerbated by longer incubation times.[49,50]



LY2109761

4. Conclusion

Heat shock proteins participate in the modulation of hepatic stellate cells (HSCs) activation, fibrogenesis, and collagen synthesis making them suitable targets for the amelioration of chronic liver illness. To treat chronic liver-related disorders, there are numerous natural, conventional, and synthetic medications that either positively or negatively regulate HSPs. The family of HSPs plays a pivotal function in the earlier diagnosis and as a biomarker for the treatment of liver-associated disease.

5. ACKNOWLEDGEMENTS

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