Implication of advanced glycation endproducts (AGEs) related to their receptor RAGE and glyoxalase-I (Glo-I) in chronic liver disease and hepatocellular carcinoma (HCC)

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Abstract

The glyoxalase system is formed by the enzymes glyoxalase-I (Glo-I) and glyoxalase-II (Glo-II) and is responsible for the detoxification of methylglyoxal (MGO). MGO is a by-product in glycolysis, threonine-catabolism and ketone bodies pathway leading to formation of advanced glycation endproducts (AGEs) and oxidative stress. AGEs bind to their receptor RAGE and activate pro-inflammatory transcription factors such as NF-κB by means of ERK1/2, PI3K, JNK and others. This review focuses on implication of Glo-I/AGE/RAGE system in chronic liver disease and HCC. Recent work showed importance of AGEs and RAGE in the latter. Both have been upregulated in fibrosis and silencing of RAGE reduced fibrosis and tumor growth of HCC. In contrast, Glo-I was demonstrated to be involved in development and progression of cirrhosis and new data offer Glo-I as an innovative target for antifibrotic therapy.
In a conclusion, there is growing evidence regarding involvement of Glo-I/AGE/RAGE system in chronic liver diseases with an interesting new therapeutic opportunity. These findings need further elucidation in preclinical and clinical studies.

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Key words: ethyl pyruvate, cirrhosis, fibrosis, methylglyoxal, AGEs, CCl_4.

Abbreviations:

AGEs	advanced glycation endproducts
AKT	protein kinase B
EP	ethyl pyruvate
ET-1	endothelin-1
Glo-I	glyoxalase-I
Glo-II	glyoxalase-II
GSH	L-glutathione
HCC	hepatocellular carcinoma
HEP	hepatocytes
HSC	hepatic stellate cells
JAK2	Januskinase 2
JNK	c-Jun N-terminal kinase
KC	Kupffer cells
LSEC	liver sinusoidal endothelial cells
MAPK	mitogen-activated protein kinase
MCD	methionine cholin deficient diet
MG-H1	5-hydro-5-methylimidazolone
MGO	methylglyoxal
NAFLD/NASH	non-alcoholic fatty liver disease / steatohepatitis
NF-κB	nuclear factor-κB
NO	nitric oxide
PI3-K	phosphoinositide 3-kinase
RAGE	receptor for advanced glycation endproducts
sRAGE	soluble form of RAGE
ROS	reactive oxygen species
STAT1	signal transducer and activator of transcription-1
TGF-β	transforming growth factor beta
THP	tetrahydropyrimidine

1. Introduction

Oxidative stress (ROS) leading to repetitive liver inflammation is responsible for development of chronic liver disease. The initial damage of hepatocytes is followed by release of pro-inflammatory cytokines and finally activation of hepatic stellate cells (HSC). Activated HSC transform to myofibroblasts, lead to deposition of collagen and finally fibrosis and cirrhosis. Several molecular mechanism are involved in this complex interplay, nevertheless the critical step is the activation of HSC by ROS. This review will focus on the glyoxalase-I (Glo-I) and related advanced glycation endproducts (AGEs) with their receptor RAGE in generation and detoxification of ROS. Recent work of Glo-I and (R)AGE in chronic liver disease with key aspect to fibrosis and cirrhosis will be highlighted.

2. Development of chronic liver disease and cirrhosis

End stage liver diseases are mainly caused by viral hepatitis, alcoholism, nonalcoholic fatty liver disease or steatohepatitis (NAFLD/NASH) or rare autoimmune and hereditary disorders. Thereby, liver cirrhosis belongs to the global burden of diseases responsible for more than one million deaths p.a. [1]. Cirrhosis is characterized by altered liver anatomy and reduced liver function. Structural alterations comprise the appearance of regenerative nodules, hepatocyte ballooning, accumulation of fibrotic tissue, disturbed microcirculation, angiogenesis and sinusoidal collapse with defenestration and development of a basement membrane [2]. Beside of the reduced liver function, these pathological alterations lead to elevation of intrahepatic resistance indicated by increased portal pressure with development of ascites and esophageal varices [3,4]. Nevertheless, portal hypertension is being caused by both, structural alterations of liver microarchitecture and hepatic endothelial dysfunction. The latter is characterized by an imbalance of vasoactive components. In fact, there is an hyperresponsiveness and overproduction of vasoconstrictors (mainly endothelin-1 (ET-1)) and an hyporesponsiveness and reduction of vasodilators (mainly nitric oxide (NO)) in the vascular bed of the liver [5–7]. Despite this hypoactive endothelium in hepatic microcirculation, portal hypertension leads to arterial vasodilation, formation of collateral vessels and hyporesponsiveness to vasoconstrictors due to hyperactive endothelium in splanchnic and systemic circulation with increased NO production. Finally these alterations result in elevated blood flow to portal vein and a vicious circle of disease [8–11].

The underlining molecular mechanism for development of fibrosis, cirrhosis and portal hypertension have intensively investigated over the last years. Hepatic stellate cells (HSC) are the main driver for accumulation of fibrosis and increased intrahepatic vascular resistance. HSC are pericytes and are quiescent but became activated upon various stimuli and transform to myofibroblasts [12]. This activation process is a complex process involving parenchymal and nonparenchymal cells and triggered via inflammatory processes [13]. Direct deterioration of hepatocytes (HSC) result in the release of ROS, DNA and damage-associated molecular pattern (DAMP) leading to activation of Kupffer cells (KC) production of pro-inflammatory cytokines such as TNF-α and IL-6 as well as profibrotic factors [14–16]. As a consequence of these induced inflammatory processes, activated KC stimulate HSC subsequently leading to production and deposition of collagen [17]. This stimulation can be effected directly by the deleterious agent [18] or via TGF-β dependent mechanisms [19] leading to secretion of TNF-α, IL-6, TIMP-1, MCP-1, collagen-I and α-SMA [20–22]. As mentioned above, pro-inflammatory factors (TNF-α, IL-1β, IL-6) are also involved in the activation of HSC. In this regard activation of

rho kinase, transcription factor NF-κB and subsequent overexpression of pro-inflammatory cytokines are important pathways [23–25].

Another important key player in development of fibrosis are liver sinusoidal endothelial cells (LSEC). They form the first line of defense protecting the liver from injury. Inflammation by LPS or ROS resulted in dysfunction of LSEC [26]. In this regard, disturbed regulation of NO-production in cirrhosis depends on activity of endothelial NO-Synthase (eNOS) and increased degradation due to phosphodiesterases, i.e. PDE-5 [27]. Although eNOS expression is upregulated in sinusoidal area in cirrhosis, eNOS activity has been shown to be reduced by caveolin-eNOS-binding [28] and was diminished by several post-translational modifications of the endothelial nitric oxide synthethase (eNOS) [9]. In contrast, in splanchnic circulation eNOS is upregulated [9] with increased enzyme activity in portal hypertension and regulated by phosphorylation of protein kinase B (Akt) [29]. Finally, all these alterations result in a hyperdynamic circulation with elevated blood flow to portal vein and further increase of portal pressure [8–10].

In a conclusion, cirrhosis demonstrates the end stage of liver disease with disturbed liver architecture and impaired liver function. Generation of ROS and stimulation of various inflammatory pathways are a critical step in activation of HSC as the main driver for fibrosis. Despite these findings, the use of antioxidants (vitamin E, N-acetylcysteine, coenzyme Q and others) in patients with alcoholic liver disease have failed to show an efficacy in improving disease conditions [30–32].

3. Glyoxalase-system

An important component in regulation and formation of ROS and oxidative stress comprises the glyoxalase-system. This enzymatic system was discovered firstly in 1913 from the groups of Dakin / Dudley and Neuberg [33]. The glyoxalase-system constitutes of two cytosolic enzymes, glyoxalase-I (Glo-I, EC 4.4.1.5) and glyoxalase-II (Glo-II, EC 3.1.2.6.). Glo-I catalyzes the conversion of α-oxo-aldehydes like methylglyoxal (MGO) into the hemithioacethal S-D-Lactoylglutathion using L-glutathione (GSH) as cofactor. Further substrates of Glo-I are hydroxypyruvaldehyde, hydroxypyruvataldehydphosphate, glyoxal, phenylglyoxal, 4,5-dioxovalerate, alkyl- and arylglyoxales [34–37]. Glo-II hydrolyses the reaction of S-D-Lactoylglutathion to H_2O and D-lactate with regeneration of GSH (fig. 1). Thereby demonstrates Glo-I rate limiting step [36,38].

The glyoxalase enzymes are ubiquitary found in all animate beings and are mainly located in the cytosol and partly in mitochondria [39]. Their predominant cellular function is the detoxification of MGO. MGO is the main subtrate of Glo-I [40] and demonstrates a reactive carbonyl compound that is formed as a by-product in glycolysis [41], ketone body metabolism and threonine catabolism [42–44]. MGO could regulate cellular processes in under physiological conditions [45,46] but leads to cell cytotoxicity in high concentrations through reaction with nucleotids, phospholipids and proteins [47,48] resulting in formation of „advanced glycation endproducts“ (AGEs). MGO lead to production of ROS via AGEs and binding to their receptor RAGE or non-enzymatic via direct reaction with hydrogen peroxide [49].

Important MGO-derived AGEs are the non-fluorescent products 5-hydro-5-methylimidazolone (MG-H1) and tetrahydropyrimidine (THP) as well as the fluorescent product argpyrimidine [50,51]. Other non-MGO-derived AGEs comprise N^{ε}-carboxymethyllysine (CML), pyrraline or pentosidine [52]. The effects of AGEs are mediated by their receptor system, which could be generally divided into two categories. The receptor for AGEs (RAGE) facilitates generation of ROS, inflammation,

angiogenesis and proliferation [53,54]. In contrast, AGE receptors (AGE-Rs), for instance AGE-R1, are responsible for detoxification and clearance of AGEs [55]. Upon binding of AGEs to RAGE various signal transduction pathways are activated. Recent studies showed involvement of the extracellular signal-regulated kinase 1/2 (ERK1/2), phosphoinositide 3-kinase (PI3-K) / protein kinase B (AKT), Januskinase 2 (JAK2) and RhoGTPases finally resulting in activation of NF-κB and production of pro-inflammatory cytokines (see fig. 2) [56]. In addition, stimulation of RAGE resulted in activation of transforming growth factor (TGF-β) pathway and induces vascular endothelial growth factor (VEGF) overexpression [54].

Glo-I is a dimer and consists in mammalian of two identical subunits with a molecular mass of 43-48 kDa [57]. Each subunit contains an zinc ion into its active center, whereas the apoenzyme remains catalytically inactive [40,58]. Spatial analyses revealed octahedral arrangement of Glo-I [51,59]. Protein sequence of Glo-I consists of 184 amino acids with posttranslational modification of n-terminal met [59]. Furthermore, association of distinct Glo-I phenotypes and Glo-I SNPs with diabetes [60], cardiovascular diseases [61] schizophrenia [62], autism [63,64], anxiety [65] and cancer [66,67] was observed. These findings led to preliminary anti-tumor effects of Glo-I inhibition by siRNA or enzymatic inhibition in different cancer models [68–71] and an Glo-I inducer formula showed improved glycemic control and vascular function in 29 obese patients [72]. Furthermore, several anti-inflammatory and antitumor agents showed inhibitory effects to Glo-I, e.g. S-ρ-bromobenzylglutathione or S-ρ-bromobenzylglutathionecyclopentyldiester [69,73], methotrexate [74], indomethacin [75], troglitazone [76] and flavanoids [77,78].

Glo-II is a monomer with two different domains and a molecular mass between 18 und 29 kDa [79]. Gene locus of Glo-II is determined on chromosome 16 [80] with expression of only one phenotype [81]. The function of Glo-II is conversion of S-D-Lactoylglutathion to D-lactate and regeneration of glutathione.

In summary, the glyoxalase system is essential for detoxification of MGO to prevent formation of AGEs and oxidative stress and is involved in different pathophysiological inflammatory processes.

4. Glo-I/AGE/RAGE in fibrosis, cirrhosis and NAFLD/NASH

4.1.Glo-I:

Despite of the essential role of Glo-I for prevention of MGO-induced inflammation, data about Glo-I in fibrosis, cirrhosis or non-alcoholic fatty liver disease (NAFLD/NASH) are lacking. Therefore, our group analyzed Glo-I in a CCl_4-model of cirrhosis [82,82]. Wistar rats were treated with inhalative CCl_4 three times a week to induce early cirrhosis without ascites after 8 weeks or advanced cirrhosis with ascites after 12 weeks. Furthermore, we isolated primary liver cells from cirrhotic and noncirrhotic livers by means of portal vein perfusion and analyzed Glo-I. Finally, we determined the effect of Glo-I enzyme modulation via ethyl pyruvate (EP, see below) *in vivo*. Glo-I could be detected in HEP, HSC and LSEC with highest expression on protein and mRNA levels in HEP. In CCl_4-model of cirrhosis, Glo-I expression was reduced in early and advanced cirrhosis in both, whole liver and primary liver cells on protein and mRNA levels (fig. 3 A). We observed a greater reduction of Glo-I with increasing severity of liver disease (8 weeks vs. 12 weeks CCl_4-treatment). Therefore, we hypothesized that reduced expression of Glo-I is accompanied by elevated levels of MGO, as mentioned before [69]. Indeed, our analysis revealed significantly elevated levels of MGO in cirrhosis measured via ELISA (fig. 3 B). Furthermore, we could show that, beside of reduction of Glo-I, advanced cirrhosis showed elevation of RAGE (“Author” et al., AASLD

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To get further insights in participation of Glo-I in cirrhosis we performed *in vitro* and *in vivo* experiments with the anti-inflammatory drug EP modulating Glo-I. EP is an α-oxo-carbonic acid and ester of pyruvate. EP was used due to anti-inflammatory effects of pyruvate but low stability in aqueous solution [83]. Therefore, EP constitutes a more stable compound and exert anti-inflammatory and protective effects in ROS-mediated models of ischemia and reperfusion [84–86], hemorrhagic shock [87], septic shock [88,89], cecal ligation and perforation [90], acute renal failure [91,92], pancreatitis [93–96], thermal injury [97], brain injury [98–102], cardiac injury [103,104], retinal damage, uveitis and cataract [105–110]. Furthermore, effect of EP on RAGE was analyzed in several studies showing reduction of RAGE expression upon EP treatment [111,112]. The molecular basis for the reduced production of TNF-α, IL-6, HMGB1, iNOS and NO as well as the prolonged survival in animals treated with EP was not fully elucidated. However, our former work demonstrated EP as an inhibitor of specific Glo-I activity providing a new mechanism for anti-inflammatory effects of EP [113]. Since EP showed protective effects in acute liver failure [114–117] and development of fatty liver [118] we analyzed effect of EP on activation of HSC stimulated with LPS, as it might occur in an initial stadium of cirrhosis. Stimulation of HSC with LPS for 24h led to increased levels of α-SMA and collagen-I indicating activation of HSC and production of collagen deposit. This stimulation could be abrogated by modulation of Glo-I activity by means of EP. Underlining mechanisms involve stimulation of Nrf2 as well as reduction of NF-κB and ERK/pERK by EP [82]. Furthermore, we used EP *in vivo*: Wistar rats were treated with CCl_4 for 12 weeks and i.p.-injected either with 40mg/kg b.w. EP or saline. After 12 weeks livers were stained with Sirius red indicating collagen deposit. EP treated rats revealed significantly less Sirius red staining and consecutive less fibrosis (fig. 3 C).

In summary, targeting Glo-I with EP in cirrhosis demonstrated a promising therapeutic option and offers and innovative target in liver disease induced by oxidative stress (see fig. 2).

4.2.AGEs:

The demonstrated role of Glo-I, AGEs and RAGE for inflammatory processes also would suggest an involvement of AGEs in inflammatory liver disease. Several groups analyzed AGEs in liver fibrosis, cirrhosis and NASH. Ahmed et al. examined protein glycation, oxidation and nitrosation marker residues as well as free adducts in portal, hepatic and peripheral venous blood plasma of cirrhotic patients. They found elevated extraction of methylglyoxal-modified proteins in cirrhotic subjects compared to controls [119]. These findings were supported by another work measuring levels of AGEs in blood plasma of cirrhotic patients. Significantly elevated concentrations of fluorescent AGEs and CML were found in cirrhosis. Also, CML levels correlated with severity of disease [120]. In addition, Yagmur et al. found increased concentrations of CML in fibrosis and cirrhosis [121] and AGEs measured by fluorescence spectroscopy were also significantly elevated in cirrhosis compared to controls [122]. On the other hand, *in vitro* treatment of HSC with AGEs resulted in enhanced production of oxidative stress providing evidence of AGEs-involvement in fibrosis [123]. Conversely, oxidative stress was found to elevate levels of CML in rats [124]. Thereby, incubation of HSC with AGEs led to elevation of α-SMA, TGF-β and collagen-I [125]. In addition, treatment of rat hepatocyte cultures with AGEs resulted in reduced cell viability and administration of ethanol to Wistar rats led to elevated levels of AGEs in rat livers [126]. In a translational study, a positive correlation of CML-AGEs with liver stiffness as indicator for fibrosis in patients with chronic hepatitis C was found (r=0.5731,

p<0.001). *In vitro* data revealed in this work enhanced cell proliferation of HSC treated with BSA-AGEs (CML) and increased production of α-SMA. Furthermore, AGEs were found to induce autophagy which subsequently contributes to the fibrosis in patients with chronic hepatitis C [127]. These results were supported by the finding, that inhibition of CML resulted in attenuation of CML-induced levels of α-SMA and ROS in HSC [128]. In contrast, in another study intraperitoneally administration of AGE-rat serum albumin (CML) revealed increased levels of α-SMA without influence on fibrosis. However, additional administration of AGE-rat serum albumin to rats underwent bile-duct ligation for induction of fibrosis showed increased hydroxyproline, Sirius red content and α-SMA indicating elevated fibrosis [129].

AGEs have also been implicated in fibrosis in models of NASH. Hepatic steatosis showed accumulation of CML and CML was associated with grade of hepatic inflammation and gene expression of inflammatory markers (PAI-1, IL-8 and CRP) [130], AGEs have also been shown to be involved in etiology of insulin resistance and diabetes, which are risk factors for development of NAFLD [131]. Also, rats fed with a diet rich in AGEs showed elevated oxidative stress and hepatic inflammation leading to NASH [132]. Additionally, high dietary AGEs increased hepatic AGEs levels and induced liver injury, inflammation and liver fibrosis via oxidative stress in activated HSC [133]. Recently, the molecular basis for involvement of AGEs in NASH was discovered. AGEs induce NOX2 leading to downregulation of Sirt1/Timp3 finally resulting in activation of TNF-α converting enzyme and inflammation [134].

Having the growing evidence of AGEs in fibrosis and chronic liver disease in mind, several studies analyzed the effect of AGEs-reduction on inflammation and fibrosis in NASH. Tang et al. found that the anti-inflammatory drug curcumin eliminated the effects of AGEs in HSC by interrupting leptin signaling and activating transcription factor Nrf2, which led to the elevation of cellular glutathione and the attenuation of oxidative stress [135]. Also, curcumin showed reduced AGEs-induced activation and proliferation of HSC and induced gene expression of AGE-clearing receptor AGE-R1 [136]. In another study the LDL-lowering drug atorvastatin decreased levels of AGEs in patients with NASH and dyslipidemia leading to improve of steatosis and nonalcoholic fatty liver disease activity score [137]. Miura et al. could further show, that combination therapy of telmisartan and nateglinide reduced levels of AGEs in rats leading to amelioration of insulin resistance [138]. Another approach evaluated effects of aqueous extracts from Solanum nigrum (AESN). AESN could reduce the AGE-induced expression of collagen-II, MMP-2 and α-SMA in HSC. Also, AESN improved insulin resistance and hyperinsulinemia and downregulated lipogenesis finally prevention fibrosis [139].

Although the results of the aforementioned studies giving evidence for involvement of MGO-related AGEs in chronic liver disease, mainly CML was investigated. Therefore, it should be considered that CML-AGEs are rarely produced via reaction of MGO and are more likely to be formed in lipoxidation and glycoxidation independent of MGO [140].

4.3.RAGE:

RAGE is a pattern recognition multi-ligand cell surface receptor that belongs to the immunoglobulin superfamily with a molecular mass of 47 to 55 kDa. RAGE expression is usually low but elevated under inflammatory conditions such as diabetes, cardiovascular diseases or cancer [141]. RAGE has been shown to be activated by MGO- and non-MGO-derived AGEs and activation of RAGE leads to intracellular signaling cascades resulting in inflammation, proliferation and angiogenesis mediated by NF-κB [142]. Several studies

analyzed relevance of RAGE-activation in fibrosis: Goodwin et al. generated AGE-rat serum albumin (mainly CML) and illustrated that treatment with AGE-rat serum albumin resulted in raised oxidative stress. Interestingly, levels of RAGE, α-SMA, hydroxyproline and Sirius red (indication of fibrosis by the latter three) were stimulated in a fibrosis model of bile-duct ligation (BDL) if the animals receive additional AGE-rat serum albumin [129]. Another study confirmed predominant expression of RAGE in HSC. RAGE was stimulated in HSC during transformation to myofibroblasts and RAGE was colocalized with α-SMA and induced by TGF-β. RAGE was predominantly found in filopodial membranes of myofibroblasts suggesting a role of RAGE in spreading and migration of activated HSC in fibrogenesis [143]. Also, elevated expression of RAGE was confirmed in activated HSC and LSEC in a fibrosis model of bile-duct-ligation. RAGE-expression significantly raised through AGE-serum albumin and TNF-α but did not alter HSC proliferation, apoptosis or fibrosis signal transduction [144]. Serban et al. further analyzed regulation and crosstalk of RAGE in fibrosis. They found that AGEs-induced RAGE upregulation resulted in induction of TGF-β, TNF-α and IL-8. Furthermore, it was propagated that there is an inhibitory crosstalk between TGF-β and RAGE since RAGE also stimulated the anti-inflammatory cytokines IL-2 and IL-4 [145].

To further analyze role of RAGE in fibrosis, recent studies investigated effect of RAGE inhibition. Firstly, the anti-inflammatory drug curcumin (also reducing AGEs, see above) inhibited the AGEs-induced gene expression of RAGE via elevation of PPAR-γ [146]. Furthermore, RAGE expression was diminished by means of RAGE siRNA in primary rat HSC resulting in downregulation of IL-6, TNF-α and TGF-β [147]. The authors of the latter study conducted a subsequent *in vivo* approach analyzing effect of RAGE siRNA in an olive-oil model of fibrosis. RAGE siRNA was injected twice weekly in the tail vein of Sprague-Dawley rats. After six weeks reduced expressions of RAGE, TNF-α, IL-6, extracellular matrix, hyaluronic acid and procollagen III were found. Also, activation of HSC and NF-κB was reduced in siRNA treated animals attenuating the initiation and progress of fibrosis [148]. Additional studies revealed protective effects of anti-RAGE antibodies in BDL-induced acute liver injury [149,150].

Beside it's implication in BDL- and pharmacological models of fibrosis, RAGE has been involved in development of NAFLD. Methionine cholin deficient (MCD) diet caused steatosis and significantly increased RAGE, pro-inflammatory cytokines and fibrosis [133]. Recently, fatty acids stimulated CML accumulation and subsequently elicit RAGE induction [130]. Another group found upregulation of RAGE in the liver of aged mice with consecutive elevated oxidative stress shown by analysis of malondialdehyde. Blocking of RAGE by anti-RAGE-antibody revealed in this work prolonged survival of animals [151].

These findings suggest that activation of RAGE is a major driver for fibrosis and inhibition of RAGE could prevent initiation and progress of extracellular matrix deposition.

5. Glo-I/AGE/RAGE in HCC

Hepatocellular carcinoma (HCC) constitutes the sixth most cancer disease and third most cause of cancer-related mortality [152]. About 90% of HCC base upon development of cirrhosis, therefore cirrhosis demonstrated the most important risk factor for HCC [153]. During tumorigenesis, dysregulation of cell proliferation, invasion, metastasis and angiogenesis occur. These alterations were indicated, amongst others, by elevated expression of transcription factors IGF and FGF [154], Snail [155], PDGF [156] and VEGF [157].

Recent work analyzed role of Glo-I in

hepatocellular carcinoma (HCC). Glo-I mRNA was upregulated in HCC tissue and Glo-I siRNA knockdown resulted in reduced proliferation of Hep3B, SK-HEP-1 and SMMC-7721 HCC cell lines and was accompanied by elevated levels of MGO [158]. Another study revealed genetic amplification and upregulation of Glo-I. Knockdown of Glo-I by means of sh-RNA led to inhibition of tumor growth and induction of apoptosis in primarily cultured HCC [159]. Furthermore effects of the Glo-I modulator EP on HCC we studied. EP treatment on SMMC-7721, HepG2, and HCC-LM3 cell lines showed reduced proliferation indicated by MTT assay and induced apoptosis in flow cytometry and TUNEL assay. EP also reduced tumor volume in xenograft model and lowered levels of HMGB1, RAGE, MMP9 and Akt [160]. Nevertheless, distinct role of Glo-I in HCC remains preliminary and need to be confirmed in additional studies.

In contrast, AGEs and RAGE have intensively studied in HCC: serum levels of AGEs were found to be raised in patients with HCC without hepatitis B or C infection. AGEs were significantly higher in HCC patients compared with NASH and control subjects (9.1±2.7, 5.2±1.7, 3.5±1.2 U/ml. $p<0.05$) [161]. Furthermore, levels of the soluble form of RAGE (sRAGE) were shown to predict tumor progression in HCC patients undergoing transarterial chemoemobilisation (TACE) in a first proof-of-concept study [162]. Another translational study confirmed overexpression of RAGE and sRAGE in HCC in a small cohort of 10 patients and showed reduced cellular growth and DNA synthesis upon RAGE knockdown by means of siRNA. Also, stimulation of RAGE with the ligand HMGB1 induced cell proliferation and activation of NF-κB in Huh7 cells [163]. Several further studies showed importance of RAGE for proliferation [164], angiogenesis [165] and invasion [166] of HCC and confirmed reduced tumor growth by means of RAGE inhibition [167,168]. In contrast, in a case-control-cohort mainly in hepatitis-related HCC, levels of sRAGE and CML-AGEs were inversely associated with HCC [169]. This study showed some limitations, mainly men and smokers were included. Nevertheless, further analysis particularly in larger populations is necessary.

In a conclusion, Glo-I is responsible for detoxification of MGO and reveals essential role for prevention of MGO-induces oxidative stress through formation of AGEs and binding to RAGE. Recent work highlighted beside importance of RAGE and AGEs in fibrosis and HCC the role of Glo-I cirrhosis. In this regard, the Glo-I/AGE/RAGE system indicates an innovative and promising target in developing cirrhosis and chronic liver disease.

Title and legends to all figures

Figure 1: Glyoxalase-system.

Glyoxalase I and glyoxalase II comprise the glyoxalase system for detoxification of MGO. Glutathione is necessary as cofactor and is regenerated by Glo-II. Adapted from [37].

Figure 2: Impact of Glo-I and (R)AGE in cirrhosis.

MGO reacts with proteins, nucleotids and lipids leading to formation of AGEs. AGEs bind to RAGE and activate several signal pathways (including MAPK (ERK1/2, p38, JNK), PI3-K/AKT and JAK2/STAT1) finally leading to activation of NF-κB. In a consequence, the induced production of TGF-β and pro-inflammatory cytokines activate quiescent stellate cells. HSC transform to myofibroblasts and produce profibrotic factors and collagen. The collagen deposition in the liver will lead to fibrosis and finally cirrhosis. Reduction of Glo-I will perpetuate both, initiation and progression of cirrhosis due to increase of MGO and a vicious circle of disease. MGO: methylglyoxal. AGEs: advanced glycation endproducts. RAGE: receptor for advanced glycation endproducts. Glo-I: glyoxalase-I. HSC: hepatic stellate cells. MAPK: mitogen-activated protein kinase. PI3-K: phosphoinositide 3-kinase. AKT: protein kinase B. JAK2: Januskinase 2. STAT1: signal transducer and activator of transcription-1. JNK: c-Jun N-terminal kinase: NF-κB: nuclear factor-κB.

Figure 3: Glyoxalase-I in CCl_4-induced cirrhosis.

A, Glo-I expression was reduced in early (8 wk CCl_4-treatment) and advanced (12 wk CCl_4-treatment) cirrhosis in Western blot. Wistar rats were treated three times per week with inhalative CCl_4 for induction of cirrhosis. **B**, MGO levels were significantly elevated in cirrhosis, indicated by ELISA-analysis. **C**, Wistar rats were treated with CCl_4 and i.p. EP or saline from week 8-12. Sirius red staining indicated significantly less fibrosis in EP treated animals. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Adapted from [82].

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Figures

Figure 1: Glyoxalase-system.

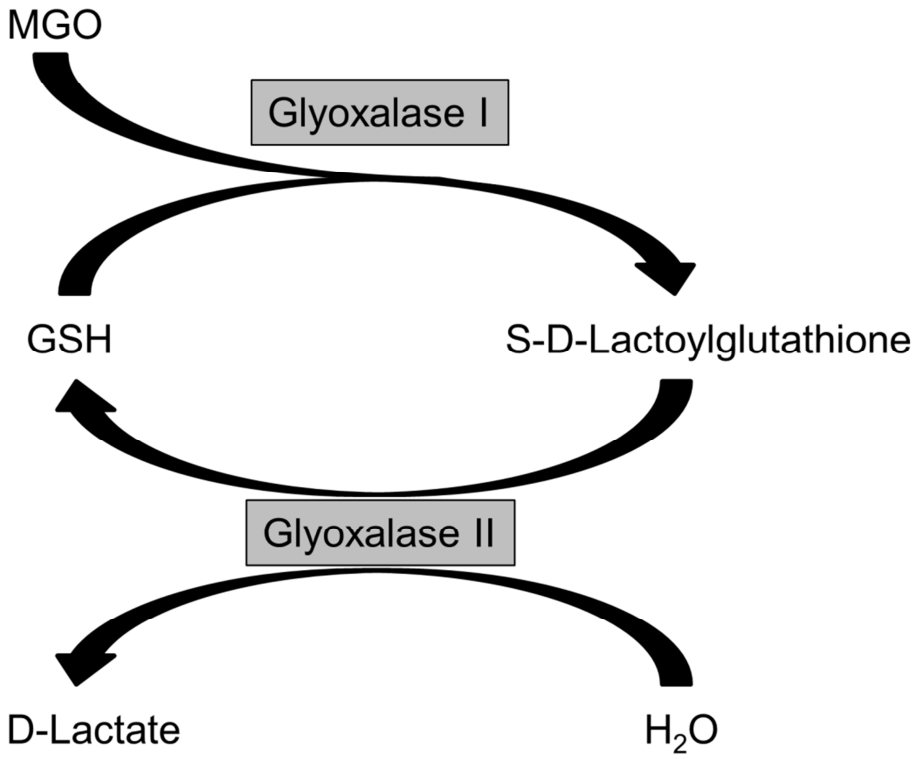

Figure 2: Impact of Glo-I and (R)AGE in cirrhosis.

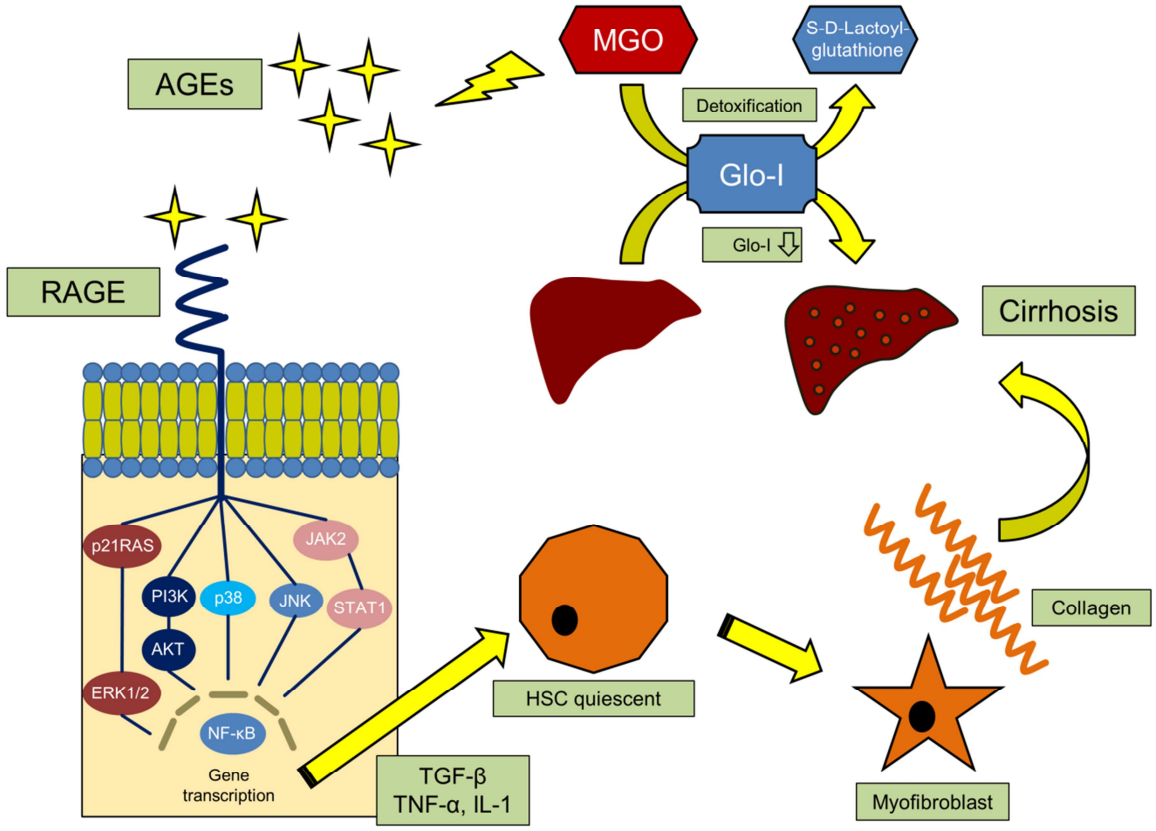

Figure 3: Glyoxalase-I in CCl_4-induced cirrhosis.