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# Impact of Glyoxalase-I (Glo-I) and Advanced Glycation End Products (AGEs) in Chronic Liver Disease

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## Abstract

Inflammation caused by oxidative stress (ROS) is a main driver for development of chronic inflammatory liver disease leading to fibrosis and cirrhosis. An important source of ROS constitutes methylglyoxal (MGO). MGO is formed as a by-product in glycolysis, threonine catabolism, and ketone bodies pathway leading to formation of advanced glycation end products (AGEs). AGEs bind to their receptor for AGEs (RAGE) and activate intracellular transcription factors, such as nuclear factor- $\kappa$ B (NF- $\kappa$ B), resulting in production of pro-inflammatory cytokines and ROS. The enzymes glyoxalase-I (Glo-I) and glyoxalase-II (Glo-II) form the glyoxalase system and are essential for the detoxification of methylglyoxal (MGO). This chapter highlights Glo-I and (R)AGE in chronic liver disease with focus on fibrosis and cirrhosis. AGEs and RAGE have been shown to be upregulated in fibrosis, and silencing of RAGE reduced the latter. In contrast, recent study highlighted reduced expression of Glo-I in cirrhosis with consecutive elevation of MGO and oxidative stress. Interestingly, modulation of Glo-I activity by ethyl pyruvate resulted in reduced activation of hepatic stellate cells and reduced fibrosis in CCl<sub>4</sub> model of cirrhosis. In conclusion, Glo-I and R(AGE) are important components in development and progression of chronic liver disease and constitute interesting therapeutic target.

**Keywords:** ethyl pyruvate cirrhosis, fibrosis, methylglyoxal, AGEs

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## 1. Introduction

Oxidate stress (reactive oxygen species, ROS) with consecutive and repetitive inflammation is responsible for development of chronic liver disease. Different etiologies of liver disease lead to damage of hepatocytes, release of pro-inflammatory cytokines, and finally activation

of hepatic stellate cells (HSC). Activated HSC transform to myofibroblasts and lead to deposition of collagen, which in turn result in fibrosis and finally cirrhosis. Several molecular mechanisms are involved in this complex interplay, nevertheless the critical step is the activation of HSC by ROS. This chapter focuses on the glyoxalase-I (Glo-I) and related advanced glycation end products (AGEs) with their receptor for AGEs (RAGE) playing an important role in generation and detoxification of ROS. Current knowledge of Glo-I and (R)AGE in chronic liver disease with key aspect to fibrosis and cirrhosis will be highlighted.

## 2. Pathogenesis of fibrosis 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. The followed repetitive liver injury caused inflammation, finally resulting in fibrosis and irreversible cirrhosis. Thereby, liver cirrhosis belongs to the global burden of disease responsible for more than one million deaths p.a. [1]. In cirrhosis, altered liver anatomy and reduced liver function are pathognomonic. Development of cirrhosis is characterized by 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]. These alterations of liver architecture lead to reduced liver function and elevation of intrahepatic resistance demonstrated 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 underlying molecular mechanism for development of fibrosis, cirrhosis, and portal hypertension has been intensively investigated over the last decades. Since the liver is formed by parenchymal cells (mainly hepatocytes (HEP)) and nonparenchymal cells (Kupffer cells (KC), hepatic stellate cells (HSC), and liver sinusoidal endothelial cells (LSEC)), both are involved in the development of fibrosis and cirrhosis. Nevertheless, HSC are the main cell type responsible for accumulation of fibrosis and increased intrahepatic vascular resistance. HSC are pericytes surrounding the sinusoids in the space of Disse. HSC are quiescent but became activated upon various stimuli and transform to myofibroblasts [12]. This activation process is a complex interplay between parenchymal and nonparenchymal cells and triggered via inflammatory processes [13]. For instance, deleterious agents (alcohol, LPS) have direct hepatotoxic effects to hepatocytes and trigger the production of reactive oxygen species (ROS). The release of ROS, DNA, and damage-associated molecular pattern (DAMP) leading

to activation of KC and innate immune system followed by subsequent production of pro-inflammatory cytokines such as TNF- $\alpha$  and IL-6 as well as pro-fibrotic factors [14–16]. Also, alcohol consumption increases permeability of the gut resulting in increased levels of portal endotoxins (LPS) with consecutive activation of KC resulting in liver injury and inflammation [17, 18]. Furthermore, inflammation triggers the classical complement pathway activation via C1q [19], followed by production of pro-inflammatory cytokines, and inhibits components of innate immune system. As a consequence of these induced inflammatory processes, activated KC stimulate HSC subsequently leading to fibrosis [20]. This stimulation can result directly by the deleterious agent [21] or via transforming growth factor beta (TGF- $\beta$ )-dependent mechanisms [22] leading to secretion of TNF- $\alpha$ , IL-6, TIMP-1, MCP-1, collagen-I, and  $\alpha$ -SMA [23–25] and finally collagen deposition.

As mentioned above, pro-inflammatory factors (TNF- $\alpha$ , IL-1 $\beta$ , IL-6) are also involved in the activation of HSC. In this regard, activation of the transcription factor nuclear factor- $\kappa$ B (NF- $\kappa$ B) and subsequent overexpression of pro-inflammatory cytokines are important pathways. NF- $\kappa$ B, thereby, is activated by growth factors, cytokines, bacterial and viral factors, and ROS and regulates by itself pro-inflammatory cytokines (like COX-2 or IL-6) [26, 27].

Beside the production of collagen and accumulation of fibrotic tissue, HSC are involved in increased intrahepatic vascular resistance not only via structural changes. Transformation of HSC to myofibroblasts was accompanied by stimulation of rho kinase leading to activation of contractile filaments of HSC and subsequently vasoconstriction of sinusoids [28].

Another key player in the development of fibrosis comprises LSEC. They form the first line of defense protecting the liver from injury. Inflammation by LPS or ROS resulted in dysfunction of LSEC [29] indicated by disturbed sinusoidal microcirculation, defenestration, hypoxia, and pathological angiogenesis [30]. In contrast, both direct deterioration of LSEC and vasoconstriction of HSC result in impaired release of vasodilators from LSEC leading to a vicious circle of disease. In this regard, disturbed regulation of NO production in cirrhosis depends on activity of endothelial NO synthase (eNOS) and increased degradation due to phosphodiesterases, that is, PDE-5 [31]. Although eNOS expression is upregulated in sinusoidal area in cirrhosis, eNOS activity has been shown to be reduced by caveolin-eNOS binding [32] and was diminished by several post-translational modifications of the endothelial NO synthase (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) [33]. Beside upregulation of eNOS, production of NO is also related to induction of the inducible form of the NO synthase, iNOS. iNOS is mainly stimulated by the presence of endotoxin and pro-inflammatory cytokines, all of whom occur in development of cirrhosis [34]. Indeed, recent study showed stimulation of iNOS rather than eNOS in splanchnic circulation by LPS, indicating an important role of iNOS in portal hypertension after bacterial translocation to mesenteric vessels [35]. Finally, all these alterations result in a hyperdynamic circulation with elevated blood flow to portal vein and further increase of portal pressure [8–10].

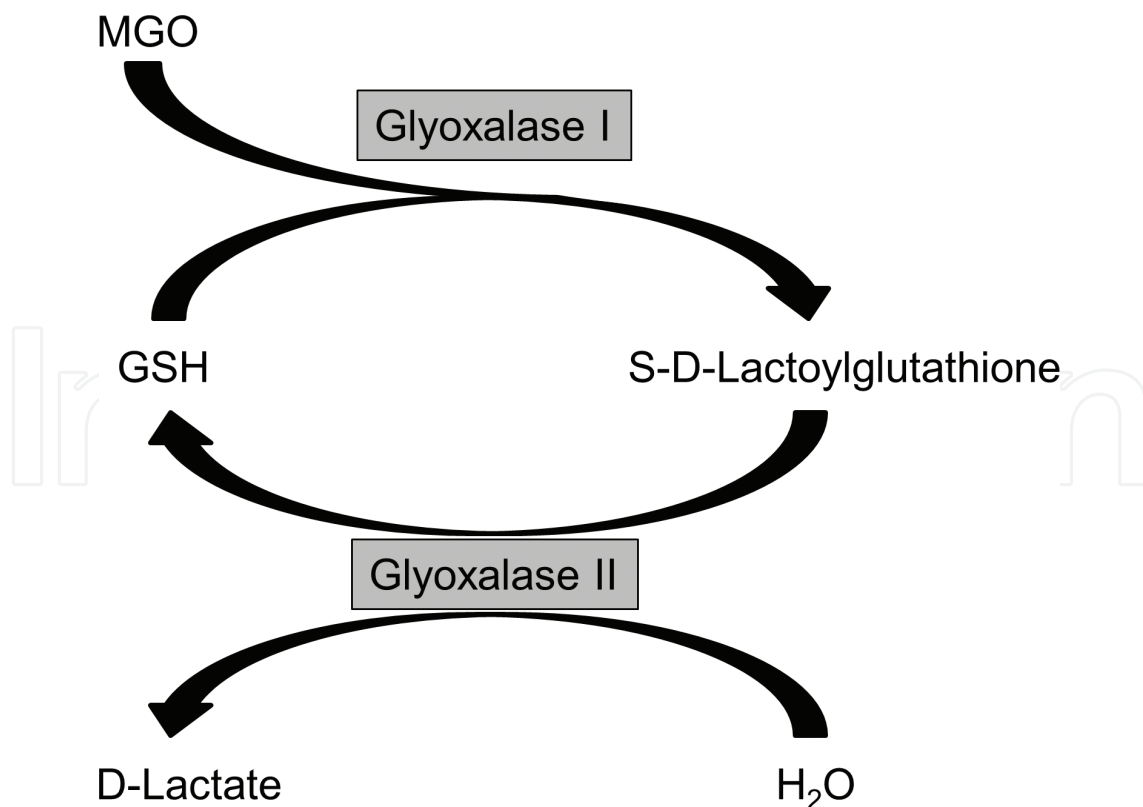
In conclusion, cirrhosis demonstrates the end stage of liver disease with disturbed liver architecture and impaired liver function. Generation of ROS and stimulation of various inflammatory pathways are critical steps in activation of HSC as the main driver for fibrosis. Despite

these findings, the use of antioxidants (vitamin E, N-acetylcysteine, coenzyme Q, and others) in patients with alcoholic liver disease has failed to show an efficacy in improving disease conditions [36–38].

3. Glyoxalase system and R(AGE)

An important role in regulation and formation of ROS and oxidative stress comprises the glyoxalase system. This enzymatic system was first discovered in 1913 [39] and constitutes two cytosolic enzymes, glyoxalase-I (Glo-I, EC 4.4.1.5) and glyoxalase-II (Glo-II, EC 3.1.2.6.). Glo-I is responsible for the catalytic conversion of  $\alpha$ -oxo aldehydes, for instance, methylglyoxal (MGO), into the hemithioacetal S-D-Lactoylglutathione using L-glutathione (GSH) as a cofactor. Further substrates of Glo-I are hydroxypyruvaldehyde, hydroxypyruvate aldehyde phosphate, glyoxal, phenylglyoxal, 4,5-dioxovalerate, alkyl and arylglyoxales [40–43]. Glo-II hydrolyzes the reaction of S-D-Lactoylglutathione to H<sub>2</sub>O and D-lactate with regeneration of GSH (**Figure 1**). Thereby, Glo-I demonstrates the rate limiting step [42, 44], and Glo-II is of subordinate interest in inflammatory research.

MGO is the main substrate of Glo-I [45] and has been described as a reactive carbonyl compound that is formed as a by-product in glycolysis [46], ketone body metabolism, and threonine catabolism [47–49]. MGO leads to cell cytotoxicity in high concentrations through



**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 [43].



reaction with nucleotides, phospholipids, and proteins [50, 51], resulting in the formation of “advanced glycation end products (AGEs)” and reactive oxygen species (ROS) via AGEs or non-enzymatic reaction with hydrogen peroxide [52]. In this regard, MGO has shown to be involved in various inflammatory processes such as diabetes, aging, renal insufficiency, hypertension, or cancer [60–64].

Important MGO-derived AGEs are the non-fluorescent products 5-hydro-5-methylimidazole (MG-H1) and tetrahydropyrimidine (THP) as well as the major fluorescent product, argpyrimidine [53, 54]. Other non-MGO-derived AGEs comprise N<sup>ε</sup>-carboxymethyllysine (CML), pyrroline, or pentosidine [55]. The effects of AGEs have been allocated to their antagonistic receptor systems. The receptor for AGEs (RAGE) mediates generation of ROS, inflammation, angiogenesis, and proliferation [56, 57]. In contrast, AGE receptors (AGE-Rs), for instance, AGE-R1, are responsible for detoxification and clearance of AGEs [58]. 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), Janus kinase 2 (JAK2), and Rho GTPases, finally resulting in activation of NF-κB and production of pro-inflammatory cytokines (**Figure 3**) [59]. In addition, stimulation of RAGE resulted in activation of transforming growth factor (TGF-β) pathway and induced vascular endothelial growth factor (VEGF) overexpression [57].

In the last years, structure and genomic sequence of Glo-I was intensively analyzed. Glo-I is a dimer and consists in mammalian of two identical subunits with a molecular mass of 43–48 kDa [60]. Each subunit contains a zinc ion in its active center, whereas the apoenzyme remains catalytically inactive [45, 61]. The active center of Glo-I is localized between both monomers and comprises two structurally equivalent residues from each domain (Gln-33A, Glu-99A, His-126B, Glu-172B) and two water molecules indicating an octahedral arrangement [54, 62]. The protein sequence of Glo-I consists of 184 amino acids with post-translational modification of N-terminal Met [62].

Genomic analysis revealed three distinct phenotypes of Glo-I: GLO 1-1, GLO 1-2, and GLO 2-2 representing homo- and heterozygous expression of *GLO*<sup>1</sup> und *GLO*<sup>2</sup> [63, 64]. Gene locus of Glo-I is determined on chromosome six between centromere and human leukocyte antigen (HLA)-DR gene [65, 66]. Demographic studies showed higher distribution of *GLO*<sup>1</sup> in Alaska and lower *GLO*<sup>1</sup> allocation in southern and eastern Europe, America, Africa, and India [67].

Genetic sequencing identified association of distinct Glo-I phenotypes and Glo-I SNPs with diabetes [68], cardiovascular diseases [69], schizophrenia [70], autism [71, 72], anxiety [73], and cancer [74, 75]. These findings led to preliminary anti-tumor effects of Glo-I inhibition by siRNA or enzymatic inhibition in different cancer models [76–79]. In this regard, well-studied Glo-I inhibitors are S-*q*-bromobenzylglutathione or S-*q*-bromobenzyl-glutathione cyclopentyl diester [77, 80], methotrexate [81], indomethacin [82], troglitazone [83], and flavonoids [84, 85] showing anti-inflammatory and anti-tumor effects. Furthermore, an Glo-I inducer led to improved glycemic control and vascular function in 29 obese patients [86].

In a nutshell, Glo-I is responsible for detoxification of MGO and prevention of MGO-related formation of AGEs and ROS. Therefore, Glo-I and (R)AGE are involved in different pathophysiological inflammatory processes.

## 4. Glo-I and R(AGE) in fibrosis, cirrhosis, and NAFLD/NASH

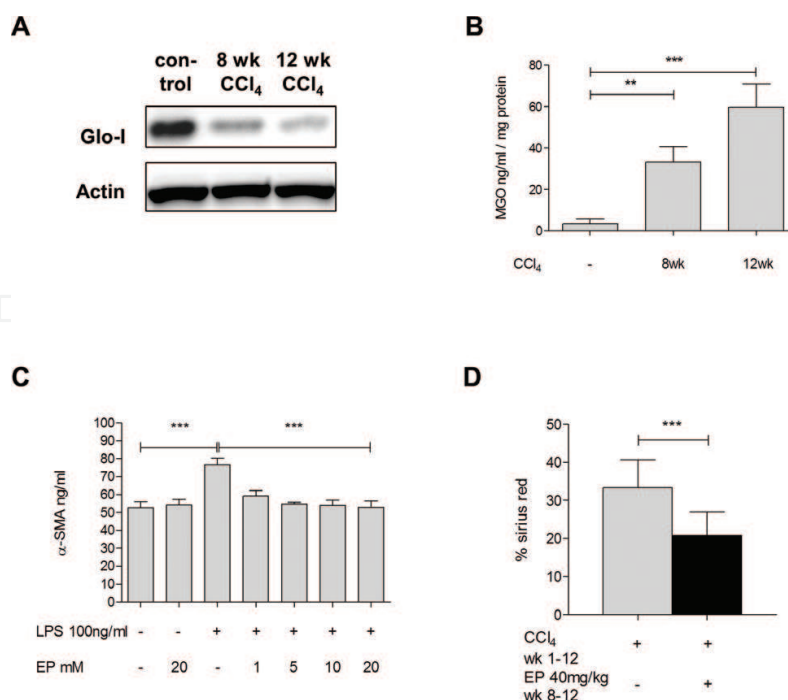
### 4.1. Glo-I

To date, although Glo-I revealed an important role in inflammation, data about Glo-I in chronic liver disease remain preliminary. In an experimental approach of CCl<sub>4</sub>-induced cirrhosis, Glo-I was analyzed *in vivo* and *in vitro* [87]. Wistar rats were treated with inhalative CCl<sub>4</sub> three times a week to induce early cirrhosis (without ascites) after 8 weeks or advanced cirrhosis (with ascites) after 12 weeks. Furthermore, primary liver cells from cirrhotic and noncirrhotic livers were isolated via portal vein perfusion and analysis of Glo-I was performed. Glo-I could be detected in HEP, HSC, and LSEC with highest expression on protein and mRNA levels in HEP. Furthermore, Glo-I expression was reduced in early and advanced cirrhosis in both whole liver and primary liver cells (**Figure 2A**). The reduction in Glo-I expression was greater with increasing severity of liver disease. Interestingly, the reduction of Glo-I was accompanied by an increase of MGO in cirrhosis (**Figure 2B**). This accumulation of MGO would lead to increased formation of AGEs and finally augment oxidative stress with ongoing inflammation in chronic liver disease [87]. So far, the reduction of Glo-I with consecutive increase of MGO would provide an explanation for perpetuating liver inflammation in advanced stages of liver disease.

Furthermore, modulation of Glo-I activity with the anti-inflammatory drug ethyl pyruvate (EP) was performed to analyze impact of Glo-I in initiation and progression of cirrhosis. EP is an  $\alpha$ -oxo-carbonic acid and ester of pyruvate. EP came in focus due to anti-inflammatory effects of pyruvate but low stability in aqueous solution [88]. Therefore, EP constitutes a more stable compound and exerts anti-inflammatory and protective effects in a lot of ROS-mediated models [89, 90]. Therefore, a possible molecular basis for the anti-inflammatory effects of EP was assumed to be the inhibition of specific Glo-I activity [91].

Since EP showed protective effects in acute liver failure [92–95] and development of fatty liver [96], effect of EP on activation of HSC, as it might occur in initial stadium of cirrhosis, was analyzed. Stimulation of HSC with LPS for 24 hours led to increased levels of  $\alpha$ -SMA, indicating activation of HSC and production of collagen deposit. This stimulation could be abrogated by modulation of Glo-I activity by means of EP (**Figure 2c**). Underlying mechanisms involve stimulation of Nrf2 as well as reduction of NF- $\kappa$ B and ERK/pERK by EP. Additional *in vivo* experiments revealed reduced collagen deposit in Wistar rats that were treated with CCl<sub>4</sub> for 12 weeks and i.p. EP [87]. Furthermore, EP-treated rats revealed significantly less Sirius red staining and consequently less fibrosis compared with controls receiving saline (**Figure 2D**).

Indeed, anti-inflammatory treatment of several diseases with EP might be a promising future clinical approach. However, EP was analyzed in a clinical trial (phase-II multicenter double-blind placebo-controlled study) in high-risk patients undergoing cardiac surgery with cardiopulmonary bypass. This trial was performed in 13 US hospitals including patients with



**Figure 2.** Glyoxalase-I in CCl<sub>4</sub>-induced cirrhosis. **(A)**, Glo-I expression was reduced in early (8 week CCl<sub>4</sub>-treatment) and advanced (12 week CCl<sub>4</sub>-treatment) cirrhosis in Western blot. Wistar rats were treated three times per week with inhalative CCl<sub>4</sub> for induction of cirrhosis. **(B)**, MGO levels were significantly elevated in cirrhosis, indicated by ELISA-analysis. **(C)**, treatment of stellate cells (HSC) for 24 hours with LPS revealed increased production of  $\alpha$ -SMA. Cotreatment with Glo-I modulator ethyl pyruvate (EP) abolished the LPS-induced effects. **(D)**, Wistar rats were treated with CCl<sub>4</sub> and i.p. EP or saline from week 8 to 12. Sirius red staining indicated significantly less fibrosis in EP-treated animals. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ . Adapted from [87].

a Parsonnet risk score  $> 15$  undergoing coronary artery bypass graft and/or cardiac valvular surgery with cardiopulmonary bypass. 102 subjects received either placebo (53) or 7.500 mg (90 mg/kg) EP (49) intravenously followed by five more doses every 6 hours. The primary endpoint was a combination of death, prolonged mechanical ventilation, renal failure, or need of vasoconstrictors. No statistically significant differences were observed between groups with regard to clinical parameters or markers of systemic inflammation [97]. Despite these disappointing results in the first clinical trial, it should be kept in mind that underlying molecular mechanisms in cardiac surgery with cardiopulmonary bypass are complex and at least partly different from ROS models showing protective effects of EP. Another clinical study design, for example, liver fibrosis, pancreatitis, septic shock, might be more promising for this interesting agent.

In summary, targeting Glo-I with EP in cirrhosis revealed an innovative therapeutic target. Nevertheless, further research needs to confirm the aforementioned results in further animal experiments and clinical trials.

## 4.2. AGEs

In contrast to straightforward evidence of Glo-I in chronic liver disease, several groups analyzed AGEs in liver fibrosis, cirrhosis, and NASH. In cirrhotic patients, limited amount of methylglyoxal-modified proteins were found to be elevated compared to controls [98].



Another study revealed increased levels of CML-AGEs in blood plasma of cirrhotic patients. Also, CML levels correlated with severity of disease [99]. Additional studies confirmed the observations of increased CML levels in fibrosis and cirrhosis [100, 101]. These clinical findings were supported by laboratory analysis: *in vitro* treatment of HSC with AGEs resulted in enhanced production of oxidative stress providing evidence of AGEs-involvement in fibrosis [102]. Conversely, oxidative stress was found to elevate levels of CML in rats [103] and incubation of HSC with AGEs led to elevation of  $\alpha$ -SMA, TGF- $\beta$ , and collagen-I [104]. In addition, treatment of rat hepatocyte cultures with AGEs reduced cell viability [105]. In an interesting translational study, CML-AGEs were positively correlated with liver stiffness in patients with chronic hepatitis C. *In vitro* data showed in this study enhanced cell proliferation of HSC treated with BSA-AGEs (CML) and increased production of  $\alpha$ -SMA. In contrast, in another study, intraperitoneal administration of AGE-rat serum albumin (CML) revealed increased levels of  $\alpha$ -SMA and fibrosis in a model of bile duct ligation [106]. Furthermore, AGEs were found to induce autophagy which subsequently contributes to the fibrosis in patients with chronic hepatitis C [107]. The finding that AGEs were elevated in fibrosis and treatment with AGEs-induced fibrosis led to an interventional approach targeting AGEs to prevent induction of chronic liver disease. Indeed, inhibition of CML resulted in attenuation of CML-induced levels of  $\alpha$ -SMA and ROS in HSC [108].

Another model to study fibrosis belongs to metabolic liver diseases: induction of NASH by means of methionine choline deficient diet (MCD). Therefore, hepatic steatosis induced by MCD 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) [109]. AGEs have also been shown to be involved in etiology of insulin resistance and diabetes [110], and rats fed with a diet rich in AGEs showed elevated oxidative stress and hepatic inflammation leading to NASH [111]. In addition, high dietary AGEs increased hepatic AGEs levels and induced liver injury, inflammation, and liver fibrosis via oxidative stress in activated HSC [112]. Another interesting study investigated the underlying mechanism of AGEs-crosstalk in NASH. AGEs induced NOX2 leading to downregulation of Sirt1/Timp3 and finally resulting in activation of TNF- $\alpha$  converting enzyme and inflammation. These pro-inflammatory cascades finally led to NASH and fibrosis [113]. Interventional studies on AGEs reduction in NASH also revealed promising results. The flavonoid curcumin eliminated the inflammatory effects of AGEs in HSC by interrupting leptin signaling and activating transcription factor Nrf2, which led to the elevation of cellular glutathione levels and the attenuation of oxidative stress [114]. In addition, curcumin decreased activation and proliferation of HSC by AGEs and induced gene expression of AGE-clearing receptor AGE-R1 [115]. The use of the LDL-lowering drug atorvastatin [116] or combination therapy of telmisartan and nateglinide [117] also decreased levels of AGEs in patients with NASH and dyslipidemia, leading to improvement of steatosis, nonalcoholic fatty liver disease activity score, and amelioration of insulin resistance. Another study evaluated effects of aqueous extracts from *Solanum nigrum* (AESN). AESN could reduce the AGE-induced expression of collagen-II, MMP-2, and  $\alpha$ -SMA in HSC. Also, AESN improved insulin resistance and hyperinsulinemia and downregulated lipogenesis, finally preventing fibrosis [118].

Having the auspicious and conclusive effects of AGEs-lowering drugs in fibrosis in mind, it should be noted that mainly CML-AGEs were investigated. Therefore, it should be considered

that CML-AGEs are rarely produced via reaction of MGO but are rather formed in lipoxidation and glycooxidation independent of MGO [119].

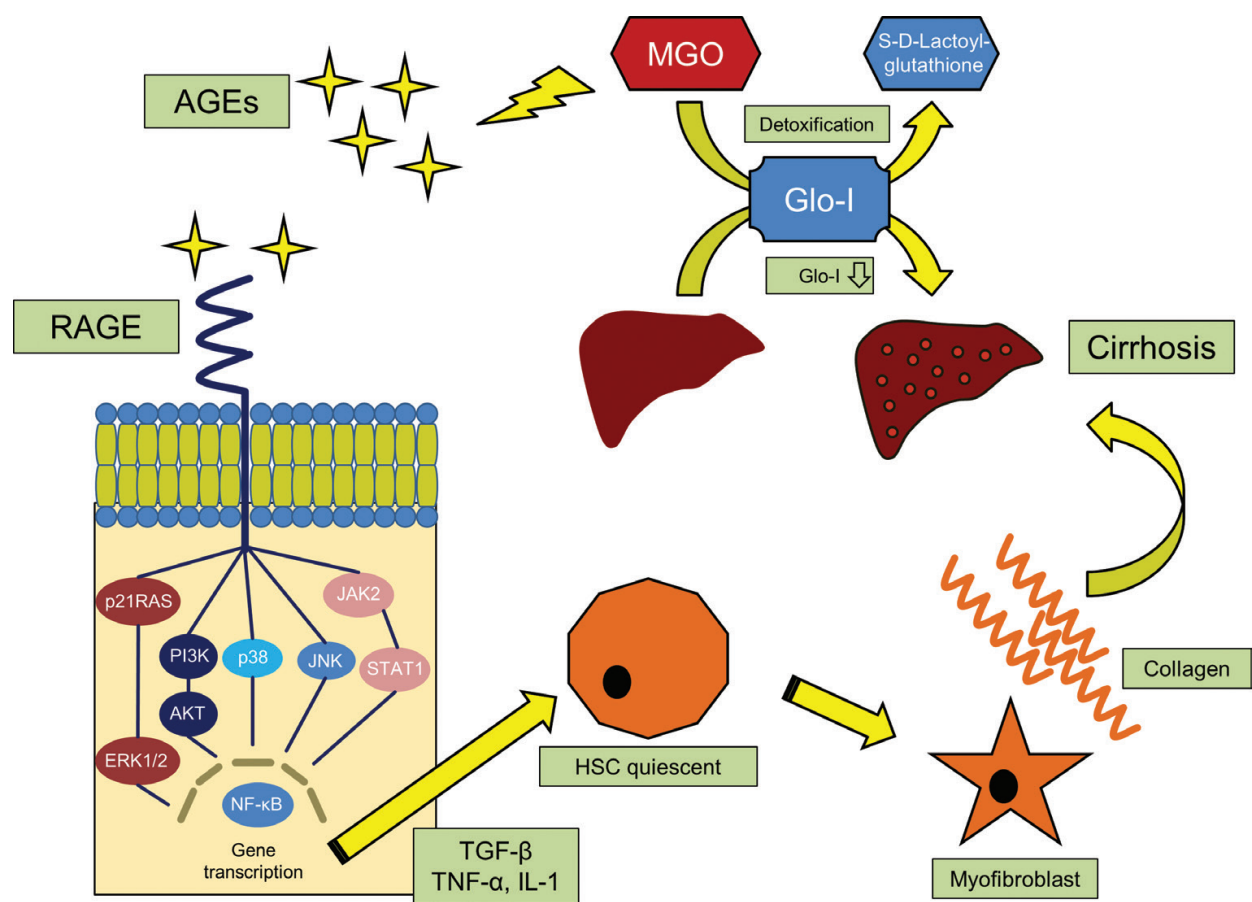
### 4.3. RAGE

The pattern recognition receptor RAGE belongs to the immunoglobulin superfamily with a molecular mass of 47–55 kDa. RAGE expression is stimulated under inflammatory conditions such as diabetes, cardiovascular diseases, or cancer [120]. RAGE has been shown to be activated by MGO- and non-MGO-derived AGEs as well as multiple ligands. Binding to RAGE results in activation of transcription factors, such as NF- $\kappa$ B [121], leading to the release of pro-inflammatory cytokines.

Indeed, several studies revealed participation of RAGE in fibrosis: Upon stimulation with AGE-rat serum albumin containing mainly CML, levels of RAGE,  $\alpha$ -SMA, hydroxyproline, and Sirius red were elevated in a fibrosis model of bile duct ligation (BDL) [106, 122]. Interestingly, RAGE was found to be predominantly expressed in HSC. RAGE was stimulated in HSC during transformation to myofibroblasts, and RAGE was colocalized with  $\alpha$ -SMA and induced by TGF- $\beta$ . In addition, RAGE was expressed in filopodial membranes of myofibroblasts suggesting a role of RAGE in spreading and migration of activated HSC in fibrogenesis [123]. Further analysis provided evidence for crosstalk of RAGE and TGF- $\beta$ : AGEs-induced upregulation of RAGE induced TGF- $\beta$ , TNF- $\alpha$ , and IL-8. Interestingly, RAGE also stimulated anti-inflammatory cytokines IL-2 and IL-4 indicating a negative feedback mechanism and inhibitory crosstalk between TGF- $\beta$  and RAGE [124]. In the next step, effect of RAGE inhibition on inflammation and fibrosis was discovered. First, curcumin was found to reduce, besides its AGEs-lowering effects, the gene expression of RAGE via elevation of PPAR- $\gamma$  [125]. Furthermore, RAGE expression was diminished by means of RAGE siRNA in primary rat HSC resulting in downregulation of IL-6, TNF- $\alpha$ , and TGF- $\beta$  [126]. In a following *in vivo* study, effects of repetitive RAGE siRNA in an olive oil model of fibrosis were analyzed. RAGE siRNA was injected twice weekly in the tail vein of Sprague-Dawley rats. After 6 weeks, reduced expressions of RAGE, TNF- $\alpha$ , IL-6, extracellular matrix, hyaluronic acid, and procollagen III were found. Also, activation of HSC and NF- $\kappa$ B was reduced in siRNA-treated animals attenuating the initiation and progression of fibrosis [127]. Additional studies revealed protective effects of anti-RAGE antibodies in BDL-induced acute liver injury [128, 129].

Growing evidence for implication of RAGE in fibrosis was found in NASH. Methionine choline deficient (MCD) diet caused steatosis and increased RAGE, inflammation, and fibrosis [112]. Recently, fatty acids stimulated CML accumulation and subsequently elicited RAGE induction [109]. 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 study prolonged survival of animals [130].

In a nutshell, various studies confirmed implication of Glo-I and (R)AGE in inflammatory liver disease and fibrosis. Especially targeting Glo-I in cirrhosis highlighted the meaning of MGO-induced liver damage and offers new therapeutic opportunities. Nevertheless, further research in this topic will uncover the exact role of Glo-I in chronic liver disease and possible translation to clinical approach (see **Figure 3**).



**Figure 3.** Impact of Glo-I and (R)AGE in cirrhosis. MGO reacts with proteins, nucleotides, 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 pro-fibrotic 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 end products, RAGE: receptor for advanced glycation end products, Glo-I: glyoxalase-I, HSC: hepatic stellate cells, MAPK: mitogen-activated protein kinase, PI3-K: phosphoinositide 3-kinase, AKT: protein kinase B, JAK2: Janus kinase 2, STAT1: signal transducer and activator of transcription-1, JNK: c-Jun N-terminal kinase, and NF-κB: nuclear factor-κB.

### Abbreviations

AGEs	advanced glycation end products
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	Janus kinase 2
JNK	c-Jun N-terminal kinase
KC	Kupffer cells
LSEC	liver sinusoidal endothelial cells
MAPK	mitogen-activated protein kinase
MCD	methionine choline deficient diet
MG-H1	5-hydro-5-methylimidazolone
MGO	methylglyoxal
NAFLD/NASH	non-alcoholic fatty liver disease/steatohepatitis
NF- $\kappa$ B	nuclear factor- $\kappa$ B
NO	nitric oxide
PI3-K	phosphoinositide 3-kinase
RAGE	receptor for advanced glycation end products
sRAGE	soluble form of RAGE
ROS	reactive oxygen species
STAT1	signal transducer and activator of transcription-1
TGF- $\beta$	transforming growth factor beta
THP	tetrahydropyrimidine

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