REVIEW

Reactive oxygen and mechanisms of inflammatory liver injury

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Abstract Reactive oxygen species (ROS) are important cytotoxic and signalling mediators in the pathophysiology of inflammatory liver diseases. They can be generated by resident and infiltrating phagocytes and/or intracellularly in every liver cell type after stimulation with cytokines. Although ROS are able to cause cell destruction by massive lipid peroxidation, in most cases, ROS are more likely to modulate signal transduction pathways by affecting redox-sensitive enzymes, organelles (e.g. mitochondria) and transcription factors. Thus, ROS can directly induce and/or regulate apoptotic and necrotic cell death. In addition, ROS can have indirect effects on the pathophysiology by supporting protease activity through inactivation of antiproteases and by modulating the formation of inflammatory mediators and adhesion molecules. Many of the effects of ROS may occur simultaneously or sequentially in the pathophysiology. Although mainly described in this review as detrimental, ROS are essential for host defence functions of phagocytes and can modulate the formation of mediators involved in regulating sinusoidal blood flow and liver regeneration. Thus, continuous efforts are necessary to improve our understanding of the role of ROS in the pathophysiology of inflammatory liver diseases and to discover therapeutic interventions that selectively target the negative effects of reactive oxygen formation.

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INTRODUCTION

There is growing evidence that resident macrophages (Kupffer cells) in addition to newly recruited monocytes and polymorphonuclear leucocytes (neutrophils) can cause liver damage in a number of disease processes. These include injury during ischaemia-reperfusion, endotoxaemia, sepsis, alcoholic hepatitis, remote trauma and haemorrhagic shock. Because a main function of macrophages and neutrophils is the destruction of invading microorganisms and removal of necrotic cells and cellular debris, these phagocytes have potent tools to kill and digest cells. This includes formation of reactive oxygen species, for example superoxide, hydrogen peroxide and hypochlorous acid, and the release of proteases such as elastase and cathepsin G. In addition to these vascular sources of cytotoxic mediators, pro-inflammatory cytokines, for example tumour necrosis factor (TNF)-α, can induce the formation of reactive oxygen species in hepatocytes. Moreover, ischaemic cell damage can lead to an intracellular oxidant stress during reoxygenation generated by mitochondria and xanthine oxidase. Although there is overwhelming evidence that reactive oxygen species (ROS) play a significant role in a number of liver diseases, the detailed mechanisms of reactive oxygen involvement are still controversial. Because of the diverse roles ROS may play, elucidating injury mechanisms will be critical for identification of therapeutic interventions. This review will discuss some of the important mechanisms of ROS-induced injury during inflammation of the liver.

Lipid peroxidation

One of the oldest and still popular hypotheses of reactive oxygen-induced cell injury is killing by lipid peroxidation (LPO). The mechanisms of peroxidative
destruction of polyunsaturated fatty acids have been extensively studied in vitro. A role for this mechanism in the liver in vivo is based mainly on two observations: an increase of parameters of LPO and a protective effect of antioxidants in combination with reduced LPO. However, if quantitative comparisons are made, the severity of LPO during an inflammatory injury in vivo is not sufficient to cause direct cell damage. To selectively kill hepatocytes by LPO, a combination of oxidant stress, iron mobilization and depletion of cellular antioxidants are necessary. If these conditions come together, massive LPO ensues resulting in complete destruction of the liver within 1–2 h. However, this is rarely the case in a realistic pathophysiological situation. Is LPO then only an epiphenomenon or are there alternative mechanisms in which LPO could play a role? One possibility, which may be important under inflammatory conditions, is the fact that certain LPO products are potent chemotactic factors for neutrophils and can modulate their reactive oxygen formation. Products of LPO may also be involved in the generation of chemokines. These findings can explain the observation that after the initial burst of inflammatory mediators LPO products are the determining factor for the continuation of neutrophil recruitment and aggravation of the injury. Also, LPO products have been shown to induce fibrosis by enhancing collagen gene expression in activated stellate cells. Thus, LPO products can be important signalling molecules.

**Vascular oxidant stress and proteases**

A number of studies have shown that proteases released by neutrophils and macrophages can cause injury to hepatocytes in vivo. In addition, protease inhibitors attenuated inflammatory liver injury in vivo. Because reactive oxygen appeared to be important for neutrophil-induced injury to hepatocytes in vivo but not in vitro, this was consistent with the hypothesis that oxidants can inactivate plasma antiproteases. Reactive oxygen species generated by neutrophils in vivo create a limited zone around the phagocyte where proteases can act undisturbed on their target. On the other hand, proteases which escape this space will be neutralized by plasma antiproteases. This mechanism allows the proteases to act on the target but prevents injury at remote sites. Based on these in vivo data with isolated hepatocytes, ROS seemed to be restricted to a supportive role for protease-mediated cell injury. However, more recent in vivo data suggest that neutrophil-derived reactive oxygen may be directly responsible for liver-cell damage during endotoxaemia. In this model, neutrophils were shown to cause severe injury after transmigration and adherence to hepatocytes. During the attack by neutrophils, an intracellular oxidant stress is detectable in hepatocytes (Table 1). Antibodies directed against the neutrophil β2 integrin Mac-1 (CD11b/CD18) prevented the intracellular oxidant stress, and attenuated the neutrophil-mediated injury. This is consistent with the observation that adherence through the Mac-1 receptor to an intercellular adhesion molecule-1 (ICAM-1) expressing target cell is critical for long-lasting adherence-dependent reactive oxygen formation by neutrophils. Most importantly, animals that lack glutathione peroxidase are significantly more susceptible to the neutrophil attack. These findings strongly suggest that reactive oxygen released by neutrophils and other phagocytes can cause an intracellular oxidant stress in target cells (Table 1), which can kill hepatocytes within 1–2 h in vivo without evidence of LPO.

How can we reconcile these data with the consistent findings of neutrophil–hepatocyte co-culture experiments in which reactive oxygen appeared not to be involved in the injury mechanism? All in vivo experiments were carried out with chemotactically stimulated neutrophils and control hepatocytes. An injury was observed between 12 and 16 h. This contrasts with in vitro, this was consistent with the hypothesis that oxidants can inactivate plasma antiproteases. Reactive oxygen species generated by neutrophils in vivo create a limited zone around the phagocyte where proteases can act undisturbed on their target. On the other hand, proteases which escape this space will be neutralized by plasma antiproteases. This mechanism allows the proteases to act on the target but prevents injury at remote sites. Based on these in vivo data with isolated hepatocytes, ROS seemed to be restricted to a supportive role for protease-mediated cell injury. However, more recent in vivo data suggest that neutrophil-derived reactive oxygen may be directly responsible for liver-cell damage during endotoxaemia. In this model, neutrophils were shown to cause severe injury after transmigration and adherence to hepatocytes. During the attack by neutrophils, an intracellular oxidant stress is detectable in hepatocytes (Table 1). Antibodies directed against the neutrophil β2 integrin Mac-1 (CD11b/CD18) prevented the intracellular oxidant stress, and attenuated the neutrophil-mediated injury. This is consistent with the observation that adherence through the Mac-1 receptor to an intercellular adhesion molecule-1 (ICAM-1) expressing target cell is critical for long-lasting adherence-dependent reactive oxygen formation by neutrophils. Most importantly, animals that lack glutathione peroxidase are significantly more susceptible to the neutrophil attack. These findings strongly suggest that reactive oxygen released by neutrophils and other phagocytes can cause an intracellular oxidant stress in target cells (Table 1), which can kill hepatocytes within 1–2 h in vivo without evidence of LPO.

### Table 1 Intracellular and vascular oxidant stress in models of Kupffer cell- and neutrophil-induced liver injury

<table>
<thead>
<tr>
<th></th>
<th>Plasma GSSG (μmol/L)</th>
<th>Liver GSSG (nmol/g tissue)</th>
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</thead>
<tbody>
<tr>
<td>Controls</td>
<td>4.7±0.9 (9.4%)</td>
<td>26.3±1.4 (0.4%)</td>
</tr>
<tr>
<td>Gal/ET 5 h</td>
<td>4.2±0.6 (8.3%)</td>
<td>25.2±2.5 (0.4%)</td>
</tr>
<tr>
<td>Gal/ET (7 h) (PMN)</td>
<td>27.8±7.0* (32.2%)</td>
<td>50.0±3.2* (1.75%)</td>
</tr>
<tr>
<td>Controls</td>
<td>1.5±0.2 (19.5%)</td>
<td>27.6±1.9 (0.1%)</td>
</tr>
<tr>
<td>45 min 1/1 h RP (KC)</td>
<td>12.5±1.3* (45.2%)</td>
<td>28.3±1.4 (0.1%)</td>
</tr>
<tr>
<td>45 min 1/6 h RP (KC/PMN)</td>
<td>8.0±1.1* (77.0%)</td>
<td>76.9±9.9* (2.0%)</td>
</tr>
</tbody>
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Plasma concentrations and liver content of glutathione disulfide (GSSG; given as GSH-equivalents) were measured as indicators of oxidant stress in a mouse model of galactosamine-endotoxin (Gal/ET)-induced liver injury and in a rat model of hepatic ischaemia–reperfusion injury (IRP). Numbers in parentheses represent the percentage GSSG of total glutathione. Five hours after Gal/ET, there is no evidence of an oxidant stress. However, during neutrophil (PMN) transmigration and adherence to hepatocytes (7 h), an oxidant stress is detectable in hepatocytes and in the vasculature. In contrast, during the initial reperfusion period (1 h) only Kupffer cells (KC) are activated causing a vascular oxidant stress. At later time points (> 5 h), when neutrophils also contribute to the injury, an intracellular oxidant stress is detectable. Data are mean ± SE of n = 5 animals per group. *P < 0.05 compared with controls (data adapted from references 2, 30, 53).
vivo findings in which not only the neutrophils but also the hepatocytes are activated. Expression of ICAM-1 on hepatocytes and generation of chemotactic signals are important for neutrophil transmigration and attack. If these in vitro conditions were replicated in an in vivo experiment, activated neutrophils killed stimulated hepatocytes within 1 h. The cytokine injury, which is dependent on Kupffer cell-induced cGMP attenuated hepatic ischaemia–reperfusion free Ca²⁺. The MPT is induced by an increase of mitochondrial uncoupling and loss of the membrane potential. A significant oxidant stress causes oxidation of mitochondrial serine proteases (calpains). In addition, cytosolic calpains promote membrane blebbing via degradation of cytoskeletal proteins. The combination of these events leads to rapid necrotic cell death.

In addition to the standard enzymatic and nonenzymatic intracellular defence mechanisms in hepatocytes and non-parenchymal cells, the liver has a specific extracellular defence system to limit the impact of vascular oxidant stress. Under inflammatory conditions, in which activated complement factors stimulate Kupffer cells to produce reactive oxygen in the hepatic vasculature, complement induces the enhanced sinusoidal release of reduced glutathione (GSH) from hepatocytes (Table 1). It can be estimated that this results in close to millimolar concentrations of GSH in the space of Disse. Because GSH and N-acetyl cysteine were equally effective (Fig. 1), it can be concluded that these compounds react non-enzymatically with hydrogen peroxide, peroxynitrite and hypochlorous acid. The release of hepatocellular GSH into the vasculature attenuated the Kupffer cell-induced injury during hepatic ischaemia–reperfusion and endotoxaemia in vivo. In addition to GSH, a large number of antioxidant interventions have been used against inflammatory liver injuries. Antioxidant strategies include treatment with superoxide dismutase, catalase, the iron-chelator deferoxamine, various forms of vitamin E, coenzyme Q, and chemical antioxidants such as tirilazad mesylate, and etselen. The advantages and potential problems with these compounds as therapeutics in inflammation have been discussed elsewhere.

![Figure 1](Image)

**Figure 1** Effect of intravascular reduced (GSH) or oxidized (GSSG) glutathione and N-acetyl cysteine (NAC) on hepatic ischaemia–reperfusion injury. Plasma alanine aminotransferase (ALT) activities were measured in a two-hit model of 30 min of hepatic ischaemia and 4 h reperfusion with injection of 0.5 mg/kg endotoxin at 30 min reperfusion. To deplete hepatic glutathione, all animals were pretreated with 100 mg/kg phorone and 2 mmol/kg buthione sulfoximine. The individual groups received a continuous intravenous infusion (portal vein) of saline (0.25 mL/h per kg), or 22 µmol/kg per kg of GSH, GSSG or NAC during reperfusion. Data represent mean ± SE of n = 6 animals per group. The data indicate that high levels of SH-containing agents (approximately 1.5 mmol/L) in the hepatic vasculature protect against the oxidant stress of Kupffer cells. The fact that NAC and GSH were equally effective suggests that the reactions are spontaneous, not enzymatically catalysed (adapted from references 50, 52).
Oxidant stress and inflammatory gene transcription

In addition to directly induced cell injury and death, ROS can affect these processes indirectly by enhancing pro-inflammatory gene expression. These include cytokines (e.g. TNF-α, interleukin (IL)-1), chemokines (e.g. IL-8) and cellular adhesion molecules. Moreover, ROS can induce stress genes such as haem oxygenase-1 (HO-1), which may protect against inflammatory tissue injury. A common denominator of these genes is the fact that all of them are regulated by the transcription factor, nuclear factor-κB (NF-κB), and in some cases, activating protein-1 (AP-1). Reactive oxygen species are thought to modulate the activation of NF-κB and AP-1.59 However, the effect of oxidant stress on the activation of these transcription factors is cell-type specific, and the molecular mechanism by which reactive oxygen species modulate these processes is still controversial.60 In the liver, TNF-α formation by Kupffer cells proved to be highly sensitive to the cellular redox status.61 A number of antioxidants inhibited the endotoxin-induced nuclear translocation of NF-κB and TNF mRNA formation in isolated Kupffer cells.61 In support of these in vitro data, the radical scavenger dimethyl sulfoxide (DMSO) attenuated TNF-α formation during endotoxaemia in vivo.62 Similar effects on TNF mRNA and protein levels were observed after N-acetyl cysteine treatment.63 Moreover, TNF-α plasma levels during endotoxaemia were three times higher in animals deficient in glutathione peroxidase than in wild-type animals.64 These data indicate that TNF-α gene transcription in Kupffer cells is enhanced by oxidant stress.

Hepatocytes, with their more potent antioxidant systems, are less susceptible to oxidant stress than non-parenchymal cells. Nevertheless, TNF-α can increase intracellular reactive oxygen formation in hepatocytes.11 Consequently, DMSO inhibited TNF-α-induced NF-κB activation and intracellular adhesion molecule-1 (ICAM-1) mRNA formation in hepatocytes in vitro.62 In addition, TNF-α-induced cytokine and chemokine formation could be attenuated to varying degrees by a number of different antioxidants in Hep G2 cells.64 The induction of the stress gene HO-1 leads to formation of the antioxidant biliverdin and the vasodilator carbon monoxide (CO). The improved sinusoidal blood flow in combination with the antioxidant effect may be responsible for the protective effect of hepatocellular HO-1 induction during haemorrhagic shock and resuscitation.65 In this model, HO-1 gene transcription in hepatocytes could be reduced by antioxidants.66 The inducing effect of reactive oxygen during haemorrhage and resuscitation is mediated by the activation of AP-1 not NF-κB.67 These data suggest that in addition to modulation of cytokine-induced gene transcription, an oxidant stress can trigger the induction of protective stress genes such as HO-1 in hepatocytes.

Reactive oxygen species have been shown to stimulate exocytosis of Weibel Palade bodies and enhance the expression of P-selectin on the surface of vascular endothelial cells.68 This effect may play a role in the rapid upregulation of P-selectin in postsinusoidal venules during the early reperfusion phase after hepatic ischaemia.69 In addition, P-selectin can be transcriptionally upregulated on venular endothelium.70 Although reactive oxygen may contribute to the enhanced transcription of P-selectin and other adhesion molecules through activation of the redox-sensitive transcription factor NF-κB, the relevance of venular neutrophil adherence is still in question. In an endotoxaemia model, neutrophils critical for the injury adhered in sinusoids not in venules.31 Another important cell type in the liver is a stellate cell. Because of their contractility, stellate cells are involved in sinusoidal blood flow regulation,71 most likely as the target of CO.72 After transformation, stellate cells are the major source of excessive collagen formation leading to fibrosis. Although there is considerable evidence that reactive oxygen and LPO products can stimulate fibrosis,72,73 recent papers demonstrated that ROS enhance collagen α1 (I) gene expression in vivo,74 and that transforming growth factor β induces α1 (I) procollagen mRNA formation in stellate cells by a hydrogen peroxide-dependent mechanism.74 In addition, reactive oxygen can induce chemokine gene transcription in stellate cells.75 Activated cells are even more responsive indicating that the priming of stellate cells during chronic liver injury may further stimulate the inflammatory response.

Oxidant stress and apoptosis

Although apoptosis generally does not induce inflammation, there is evidence that under certain circumstances apoptotic cell death may promote inflammation76 or modulate an existing inflammatory response.57,77 Reactive oxygen species have been implicated in apoptosis.78 In the liver, the involvement of reactive oxygen has been suggested in apoptotic cell death of hepatocytes30–32 and endothelial cells.31,82 However, the molecular mechanisms are not well understood. One possible explanation is that oxidant stress can induce the mitochondrial membrane permeability transition, a central event of apoptosis and necrosis.83 Another potential target could be caspases, a family of cysteine proteases that is important for the initiation and progression of apoptosis.84 Caspases can be activated by low concentrations of hydrogen peroxide.85 In contrast, higher levels inhibit the enzyme activity presumably due to oxidation of critical sulfhydryl groups.85 This mechanism may be important for the delayed death of activated neutrophils during an inflammatory response.86 Thus, reactive oxygen species may induce or inhibit apoptosis depending on the experimental conditions. However, more mechanistic investigations are necessary to determine the critical cellular targets of reactive oxygen during apoptosis in liver cells.

CONCLUSIONS

Reactive oxygen species are important cytotoxic and signalling mediators in the pathophysiology of inflam-
matory liver diseases. Generated intracellularly or in the extracellular space by resident and infiltrating phagocytes, ROS are able to modulate apoptotic and necrotic cell death pathways. In addition, reactive oxygen can affect the pathophysiology indirectly by enhancing the formation of pro-inflammatory mediators and by inactivating antiproteases. Despite the many detrimental effects, reactive oxygen species are essential for host-defense functions of phagocytes and may enhance the formation of mediators regulating liver blood flow and regeneration. Thus, continuous efforts are necessary to improve our understanding of the role of oxidant stress in the pathophysiology of inflammatory liver diseases and to discover therapeutic interventions, which selectively target the negative effects of reactive oxygen formation.

REFERENCES


