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COVID-19 and the liver: an adverse outcome pathway perspective

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ABSTRACT

Liver damage is observed in up to half of hospitalized COVID-19 patients and can result either from actions of SARS-CoV-2 as such or from pharmacological treatment. The present paper introduces an adverse outcome pathway construct that mechanistically describes the pathways induced by SARS-CoV-2 leading to liver injury. This can be caused by direct binding of the virus and local actions in cholangiocytes, but may also indirectly result from the general state of hypoxia and systemic inflammation in COVID-19 patients. Further research is urgently needed to fill remaining knowledge gaps. This will be anticipated to create a solid basis for future and more targeted development of vaccines and, in particular, therapies.

1. Introduction

More than 120 million people around the world were infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and 2.7 million individuals died as a consequence of the resulting coronavirus disease 2019 (COVID-19) early 2021 (https://coronavirus.jhu.edu/). One of the most frequent complications of COVID-19 is pneumonia (Vinken, 2020a). Nevertheless, in many cases, other organs are affected by SARS-CoV-2 as well. Among those is the liver, which in general is a frequent target for disease and toxicity because of its unique location and function in the body. However, the exact role and relevance of the liver in COVID-19 remains elusive. Adverse outcome pathway (AOP) constructs could be useful tools to assist in the mechanistic elucidation of the involvement of liver pathology in COVID-19. An AOP is activated following interaction of foreign entities with a biological system, referred to as the molecular initiating event (MIE). This induces a series of key events (KEs), linked through key event relationships (KERs) at different levels of biological organization, ranging from the molecular to the organism level, resulting in the actual adverse outcome (AO) (Ankley et al., 2010; Vinken et al., 2017).

In this context, the Joint Research Centre of the European Commission started an interdisciplinary project called CIAO (modelling the pathogenesis of COVID-19 using the AOP framework), aiming at providing an overarching knowledge entry point to COVID-19 research separating signal from noise and giving scientists the possibility to quickly grasp dependencies between research fields not normally collaborating through the AOP concept (https://ec.europa.eu/jrc/en/event/webinar/intro-webinar-ciao-project). One of the many adversities scrutinized in CIAO is liver injury, which is documented in the present paper.

2. COVID-19 and the liver

2.1. Clinical features

The incidence of liver injury in COVID-19 ranges between 14.8 %–53 %, mainly manifested as abnormal aminotransferase levels that may go up to 3 times the upper limit of normal (Cai et al., 2020; Meta wea et al., 2021). However, this may not only arise from the liver, but can equally originate from other organs, such as the muscles (Bangash et al., 2020). Many patients also show an increase in gamma glutamyl transferase serum amounts to 3 times the upper limit of normal, which points to cholangiocyte injury. Increases in alkaline phosphatase serum levels seem less common (Cai et al., 2020; Shao et al., 2020). Liver biopsy specimens of deceased COVID-19 patients indicate moderate microvascular steatosis, mild lobular and portal activity (i.e. necrosis, inflammation and/or fibrosis) (Ji et al., 2020; Schmit et al., 2021; Xu et al., 2020) as well as cholestasis (Schmit et al., 2021). Overall, the incidence of liver injury is higher in patients with severe COVID-19 compared to patients with mild symptoms and may even predict mortality.

Abbreviations: ACE2, angiotensin-converting enzyme 2; AO(P), adverse outcome (pathway); CIAO, modelling the pathogenesis of COVID-19 using the AOP framework; COVID-19, corona virus disease 2019; IFN, gamma interferon gamma; KE(R), key event (relationship); MIE, molecular initiating event; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane serine protease 2; TNF, alaphatumor necrosis factor alpha.

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also occur indirectly as part of the severe systemic inflammatory response (Yang et al., 2020; Zhao et al., 2020). Liver damage in COVID-19 may be shown by a significant increase in serum levels of inflammatory markers, i.e. cytokine storm, following SARS-CoV-2 infection (in vitro, ex vivo, in silico). In this light, sepsis and associated multi-organ failure underlie the cause of death in about one third of COVID-19 patients. Further-more, liver damage in COVID-19 may be indirectly caused by oxygen deprivation in patients, which in turn results from pneumonia (Vinken et al., 2020a). This state of hypoxia is known to be permissive for the formation of microthrombi, in casu in liver, which can induce tissue damage (Pilli et al., 2018).

2.2. Mechanisms

Liver injury in COVID-19 can result either from actions of SARS-CoV-2 as such (i.e. pathology) or from pharmacological treatment (i.e. therapy). Regarding the latter, several drugs used in the clinical management of COVID-19 are known to trigger liver injury in patients, including lopinavir/ritonavir (Cai et al., 2020; Fan et al., 2020), hydroxychloroquine (Falcao et al., 2020) and remdesivir (Grein et al., 2020). Such drug-induced hepatotoxicity typically manifests as steatotic (i.e. fat accumulation) or cholestatic (i.e. bile acid accumulation) liver injury, all for which well-established AOPs are available (https://aopwiki.org/). As much as 59.7 % of the total cholangiocyte population expresses the angiotensin-converting enzyme 2 (ACE2) receptor, which facilitates cellular entry of SARS-CoV-2, a level that equals ACE2 receptor quantities measured in lung alveolar type 2 cells (Chai et al., 2020). Therefore, SARS-CoV-2 could directly target and enter cholangiocytes, resulting in viral replication and local cytopathic effects. In vitro experiments with human hepatocyte and cholangiocyte organoids indeed show infection with SARS-CoV-2 leading to virus replication (Yang et al., 2020; Zhao et al., 2020). Liver damage in COVID-19 may also occur indirectly as part of the severe systemic inflammatory response (i.e. cytokine storm) following SARS-CoV-2 infection. This is shown by a significant increase in serum levels of inflammatory markers, such as C-reactive protein, ferritin, lactate dehydrogenase, d-dimer, interleukins 2 and 6 (Chen et al., 2020; Ponti et al., 2020; Zhou et al., 2020a). In this light, sepsis and associated multi-organ failure underlie the cause of death in about one third of COVID-19 patients. Furthermore, liver damage in COVID-19 may be indirectly caused by oxygen deprivation in patients, which in turn results from pneumonia (Vinken et al., 2020a). This state of hypoxia is known to be permissive for the formation of microthrombi, in casu in liver, which can induce tissue damage (Pilli et al., 2018).

2.3. Adverse outcome pathway

A putative AOP depicting the mechanisms underlying the impact of COVID-19 on liver is proposed based on present knowledge and relevant literature data (Fig. 1). This AOP only takes pathology-related mechanisms, and thus not the adverse liver effects of therapy, into consideration. A total of 3 pathways lead to 3 liver injury, namely 1 direct pathway (i.e. triggered by the binding of SARS-CoV-2 to cholangiocyte ACE2 receptors) and 2 indirect pathways (i.e. triggered by the binding of SARS-CoV-2 to extrahepatic ACE2 receptors). In contrast to the former, the indirect pathways are preceded by a number of systemic adverse effects with MIEs located in other organs(s), which ultimately hit the liver. The data substantiating each of the KERs, and hence KEs, originate from various in silico, in vitro, ex vivo, in vivo and clinical studies (Table 1).

3. Outlook

AOPs have multiple applications, most of them in the area of toxicology and chemical risk assessment, such as in chemical categorization, test prioritization/de-risking strategies, development of in vitro and in silico assays, and establishment of integrated approaches to testing and assessment (Vinken et al., 2017). Nevertheless, AOPs also have their value in a clinical context. Indeed, AOPs can serve the development and optimization of clinically relevant animal models of disease for fundamental and translational research as well as for testing new therapeutics, and can aid the characterization of novel diagnostic and prognostic biomarkers of disease (Vinken, 2020b). In this respect, the CIAO project intends to model the pathogenesis of COVID-19 in view of facilitating the further development of vaccines and therapies (https://ec.europa.eu/jrc/en/event/webinar/intro-webinar-ciao-project). The pathways leading to COVID-19-related injury in the liver only represent a piece in this mechanistic puzzle and need to be linked to other critical mechanisms induced by SARS-CoV-2 in the body. It remains to be investigated how specific the effects of SARS-CoV-2 on the liver actually are and thus if the liver represents a primary target in COVID-19 per se. While SARS-CoV-2 directly binds and acts in cholangiocytes, the outcome of COVID-19 in liver equally results from the general state of hypoxia and systemic inflammation, which as such may affect all organs. AOPs are able to identify such knowledge gaps and therefore help to set future research priorities. By doing so, new scientific information will be generated that can be fed into the nascent AOP and that will either substantiate existing KERs and KEs, or that will identify new ones. It is anticipated that this will ultimately lead to thorough understanding of the mechanisms driving COVID-19, which in turn will form a solid basis for the further and more targeted development of vaccines and, in particular, therapeutics.

CRediT authorship contribution statement

Mathieu Vinken: Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing, Visualization.

Declaration of Competing Interest

The author declares that he has no known competing financial interests.

Fig. 1. Adverse outcome pathway describing the effects of COVID-19 on the liver (ACE2, angiotensin-converting enzyme 2; AO, adverse outcome; KE, key event; MIE, molecular initiating event).
Table 1 | Key event relationship | Data | Type of study | Reference |
| --- | --- | --- | --- |
| ACE2 receptor activation (liver) | more likely to develop liver injury | Clinical study | Chu et al., 2020 |
| Viral replication | Liver injury in COVID-19 patients is associated with systemic inflammation | Clinical study | Sun et al., 2020 |
| Viral replication | The severity of liver injury in COVID-19 patients is directly related to the incidence of sepsis | Clinical study | Zhang et al., 2020 |
| Viral replication | Abundance of cytokines in COVID-19 patients is positively related to the incidence of liver injury | Clinical study | Zhang et al., 2020 |

Table 1 (continued) | Key event relationship | Data | Type of study | Reference |
| --- | --- | --- | --- |
| ACE2 receptor activation | Hypoxia increases the risk of thrombosis | In vitro study | Pilli et al., 2018 |
| (liver) | Hypoxia promotes deep vein thrombosis | In vitro study | Brill et al., 2013 |
| Virus replication | Elevation-associated hypoxia is an independent prothrombotic risk factor | Clinical study | Zangari et al., 2013 |
| Viral replication | COVID-19 patients with hypoxia are more likely to develop liver injury | Clinical study | Fu et al., 2021 |
| Viral replication | Liver injury in COVID-19 patients is associated with hypoxia | Clinical study | Chu et al., 2020 |
| Viral replication | Livers of COVID-19 patients show thrombosis in portal and sinusoidal vessels | Clinical study | Sonzogni et al., 2020 |
| Viral replication | Livers of COVID-19 patients show thrombosis in central veins | Clinical study | Lax et al., 2020 |
| Viral replication | Liver dysfunction in COVID-19 patients is induced by microvascular thrombosis | Clinical study | Tsutsumi et al., 2021 |
| Viral replication | Thrombosis in COVID-19 patients is associated with liver dysfunction | Clinical study | Brosnahan et al., 2021 |

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