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Review

The role of apoptosis in acetaminophen hepatotoxicity

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ABSTRACT

Although necrosis is recognized as the main mode of cell death induced by acetaminophen (APAP) overdose in animals and humans, more recently an increasing number of publications, especially in the herbal medicine and dietary supplement field, claim an important contribution of apoptotic cell death in the pathophysiology. However, most of these conclusions are based on parameters that are not specific for apoptosis. Therefore, the objective of this review was to re-visit the key signaling events of receptor-mediated apoptosis and APAP-induced programmed necrosis and critically analyze the parameters that are being used as evidence for apoptotic cell death. Both qualitative and quantitative comparisons of parameters such as Bax, Bcl-2, caspase processing and DNA fragmentation in both modes of cell death clearly show fundamental differences between apoptosis and cell death induced by APAP. These observations together with the lack of efficacy of pan-caspase inhibitors in the APAP model strongly supports the conclusion that APAP hepatotoxicity is dominated by necrosis or programmed necrosis and does not involve relevant apoptosis. In order not to create a new controversy, it is important to understand how to use these "apoptosis" parameters and properly interpret the data. These issues are discussed in this review.

1. Introduction

Apoptosis, a form of cell death different from the commonly recognized necrosis, was first described in the 1970s by Kerr and coworkers (Kerr et al., 1972). However, it took another 20 years until major progress was made in understanding the specific signaling mechanisms of apoptosis including the discovery of caspases in the 1990s (Green and Kroemer, 1998; Thornberry, 1997). As a consequence, the number of publications describing apoptotic cell death in virtually every disease process including liver diseases, increased exponentially. This culminated in the prevailing hypothesis that apoptosis is the dominant form of cell death for most liver diseases (Guicciardi and Gores, 2005). A typical example of this development was hepatic ischemia-reperfusion injury, where initial studies only recognized necrosis mainly caused by inflammatory cells (Jaeschke, 1998). However, only a few years later, the injury appeared to be exclusively caused by apoptosis (Neuman, 2001; Rudiger et al., 2003). Although subsequent studies questioned these conclusions and demonstrated convincingly that cell death during hepatic ischemia-reperfusion is caused by necrosis and that apoptosis is of very limited relevance (Gujral et al., 2001; Yang et al., 2014), the idea that apoptosis is somehow important prevails until today (Cao et al., 2016). For acetaminophen (APAP) hepatotoxicity, a slightly different story developed. Whereas early studies suggested necrosis (Mitchell et al., 1973), only few studies surfaced that implicated a relevant contribution of apoptosis (El-Hassan et al., 2003; Ray and Jena, 2000; Ray et al., 1996). In addition, direct comparison between apoptosis and APAP-induced cell death showed very clearly that APAP-induced liver injury is caused by necrosis and not apoptosis (Gujral et al., 2002). Today, none of the main laboratories investigating mechanisms of APAP hepatotoxicity suggest a role for apoptosis in the pathophysiology (Iorga et al., 2017; Jaeschke et al., 2012; Hinson et al., 2010; Ramachandran and Jaeschke, 2018). However, during the last few years a rapidly increasing number of studies, especially in the natural product literature, are being published that again claim that apoptosis is an important part of the pathophysiology (e.g, Ahmed et al., 2016; Cao et al., 2018; Dong et al., 2014; Hu et al., 2017; Hong et al., 2012; Li et al., 2013; Sharma et al., 2011; Song et al., 2014; Wang et al., 2010; Wang et al., 2017; Wang et al., 2018; Zhang et al., 2017; Zhao et al., 2012). Although most of these studies do not appear to directly challenge previous reports regarding the lack of apoptosis, they mainly ignore the pertinent literature and simply conclude based on the measurement of a few, assumed apoptosis parameters that the natural product that is being tested is protecting due to its anti-apoptotic effect (e.g. Ahmed et al., 2016; Cao et al., 2018; Dong et al., 2014; Hu et al., 2017; Hong et al., 2012; Li et al., 2013; Sharma et al., 2011; Song et al., 2014; Wang et al., 2010; Wang et al., 2017; Wang et al., 2018; Zhang

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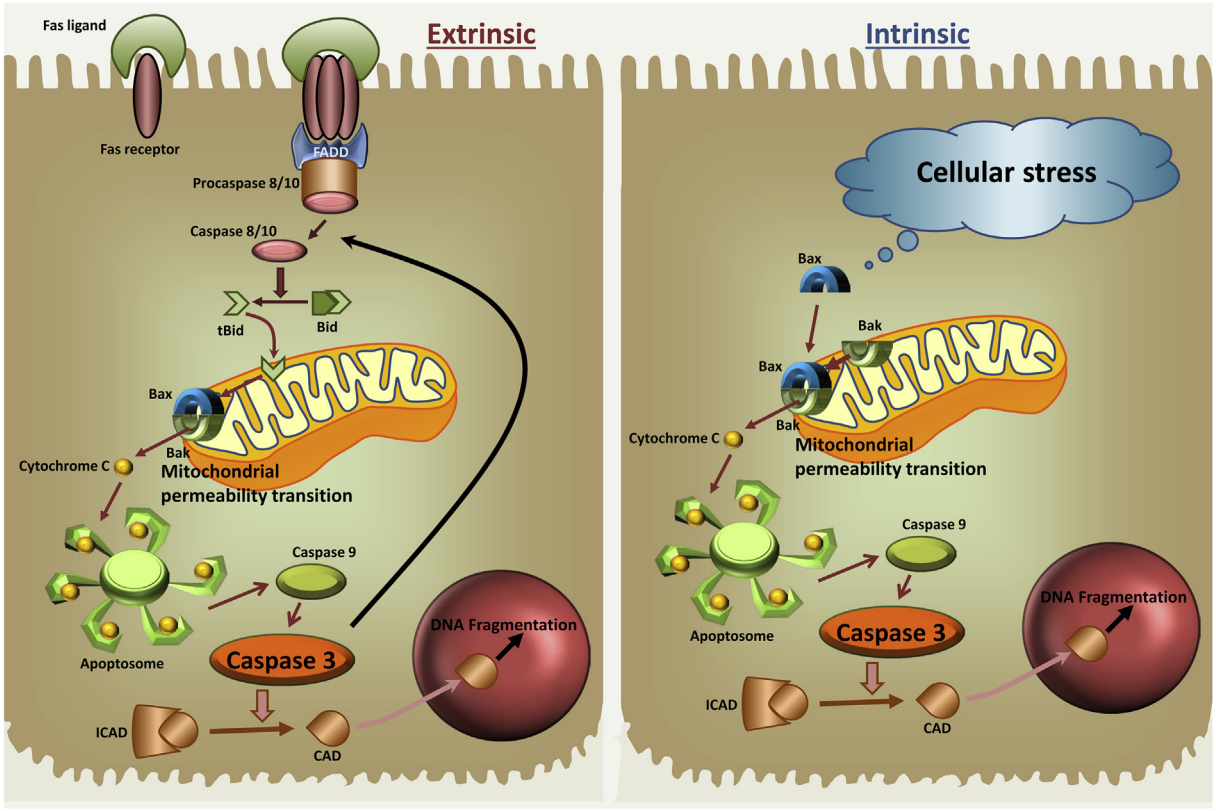

Fig. 1. Signaling Mechanisms of Apoptotic Cell death. The extrinsic pathway requires activation of a death receptor, such as the Fas receptor, which triggers the formation of a death-inducing signaling complex with FADD and procaspase-8 or -10. Generation of the active caspase then results in cleavage of Bid to tBid, which translocates to mitochondria and initiates the mitochondrial outer membrane permeabilization facilitated by Bax and Bak, which triggers the release of cytochrome c. This enables formation of the apoptosome and subsequent activation of caspase-9 and caspase-3. Active caspase-3 cleaves ICAD to release CAD, which translocates to the nucleus and initiates nuclear DNA fragmentation. Caspase 3 can also promote procaspase-8 cleavage and further amplify the pro-apoptotic signaling loop through the mitochondria. The intrinsic pathway for apoptosis is activated by cellular stress signals, which activate Bax and its translocation to the mitochondria, which then follows signaling events similar to the extrinsic pathway downstream of the MPT.

et al., 2017; Zhao et al., 2012). Unfortunately, this is perpetuating a fundamentally wrong conclusion, which not only questions the proposed mechanism of protection of a particular natural product but runs the risk that many other studies follow their lead. Therefore, the objective of this review is to re-visit the mechanisms of apoptosis and how this can be studied in APAP toxicity.

2. Signaling mechanisms of apoptotic cell death in hepatocytes

Apoptosis is characterized by very tightly regulated signaling mechanisms (Guicciardi and Gores, 2005; Schattenberg et al., 2006). There are 2 principal pathways of apoptosis induction: the extrinsic (cell death receptor) pathway and the intrinsic (mitochondrial) pathway. Both pathways trigger the activation of intracellular proteases and endonucleases, which are responsible for the breakdown of the cell (Fig. 1). In the case of the extrinsic pathway, a ligand (e.g., FasL) binds to the death receptor (e.g., Fas receptor), which triggers the trimerization of the receptor and the formation of a death-inducing signaling complex consisting of the cytoplasmic death domain of the receptor, an adapter molecule (e.g. FADD) and procaspase-8 or -10. Through autocatalytic processing, the active caspase is generated, which can cleave the pro-apoptotic Bcl-2 family member Bid to form tBid. This truncated form of Bid translocates to the mitochondria and facilities together with Bax and Bak the formation of pores in the outer mitochondrial membrane (MOMP), which enable the release of intermembrane proteins. Critical for the progression of apoptotic signaling is the mitochondrial release of cytochrome c, which facilitates the formation of the apoptosome and the activation of caspase-9 and subsequently caspase-3. The active caspase-3 can promote procaspase-8 cleavage and further amplify the pro-apoptotic signaling loop through the mitochondria and it can cleave downstream targets to continue the apoptosis pathway. One of the targets of caspase-3 is the inhibitor of caspase-activated DNase (ICAD). Cleavage of ICAD liberates CAD for translocation to the nucleus causing nuclear DNA cleavage into inter-nucleosomal fragments of 180 base pairs and multiples thereof (Nagata et al., 1998). The intrinsic pathway of apoptosis is activated mainly by internal signals such as Bax activation and its translocation to the mitochondria and then follows the same signaling events downstream of MOMP formation as the extrinsic pathway. Hepatocytes generally require mitochondria to amplify the death signal and execute apoptosis (type II cell) (Scaffidi et al., 1998). However, a very strong signal at the Fas receptor can lead to sufficient caspase-8 activation, which in turn directly activates enough caspase-3 to cause apoptotic cell death (type I cell) (Schüngel et al., 2009).

Because a number of cytokines and stress signals can activate the apoptotic pathways, it is critical to have checkpoints in the process in order to avoid accidental activation. FLICE-inhibitory proteins can prevent death receptor activation (Krueger et al., 2001) and inhibitors of apoptosis (IAPs) in the cytosol can bind to certain pro-caspases and activated caspases and prevent further activation (Marivin et al., 2012; Wang and Lin, 2013). On the other hand, Second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI (Smac/Diablo) can be released from mitochondria and inactivate IAPs to allow apoptotic signaling to progress (Fulda, 2015). The intermembrane proteins apoptosis-inducing factor (AIF) and endonuclease G can also be released through the outer membrane pores but do not