A hepatotoxic dose of acetaminophen modulates expression of BCL-2, BCL-X_L, and BCL-X_S during apoptotic and necrotic death of mouse liver cells in vivo

Abstract

The protein BCL-X_L and protein product of proto-oncogene bcl-2 act as apoptosis antagonists, and BCL-X_S serve as a dominant death promoter, including apoptosis following exposure to chemotherapeutic drugs. This investigation examined whether some aspects of the highly integrated process of acetaminophen (AAP)-induced hepatotoxicity involve down-regulation or upregulation of expression of BCL-2, BCL-X_L and BCL-X_S in mouse liver in vivo. Male ICR mice (CD-1; 35–45 g) were treated ip with a hepatotoxic dose of AAP (500 mg/kg) and sacrificed 0, 6, and 18 h later. Blood was collected upon sacrifice for determination of serum alanine aminotransferase (ALT) activity and the liver was sectioned for histopathological diagnosis of necrosis/apoptosis. Portions of liver tissues were also used for DNA extraction (for gel electrophoresis) and Western blot analysis. This study demonstrates that administration of a hepatotoxic dose of AAP to ICR mice results in severe liver injury (ALT leakage >200-fold at 6 h and >600-fold at 18 h) leading to massive cell death by apoptosis (diagnosed by nuclear ultrastructure, histopathology, and DNA ladder), in addition to necrosis coupled with spectacular changes in the BCL-X_L expression (6 and 18 h after AAP administration). Western blot analysis of the liver proteins revealed that mouse liver expresses two proteins, BCL-X_L and BCL-X_S, and does not express BCL-2. As the toxicity progressed, during 6 and 18 h post-AAP administration, the BCL-X_L protein band shifted to a slower mobility band which might represent a phosphorylated form of BCL-X_L. Appearance of this higher molecular weight BCL-X_L protein band correlated with massive apoptotic death of liver cells along with ladder-like DNA fragmentation. In the same time period, death inhibitory gene bcl-2 remained unexpressed, and the level of expression of BCL-X_S remained unaltered. Whether the consistent level of expression of BCL-X_S reflected inability of AAP to influence its expression remains unknown. Unaltered expression of BCL-X_S in the near total absence of BCL-2 expression raises questions regarding the death promoting role of BCL-X_S in vivo. The precise role of modified form of BCL-X_L remains elusive. However, this study may have demonstrated for the first time drug-induced changes in the expression of anti-apoptotic gene BCL-X_L, and a positive link between AAP-induced apoptotic death and modification of BCL-X_L protein in vivo.

Key words

Acetaminophen · Hepatotoxicity · Apoptosis · bcl-X_L expression · DNA fragmentation

Abbreviations

bcl-2 B cell lymphoma · bcl-X_L bcl-X (long form) · bcl-X_S bcl-X (short form) · ICR Institute of Cancer Research · P450 cytochrome P450

Introduction

More than 70 million prescriptions for nonsteroidal anti-inflammatory drugs (NSAIDs) are written in the US every year, and the worldwide market for NSAIDs is estimated as several billions per year. Of these, over-the-counter antipyretic and analgesic drug acetaminophen (AAP: N-acetyl-p-aminophenol, paracetamol) enjoys maximum popularity. Because of its clinical importance, AAP has been used as a powerful tool to study mechanisms of hepatotoxicity for decades. Acetaminophen poisoned humans and experimental animals display profound hepatotoxicity in addition to nephrotoxicity (Boyd and Bereczky 1966; Maruyama and Williams 1988; Ray et al. 1993, 1998; Kroger et al. 1997) and even
death (Ray et al. 1999). In 1997 alone, more than 72,000 cases were reported to US poison control centers, of which 560 were life-threatening and 65 were fatal.

AAP is metabolized to a highly reactive arylating metabolite, presumably N-acetyl-p-benzoquinone imine (NAPQI), by the cytochrome P-450-dependent mixed function oxidase system (Mitchell et al. 1973; Esterline et al. 1989; Lee et al. 1996). Therapeutic doses of this drug are safely biotransformed and eliminated as nontoxic conjugates of sulfate and glucuronic acid (Nelson 1990), and only a small portion is converted to NAPQI which is rapidly detoxified via conjugation with glutathione (GSH) and excreted (Coles et al. 1988). However, during overdoses of AAP, the capacity for its removal by hepatic sulfation is saturated, and the rate of glucuronidation is decreased (Hjelle and Klaassen 1984). Thus, the rate of formation of toxic metabolites is increased. In addition to NAPQI, reactive oxygen species (ROS) formed during AAP metabolism induces oxidative stress which in turn contributes to the cell injury process. AAP practically interferes with ongoing processes of each and every intracellular compartment: mitochondria (Ji et al. 1986; Esterline et al. 1989; Burcham and Harman 1991; Gupta et al. 1997), nucleus (Ray and Corcoran 1991; Ray et al. 1990, 1991, 1993, 1996), plasma membrane (Moore et al. 1985; Muriel et al. 1992), and cytoplasm (for review see Nelson 1995; Pumford et al. 1997). Recently, a novel pathway of AAP hepatotoxicity was proposed the potential of AAP to influence the integrity of genomic DNA directly or indirectly in a Ca^{2+}-dependent manner has gained considerable attention (Ray et al. 1993; Corcoran and Ray 1992). Using biochemical, histopathological, and ultrastructural criteria, Ray and his collaborators have subsequently shown that AAP can turn on apoptosis (programmed cell death: PCD) in addition to necrosis (unprogrammed cell death) in the liver in vivo (Ray et al. 1996). This communication extends the earlier observations at the genetic level and, for the first time, reports in vivo involvement of BCL-XL and its modulation during AAP-induced PCD.

Cell death has been classically defined as a passive degenerative phenomenon consequent to toxic injury usually preceded by irreversible morphological and biochemical changes. Two biochemically distinct mechanisms of cell death, apoptosis and necrosis, have been characterized based on morphological criteria. Following toxicant exposure, specific cells may elect to die via a self-orchestrated process, apoptosis (the programmed death), or face the consequences of necrosis (the unprogrammed death). Apoptosis is a tightly regulated active process and it occurs when cells have sufficient time to organize and participate in their own demise. Apoptotic death occurs when a cell activates an internally encoded suicide program as a result of either extrinsic or intrinsic signals. Apoptotic death is characterized by overall cell shrinkage, margination of chromatin in sharply defined masses adjoining the nuclear envelope, and nuclear and cellular fragmentation to produce apoptotic bodies. Also included during this ongoing process is the cell’s ability to regulate its own or organelar volume. This chaotic aftermath leads to shrinkage and blebbing of the cytoplasm and disintegration of the cell into condensed membrane bound fragments or apoptotic bodies. These bodies are then recognized and engulfed by phagocytes by a receptor mediated process or by neighboring cells or by macrophages for rapid removal. The loss of Ca^{2+} regulation and subsequent cleavage of DNA also appears instrumental to the process (Wylie 1980; McConkey et al. 1988; Ray et al. 1993; Schwartzman and Cidlowski 1993; Nakashima et al. 1999).

The difference between necrosis and apoptosis is that necrosis refers to a spectrum of morphologic changes that occur following the swelling and lysis of cellular organelles including the plasma membrane. The cell’s ability to regulate its own or organelar volume in necrosis is lost early during toxicity. Many drugs and chemicals are powerful inducers of apoptosis in vivo (e.g. aliphatic alcohols: Goldin et al. 1993; Manolas et al. 1997; bleomycin: Hagimoto et al. 1997; cycloheximide: Faa et al. 1994; dimethylnitrosamine: Ray et al. 1992; Pritchard and Butler 1989; DMBA: Burchiel et al. 1992; doxorubicin: Herman et al. 1997). Our studies on acetaminophen-induced liver injury concludes that a vast majority of liver cells predominantly choose to die via programmed apoptotic pathway rather than facing unprogrammed consequences of necrosis (Ray et al. 1996). Therefore, the goal of this battery of experiments was to search for the genes involved in this processes in the liver in vivo.

Because of the importance and involvement of apoptotic death in various toxic processes, considerable interest has arisen in genes that are capable of regulating this process. Apoptosis has been well documented in the nematode, Caenorhabditis elegans, where genetic studies have provided considerable insight into the mechanism of cell death (Ellis et al. 1991). It is now established that the expression of two genes, ced-3 and ced-4, is required for the cells to die (Ellis et al. 1991). The other key players implicated in the cell death process are p53, c-myc, c-fos, TRPM-2, fas, ICE (Interlukin-Converting-Enzyme) group, and bcl-2 families. Among all these, the bcl-2 family has captured the most attention of cell death researchers (Ray 1999).

The proto-oncogene bcl-2 prevents apoptosis induced by a variety of agents including exposure to chemotherapeutic drugs (Vaux et al. 1988; Miyashita and Reed 1993; Haldar et al. 1994, 1995). Changes in bcl-2 expression, therefore, may be associated with altered responses to such drugs. In vivo, BCL-2 prevents many, but not all forms of apoptotic cell death that occur during lymphoid (Hockenbery et al. 1991; Miyashita and Reed 1993) development. Expression of a bcl-2 transgene can prevent radiation- and calcium ionophore-induced apoptotic cell death in thymocytes but does not inhibit negative selection (Sentman et al. 1991). These results suggest the existence of multiple intracellular mechanisms of apoptosis, some of which can be prevented by bcl-2 and others which are unaffected by...