Original Article

Apoptosis in acute hepatic failure: Histopathological study of human liver tissue using the tunel method and immunohistochemistry

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The role of apoptosis in the occurrence of massive or submassive liver cell death was investigated. Of liver tissues removed from 49 autopsy cases of acute hepatic failure, 26 cases were fulminant hepatic failure (FHF), 13 were subacute hepatic failure (SAHF) and the remaining 10 were acute-on-chronic hepatic failure (AoCHF). Eleven cases were associated with HBV infection, 2 with HCV, 5 with non-A non-B hepatitis, 4 with drug medication and 4 with autoimmune disorder, while the etiology of 23 cases remained unknown. Examinations by the TUNEL method and immunohistochemistry using anti-Fas, -Bax, and -Bcl-2 antibodies were conducted. Most of the FHF and AoCHF cases and half of the SAHF cases showed massive or submassive hepatic lobular injury histopathologically. TUNEL positive cells were observed frequently, and the grade of lobular injury showed significant correlation with the frequency of TUNEL-positive liver cells. Bax and Fas were expressed on the remaining liver cells, while Bcl-2 was seen on the infiltrating lymphocytes. The frequency of TUNEL-positive liver cells showed a significant correlation with Bax expression and the grades of inflammatory cell infiltration, but a poor correlation with Fas and Bcl-2 expression. The results of the present study suggested that apoptosis plays an important role in massive and submassive hepatic cell death. Fas and Bcl-2 might not be involved in the apoptosis of liver cells, but the findings suggested that Bax might play a role in inducing liver cell apoptosis, though the mechanism could not be explained.

Key words: Acute hepatic failure, apoptosis, TUNEL, immunohistochemistry, Bax

Introduction

It is well known that necrosis is induced via cell membrane destruction by various inflammatory and degenerative conditions, whereas apoptosis is another process of cell death that is characterized by a series of morphological changes beginning with granular aggregation of chromatin, followed by nuclear condensation and fragmentation and finally resulting in cytoplasmic vacuolation and segmentation. The term “apoptosis” was first proposed by J.F.R. Kerr in 1972¹, with the definition of genetically programmed cell death. Apoptosis was predicted to be the physiological counterpart of mitosis and was believed to eliminate the excessive cells in organogenesis of the embryo. However, many in vivo and in vitro studies have revealed that various pathological stimuli, such as low doses of irradiation and drugs and infectious microorganisms can induce similar morphological changes². The term “apoptosis” is now, therefore, used not only to cover genetically programmed cell death, but also cell death in a broad spectrum of diseases including
degeneration, inflammation, cancer growth and immune reaction. J.F.R. Kerr examined human chronic active hepatitis histologically and demonstrated the morphological characteristics of apoptotic liver cells in piecemeal necrosis. Since then, many investigations have confirmed that apoptosis plays an important role in inducing hepatic cell death in human acute and chronic hepatitis of viral, alcoholic, drug-induced and autoimmune diseases. However, so far, no direct evidence has been obtained to demonstrate the significance of apoptosis in the occurrence of massive liver cell death in fulminant hepatic failure.

TUNEL (Terminal deoxynucleotidyl transferase-mediated biotin-dUTP Nick End Labeling), which was introduced by Gabrieli et al. in 1992, is a procedure by which the 3'-OH terminal of fragmented double-stranded DNA in apoptotic nuclei is labeled with biotinylated deoxyuridine triphosphate (d-UTP) by terminal deoxynucleotidyl transferase (TdT). This procedure is now used widely to detect apoptotic cells histologically or cytologically. On the other hand, specific proteins such as Fas, Bcl-2 and Bax have been discovered to induce or inhibit apoptosis and their expression can be detected by immunohistochemical methods using monoclonal or polyclonal antibodies against these proteins. In the present study, autopsied liver tissues of fulminant and other types of acute hepatic failure were investigated to detect apoptotic cells with the TUNEL method. Furthermore, the expression of Fas, Bax and Bcl-2 proteins was examined immunohistochemically, and the role of apoptosis in the occurrence of massive or submassive liver cell death was considered.

**Materials and Methods**

**Materials:** Forty-nine liver tissues obtained from various types of acute hepatic failure cases autopsied in the period between 1975 and 1997 at the following institutes were used in the present study: Tokyo Medical and Dental University Hospital, International Medical Center of Japan, Kitasato University Hospital, Japan Red Cross Medical Center and Tsuchiura Kyodo Hospital. Ages of the patients ranged from 13 neonatal days to 87 years. Seventeen cases were male and 32 female. Three clinical types of acute hepatic failure were classified clinically according to the duration from the onset of jaundice to coma: fulminant hepatic failure (FHF; shorter than 4 weeks), subacute hepatic failure (SAHF; between 4-24 weeks) and acute-on-chronic hepatic failure (AoCHF; preceding chronic hepatitis or liver cirrhosis), and the case numbers were 26, 13 and 10, respectively. A case of chronic active hepatitis and a case without hepatic disease were used as the negative controls. Hepatitis B virus (HBV) was the clinically determined etiology in 11 cases, hepatitis C (HCV) in 2, non-A non-B virus in 5, drugs in 4, autoimmune disorder in 4, and unknown etiology in 23 cases. Seventy-nine cases of acute hepatic failure were initially examined, but 24 cases were not reactive to primary antibodies probably due to their post-mortem times longer than 10 hours. In addition, the results of 6 cases could not be assessed because all of their liver cells had disappeared. Therefore, the remaining 49 cases whose post-mortem times were shorter than 10 hours were finally used for the present study.

All of the liver tissues were formalin-fixed and paraffin-embedded. Sections were cut in a thickness of 4 micrometers, stained with hematoxylin-eosin (HE) and used for the TUNEL method and immunohistochemistry. As the positive controls, surgically resected skin and a hyperplastic tonsil were used for TUNEL and Fas-immunohistochemistry, a normal lymph node for Bcl-2, and breast cancer tissue for Bax.

**TUNEL method:** TUNEL was carried out using the Megstain Apoptosis Kit (Medical and Biological Laboratories Co. Ltd., Japan). Liver tissue sections were deparaffinized with xylene and washed with concentration-degraded alcohol and distilled water. After rinsing with phosphate-buffered saline (PBS, pH7.2) at 37°C, the sections were treated with proteinase K at 37°C for 30 minutes, then washed with distilled water twice, for 5 minutes each. The tissue sections were treated with 3% H2O2 methanol solution for 5 minutes at room temperature to inactivate intrinsic peroxidase, after which they were rinsed with PBS. Next, the sections were rinsed with terminal deoxynucleotidyl transferase (TdT) buffer for 10 minutes at room temperature, and incubated with a TdT solution (0.3 equivalent U/μl TdT and 0.04 nmol/μl biotinylated dUTP in TdT buffer) in a moist chamber for 1 hour at 37°C. After rinsing in 30 mM sodium citrate and 300 mM NaCl buffer solution for 15 minutes at room temperature and washing with distilled water, the tissue sections were finally treated with peroxidase-conjugated streptavidin (DAKO, Denmark) for 30 minutes at 37°C. The positive reactions were visualized with a 0.3% diaminobenzidine (DAB; Wako, Japan) solution, and the tissues counterstained with 2% methyl green. A similar process without TdT was conducted for the neg-
ative control.

Immunohistochemistry: After deparaffinization, the tissue sections were treated with 3% H2O2 methanol solution to block intrinsic peroxidase. For retrieval of Bcl-2 and Bax antigenicity, the sections were pretreated with microwaves 3 times, for 5 minutes each, in citrate buffer at 95°C. After blocking the non-specific reaction with bovine serum albumin, the tissue sections were incubated with rabbit polyclonal anti-Fas antibodies (Nichirei Co., Japan) for one night at 4°C, rabbit polyclonal anti-Bax antibodies (Pharmingen Co., USA) and mouse monoclonal anti-Bcl-2 antibody 124 (DAKO, Denmark) for 30 min. at 37°C, respectively. The anti-Fas antibodies used are specific to the intracellular domain of Fas. Next, the sections were treated with a peroxidase-labeled polymer reagent in Envision kit (DAKO, Denmark) for 1 hour at room temperature. Finally, the positive reaction was visualized by 0.3% DAB and the sections were counterstained with 2% methyl green solution or Carazzi’s hematoxylin. A similar process without the primary antibody was carried out for the negative control.

Observation and Grading: The histopathologic characteristics of the liver were observed using the HE-stained specimens, focusing in particular on the degree of hepatic lobular injury and mononuclear inflammatory cell infiltration. The degree of hepatic lobular injury was classified into 4 grades from − to 3+: −: no or minimal, 1+: focal or zonal injury, 2+: interlobular or submassive confluent injury and 3+: massive panlobular injury. The degree of inflammatory cell infiltration was also classified into 4 grades from − to 3+: −: non or minimal, 1+: slight, 2+: moderate and 3+: marked. The frequency of TUNEL-positive liver cells (TUNEL positivity) was classified into 4 grades from − to 3+: −: a few in a specimen, 1+: a few per one 200x field, 2+: several in every remaining lobule and 3+: many in clusters. Positivity or negativity of Fas, Bax and Bcl-2 was determined by comparing with the stainability of the background hepatic or inflammatory cells of the control liver tissues and positive cells in the lymph node, skin, tonsillar and breast cancer tissues. Percentages of the cases which showed − ~ 3+ lobular injury, inflammatory cell infiltration, and TUNEL positivity were then calculated. The percentages of Fas- Bcl-2- and Bax-positive cases (Fas, Bcl-2 and Bax positivity) were calculated in the 3 clinical types of acute hepatic failure and the groups showing different grades of lobular injury or TUNEL positivity, respectively.

Statistical analysis: The Kruskal-Wallis test was applied to statistical analysis of the differences in the grades of hepatic lobular injury, the grades of inflammatory cell infiltration, TUNEL or Bax positivity among the 3 clinical types and the clinically determined 5 etiology groups. The cases of HCV and non-A non-B hepatitis were examined altogether because of the following two reasons. The first was that the numbers of cases were too small for the statistical analysis. The second reason was that the diagnosis of non-A non-B hepatitis had been used for most cases of HCV hepatitis before 1989 when the detection of anti-HCV antibody came into practice in Japan. Spearman’s correlation coefficient obtained by the rank test was used for the statistical analysis of the correlation between the grades of lobular injury and inflammatory cell infiltration or TUNEL positivity, between the grades of inflammatory cell infiltration and TUNEL positivity, and between TUNEL and Bax positivity.

Results

Histological appearance

The liver tissues of 49 cases of acute hepatic failure showed various degrees of lobular injury while no or minimal lobular injury was seen in the 2 control cases. Most parts of the lobules were destroyed and a small number of the liver cells remained in the cases of 3+ lobular injury (Fig.1a). More than half of the lobules had disappeared in the cases of 2+ injury (Fig.1b), and most of the lobules were preserved in the cases of 1+ injury (Fig.1c). The liver tissues in all of the FHF and 9 out of 10 cases (90%) of the AoCHF cases demonstrated 3+ massive or 2+ submassive hepatic lobular injury. On the other hand, 6 out of 13 cases (46%) of SAHF showed 3+ or 2+ lobular injury, while the remaining 7 cases showed 1+ injury. The grades of lobular injury were significantly higher in the FHF and AoCHF cases than the SAHF cases by Kruskal-Wallis test (Fig.2).

In the injured hepatic lobules, the structure of the hepatic cell cords and sinusoids was destroyed, liver cells had degenerated and hemorrhage was prominently observed. Acidophilic bodies or Councilman bodies showing shrunken eosinophilic cytoplasm with or without pyknotic nuclei were observed frequently in the remaining areas of the injured hepatic lobules, in particular at the boundary zone between the injured and remaining areas (Fig.1b). Degenerative liver cells showing crescentic or ring-like nuclear chromatin condensation, karyorrhexis (chromatin fragmentation) and phagocytosis by adjacent hepatic cells or
macrophages, were observed in the injured hepatic lobules. These findings were markedly observed in the cases showing 3+ or 2+ lobular injury, but were inconspicuous in 1+ cases.

Inflammatory cells, mainly lymphocytes, infiltrated the portal areas in various grades (Fig.1). Lymphocytes were rarely seen in the areas of massive or submassive hepatic lobular injury. Among the 49 cases examined, 18 cases showed 3+, 27 cases 2+ and 4 cases 1+ mononuclear cell infiltration, while the 2 control cases showed no or minimal mononuclear cell infiltration. However, no significant difference was noted in the grades of inflammatory cell infiltration among the 3 clinical types of acute hepatic failure.

In the AoCHF cases, fibrosis of the portal areas in various degrees from portal enlargement to liver cirrhosis was observed (Fig.1c), which suggested that chronic hepatitis had preceded the acute hepatic failure in these cases. Proliferation of small bile ducts was observed frequently in the portal areas in some of the cases.

**TUNEL-positive cells**

TUNEL-positive liver cells were identified in 26 cases by their dark brown-colored nuclei. Some of the TUNEL-positive liver cells showed coarse granular, pyknotic or fragmented chromatin, but others had round nuclei with no or minimal morphological changes (Fig.3). The cytoplasm of most of the TUNEL-positive liver cells was not stained, but some showed dark brown cytoplasm. The positive liver cells were found frequently in the boundary areas between the injured and remaining lobules, and a few positive liver cells were found occasionally in the remaining lob-
A histogram showing the grade of lobular injury (−: non or minimal, 1+: focal or zonal, 2+: submassive, 3+: massive) in the 3 different types of hepatic failure (N: normal, CA: chronic active hepatitis, SA: subacute hepatic failure, AoC: acute-on-chronic hepatic failure, F: fulminant hepatic failure). The vertical axis indicates the percentage of cases that showed −, 1+, 2+, and 3+. The number of cases for each type of hepatic failure is shown in the legend (N:20, CA:14, AoC:10, F:26). Type of Hepatic Failure: number of cases.

Fig. 2. A histogram showing the grade of lobular injury (−: non or minimal, 1+: focal or zonal, 2+: submassive, 3+: massive) in the 3 different types of hepatic failure (N: normal, CA: chronic active hepatitis, SA: subacute hepatic failure, AoC: acute-on-chronic hepatic failure, F: fulminant hepatic failure). The vertical axis indicates the percentage of cases that showed −, 1+, 2+, and 3+ lobular injury. FHF and AoCHF cases showed significantly higher grades of lobular injury than SAHF cases by the Kruskal-Wallis test (p<0.005).

ules. TUNEL-positive inflammatory cells were also found frequently in the liver tissues, which were round and smaller than the liver cells, had scarce cytoplasm, and were located outside the hepatic cell cords (Fig.3b). The frequency of TUNEL-positive liver cells (TUNEL positivity) was assessed as 3+ in 8, 2+ in 10, and 1+ in 10 cases, whereas the remaining 21 cases and the 2 control cases showed no or a few positive cells and they were assessed as −.

TUNEL-positive liver cells were observed in 18 out of 26 (69%) cases of FHF, 6 out of 10 cases (60%) in AoCHF and 4 out of 13 cases (31%) of SAHF. The TUNEL positivity was significantly higher in FHF and AoCHF than SAHF by Kruskal-Wallis test (Fig.4). On the contrary, TUNEL positivity did not show any significant difference among the 5 etiological groups (Fig.5). TUNEL-positive liver cells were identified in 6 out of 7 (86%) cases of 3+ lobular injury, 19 out of 34 (56%) cases of 2+ and 3 out of 8 (38%) cases of 1+. TUNEL-positive liver cells were identified in 13 out of 18 cases (72%) of 3+ inflammatory cell infiltration, 14 out of 27 cases (52%) of 2+ and 1 out of 4 cases (25%) of 1+. Significant correlations were seen between TUNEL positivity and the grades of lobular injury or inflammatory cell infiltration with Spearman's correlation coefficient determined by the rank test (Fig.6, 7).

Immunohistochemistry for Fas, Bax and Bcl-2

Both Bax and Fas were more strongly expressed in the injured liver tissues than in the 2 control cases in which the liver cells were stained in a weak background level. Bax was expressed in 24 out of 49 cases in which the cytoplasm of the liver cells was positive and the nuclei were not stained in every case. The cell membrane or cytoplasm of small bile ducts proliferating in the portal areas was positive in 2 cases (Fig.8a). Fas was expressed in 15 cases, in which the cytoplasm of the liver cells was stained positively in 8 cases, while macrophages were stained in 5 cases and proliferated small bile ducts in 2 cases (Fig.8b). Bcl-2 was expressed on the infiltrating lymphocytes in 19 cases (Fig.8c), but not on the liver cells in any of the cases.

Percentage of Bax-positive cases on the liver cells (Bax positivity) was significantly higher in the FHF and AoCHF than the SAHF groups by the Kruskal-Wallis test (Fig.9). However, Bax positivity was not significantly different among the 5 groups of different etiology. Bax positivity in the groups of 3+, 2+, 1+ and -TUNEL-positive cases were 70%, 68%, 50% and 25%, respectively, and Bax positivity was significantly higher in the groups of 3+ and 2+ TUNEL-positive cases than in 1+ cases by Spearman's correlation coefficient determined by the rank test (Fig.10). On the other hand, Fas and Bcl-2 expression did not show any statistical correlation with the degree of hepatic lobular injury or TUNEL positivity, and did not show significant differences either among the 3 clinical types of acute hepatic failure or the 5 etiological groups.

Discussion

In the present study, the liver tissue in all of the FHF and most of the AoCHF cases demonstrated 3+ massive or 2+ submassive hepatic lobular injury. On the other hand, 6 out of 13 cases of SAHF showed 3+ or 2+ lobular injury, while the remaining 7 case showed 1+ injury. The TUNEL-positive liver cells, in which fragmented double-stranded DNA in apoptosis was stained, were identified in most of the cases showing massive or submassive lobular injury. The degree of hepatic lobular injury showed significant positive correlation with TUNEL positivity. Furthermore, TUNEL positivity showed significant positive correlation with Bax positivity. Our results, therefore, suggested that apoptosis plays an important role in the occurrence of massive or submassive hepatic lobular injury in acute hepatic failure, irrespective of the etiology. Bax also appeared to be related to liver cell apoptosis.

Acidophilic or Councilman bodies identified with
Fig. 3. Histological pictures of TUNEL-positive liver cells.

(a) Abundant (grade 3+) TUNEL-positive liver cells showing brown nuclei are observed in the hepatic lobule (a case of FHF of unknown etiology in a 49-year-old female). The TUNEL-positive liver cells have wide cytoplasm and form the liver cell cords. The TUNEL-negative cells show pale or gray nuclei (x200).

(b) Relatively many (grade 2+) TUNEL-positive liver cells are scattered in the remaining lobules (a case of FHF of unknown etiology in a 58-year-old female). TUNEL-positive inflammatory cells (arrowheads) can be observed in the sinusoids, which have smaller nuclei and narrower cytoplasm than the liver cells (x200).

(c) A small number (grade 1+) of TUNEL-positive cells (arrowheads) are observed in the remaining part of the injured lobule (a case of FHF of unknown etiology in an 18-year-old female case, x200).

Fig. 4. A histogram showing TUNEL positivity (−: a few in a specimen, 1+: a few per one X200 field, 2+: several in every remaining lobule, 3+: many in clusters) in the 3 different clinical types of acute hepatic failure (N: normal, CA: chronic active hepatitis, SA: subacute hepatic failure, AoC: Acute-on-chronic hepatic failure, F: fulminant hepatic failure). The vertical axis indicates the percentage of cases that show − 3+ TUNEL positivity. The TUNEL positivity is significantly higher in FHF and AoCHF than SAHF by Kruskal-Wallis test (p<0.05).

Fig. 5. A histogram showing TUNEL positivity (−: a few in a specimen, 1+: a few per one X200 field, 2+: several in every remaining lobule, 3+: many in clusters) in the 5 groups of different etiology including unknown cause (AI: autoimmune, D: drug, HB: hepatitis B virus, NANN: hepatitis C or non-A non-B viruses, UK: unknown). The vertical axis indicates the percentage of cases that showed − 3+ lobular injury. No significant difference was observed among the 5 groups by Kruskal-Wallis test.
Fig. 6. A histogram showing the relationship between TUNEL positivity (−: a few in a specimen, 1+: a few per one X200 field, 2+: several in every remaining lobule, 3+: many in clusters) and the grade of lobular injury (−: non or minimal, 1+: focal or zonal, 2+: submassive, 3+: massive). The vertical axis shows the percentage of cases that showed −→−3+ TUNEL positivity. The TUNEL positivity was significantly highest in the cases of 3+ lobular injury, higher in the cases of 2+ injury and lowest in the cases of 1+ injury by Spearman’s correlation coefficient determined by the rank test (p<0.05). (rs = 0.43)

Fig. 7. A histogram showing the relationship between TUNEL positivity (−: a few in a specimen, 1+: a few per one X200 field, 2+: several in every remaining lobule, 3+: many in clusters) and the grade of inflammatory cell infiltration (−: non or minimal, 1+: slight, 2+: moderate, 3+: marked). The vertical axis shows the percentage of the cases that show −→−3+ TUNEL positivity. The TUNEL positivity was significantly highest in the cases of 3+ inflammatory cell infiltration, higher in the cases of 2+ infiltration and lowest in the cases of 1+ infiltration by Spearman’s correlation coefficient determined by the rank test (p<0.05). (rs = 0.39)

Fig. 8. Histological pictures of immunohistochemical staining of Bax, Fas and Bcl-2. The positive cells show brown cytoplasm. Bax (a, left, x200) and Fas (b, upper right, x200) are expressed on the liver cells in the hepatic lobule (L) and small bile ducts (arrowheads). Bcl-2 (c, lower right, x200) is expressed on the lymphocytes in a small follicle in the portal area.

condensed eosinophilic cytoplasm with or without pyknotic nuclei were observed frequently in the liver tissues. The acidophilic body seen in the HE-stained liver tissue had been considered to be a solitary liver cell necrosis in acute or chronic active hepatitis. However, J.F.R. Kerr demonstrated with electron microscopy that the acidophilic body was a morphological manifestation of the cell in apoptosis in human and experimental animal liver tissues\(^3\), and the acidophilic body is now presumed to be the apoptotic body. In addition to
the acidophilic body, other types of apoptotic cells have been reported to show crescentic or ring-like chromatin condensation, karyorhexis and nuclear fragmentation. However, not only the acidophilic bodies, but also the other types of apoptotic cells could not be discriminated completely from cells showing coagulative necrosis or degeneration. Therefore, the TUNEL method was employed to detect apoptotic cells precisely in the present study.

Many studies using the TUNEL method have revealed the role of apoptosis in the occurrence of various diseases of humans and experimental animals. TUNEL-positive cells were detected in the human lymph node of necrotizing lymphadenitis, in the rat heart of experimental acute myocardial infarction with coronary artery ligation and in the rat pancreas of caerulein-induced acute pancreatitis. However, TUNEL has been reported to be positive on necrotic cells as well as apoptotic cells. Autolysis and tissue processing of fixation, embedding and sectioning might increase TUNEL positivity. Such false-positive cells, nevertheless, can be discriminated from truly "apoptotic" TUNEL-positive cells by the morphological features of the nuclei, cytoplasmic stainability, and the distribution pattern in the tissue. As for cytoplasmic stainability, it was reported that the nucleus of "apoptotic" TUNEL-positive cell was singly stained and the cytoplasm was not, whereas both the nucleus and the cytoplasm of "necrotic" TUNEL-positive cells were stained. Most of the liver cells positive for TUNEL on the nuclei, therefore, could be identified as "apoptotic"

TUNEL-positive cells.

The frequency of TUNEL-positive cells (TUNEL positivity) was significantly higher in the cases of 3+ and 2+ than 1+ lobular injury. In addition, the percentage of cases of 3+ or 2+ lobular injury was significantly higher in FHF and AoCHF than in SAHF cases. These findings suggested that apoptotic cells were identified at a higher frequency in the more injured liver tissues of the severer type of hepatic failure. However, apoptotic cells have been reported to disappear with rapid draining by the blood flow or phagocytosis by neighboring liver cells or macrophages within a few hours. Accordingly, apoptotic liver cells were predicted to have disappeared in the TUNEL-negative cases of 3+ or 2+ lobular injury. It is, therefore, concluded that apoptosis plays an important role in massive or submassive lobular injury.

Liver cell apoptosis can be induced by different causative factors, such as direct viral cytotoxicity, cell-mediated immune response, drug, alcohol and even bile salt. As for viral hepatitis, cytotoxic T lymphocyte-associated liver cell apoptosis via the Fas-Fas ligand system has been reported to play a significant role. The grades of lymphocyte infiltration were significantly well correlated with TUNEL positivity, and it was predicted that liver cell death was due to a cell-mediated immune response. However, the grade of inflammatory cell infiltration had no significant correlation with the
grade of lobular injury in the present study. Moreover, Fas expression did not show any correlation with the degrees of hepatic lobular injury and TUNEL positivity. The cell-mediated apoptosis involving the Fas-Fas ligand system, therefore, may not play a significant role in the occurrence of massive or submassive lobular injury in acute hepatic failure.

Bax proteins were more strongly expressed on the liver cells of FHF and AoCHF than SAHF. Bax positivity was significantly correlated with TUNEL positivity, and Bax appeared to induce liver cell apoptosis in the present study. Bax is considered to accelerate apoptosis via competition with Bcl-2 which is a major inhibitor of apoptosis. Human liver cells, however, do not express Bcl-2 under physiological conditions. Therefore, the role of Bax in liver cell apoptosis remained unclear.

The results of the present study suggested that apoptosis plays an important role in the occurrence of massive or submassive hepatic lobular injury. Fas and Bcl-2 were not involved in the process of liver cell apoptosis. Although Bax expression was positively correlated with the grades of lobular injury and TUNEL positivity, the mechanism could not be explained. Another pathway activated by Bax might induce apoptosis of the liver cells.

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