

, Tdt (terminal dioxynucleotidyl transferase)
 cytokine digoxigenine
 , anti-digoxigenine-peroxidase DAB
 cytokine (3,3-diaminobenzidine) . Apoptosis
 cytokine 1 (interleukin 1, (400) apoptosis
 IL-1), 6 (interleukin 6, IL-6),
 (tumor necrosis factor, TNF) .³⁾ Cytokine IL-6
 , TNF, IL-1 cytokine , ,
 , cytokine LSAB (Labeled Streptavidin-biotin, DAKO, Cartenteria,
 .⁶⁾ TNF USA)kit Hsu ⁹⁾ ,
 (cytotoxicity) , DNA 가 programmed cell IL-1, IL-4 IL-6 TNF
 death , apoptosis .⁶⁾ 5 μm
 4(interleukin 4, IL-4) H₂O₂
 .⁷⁾ (endogenous peroxidase) ,
 (phosphate buffered saline, PBS, pH 7.2)
 IL-4 IL-6 30 , TNF 1 , IL-1 10
 apoptosis , PBS
 apoptosis cytokine (antirabbit IgG antibody)
 .⁸⁾ 20 PBS 가
 cytokine apoptosis streptavidin 20 AEC
 . (3-amino-9- ethylcarbazole) . Mayer's
 hematoxylin
 IL-1, IL-4, IL-6 TNF
 grade .
 PBS ,
 가 가 grade 3,
 가 grade 2,
 grade 1 .
 IL-6 IL-6
 (IL-6 dependent murine hybridoma subclone) B 9.55
 IL-6 . B 9.55
 10% 37.5
 72 , 6
 0.5 μCi ³H-thymidine (specific activity;
 , 1/2 10%
 10%
 5 μm
 hematoxylin-eosin
 . Apoptosis (400)
 apoptosis , in situ nick
 end labelling method , proteinase K
 Apoptag kit (Oncor, Gaithersburg, USA)

2 : Apoptosis cytokine

84.8 Ci/mmol) . DNA liquid Kupffer cell (Fig. 1C, Fig. 2C).
 , scintillation counter (Beckman, LS 6000A, Brea, USA) , 7 apoptosis 2.3
 IL-6 cytokine In situ detection method , 1
 , apoptosis , IL-6 , (Fig. 2A, B) 8.2 , 14.8
 , group ttest apoptosis가 3 (Fig. 2C, D) 9.4
 , 0.05 가 1 7 , 14
 (Table 1)
 IL-1 1 3
 , (Fig. 3A). IL-4
 가 (Fig. 3B). TNF IL-1 1 3
 가 (Fig. 3C, D). IL-6 1 3
 (Fig. 1A), 1
 sinusoidal space가 Kupffer cell (Table 2, Fig. 4).
 (Fig. 1B). Apoptosis 1
 8.2 (Fig. 2A), 3 6.4

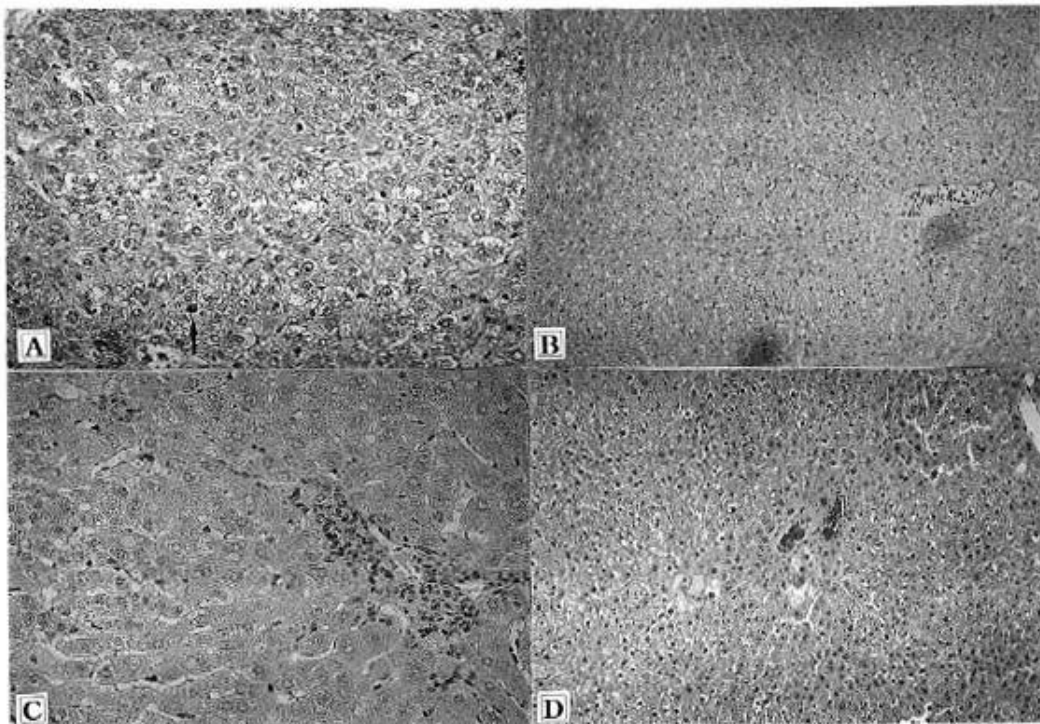


Fig. 1. Histopathologic findings in control and irradiated liver tissue. A) Hepatic lobular architecture is well preserved in normal control tissue (H&E stain, x100). B) Hydrophic swelling of hepatocytes and increased apoptotic bodies (arrow) are observed in postirradiation 1 day (H&E stain, x200). C) Postirradiation 3 days revealed mild infiltration of inflammatory cells & Kupffer cell mobilization (H&E stain, x200). D) Mild fibrotic change is shown in postirradiation 7 days (H&E stain, x100).

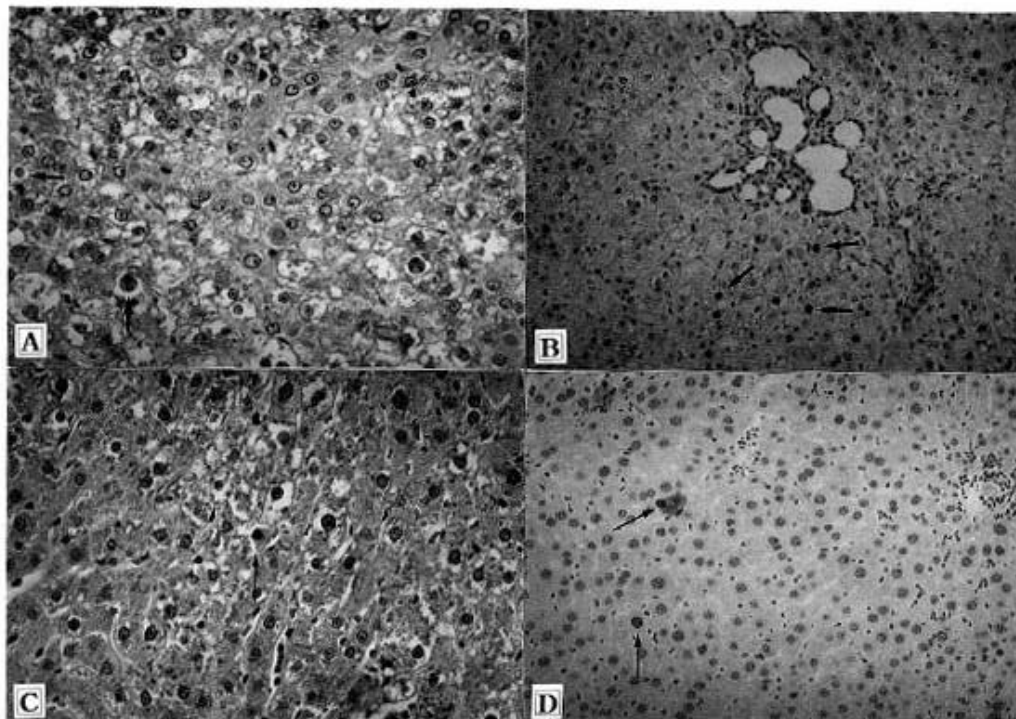


Fig. 2. Apoptotic bodies(arrows) are frequently seen in postirradiation 1 and 3 days.A) Apoptotic bodies in light microscopic findings in postirradiation 1 day (H&E stain, ×200).B) Apoptotic bodies detection with In situ Nick end labelling method in postirradiation 1 day(In situ detection, ×200).C) Apoptotic bodies in light microscopic finding in postirradiation 3 days (H&E stain, ×200).D) Apoptotic bodies in In situ detection in postirradiation 3 days(×200).

Table 1. Number of Apoptotic Cells Induced by 8 Gy irradiation

method	control	post-irradiation day			
		1 day days	3 days 14 days	5 days	7
Light microscope	1.5± 2.3±	8.2± 2.4±	6.4±	3.5±	
In situ detection	1.4±	14.8±	9.4±	6.7±	

*p<0.05, No. of apoptosis : mean±SD.

Table 2. Immunohistochemical Stain of Cytokines

	control	post-irradiation day				
		1 day	3 days	5 days	7 days	14 days
IL-1	-	+	+	-	-	-
IL-4	-	-	-	-	-	-
IL-6	-	+++	+++	++	+	+
TNF	-	++	+	-	-	-

+ : Focal stain, ++ : Diffuse weakly stain, +++ : Diffuse strongly stain

IL-6 가 , 1 가 , (Table 3). IL-6 1 가 , 14 가 (Table 4). (Table 3). IL-6

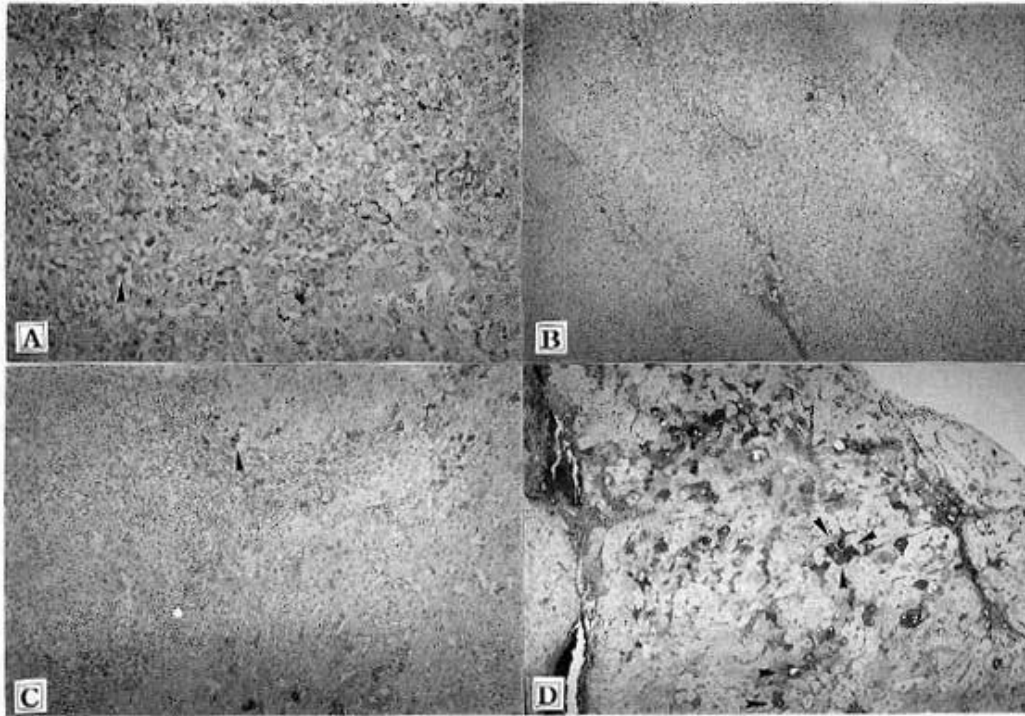


Fig. 3. Immunohistochemical stain of IL-1, IL-4, and TNF in the rat's liver. A) IL-1 is weakly expressed (arrowhead) in postirradiation 1 day (Immunostain, $\times 200$). B) IL-4 is not detected (Immunostain, $\times 200$). C) TNF expression is highest in postirradiation 1 day (Immunostain, $\times 100$). D) TNF expression is decreased in postirradiation 3 days (Immunostain, $\times 100$).

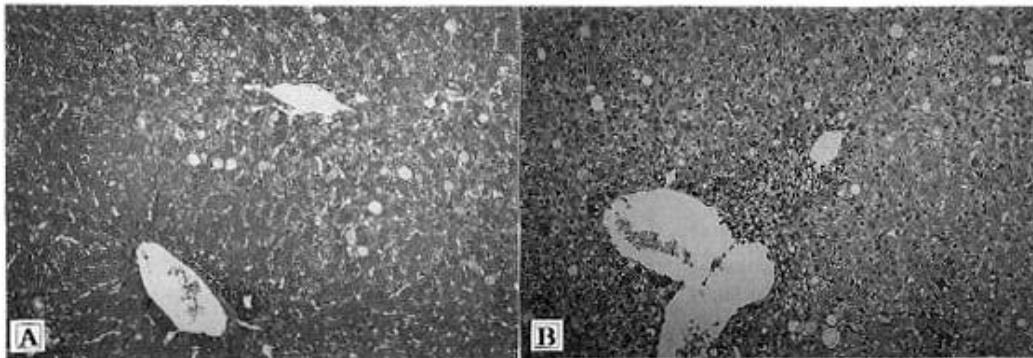


Fig. 4. Immunohistochemical stain of IL-6 in the rat's liver. A) Strong and diffuse expression of IL-6 is demonstrated in postirradiation 1 and 3 day (Immunostain, $\times 100$). B) IL-6 expression is markedly decreased in postirradiation 5 days (Immunostain, $\times 100$).

apoptosis , apoptosis

.¹⁰⁾

가

apoptosis , DNA

2 : Apoptosis cytokine

25) cytokine

(bioassay), (RIA) apoptosis cytokine

1 3 IL-6가 cytokine apoptosis

가 5 , 7 가

apoptosis

IL-6 IL-6가

가 가 IL-6

IL-6가 IL-6

가 apoptosis 가

TNF 17kD cachectin

가 21) TNF IL-6 IL-1

IL-6 26)

TNF 가

TNF spingomyelin

ceramide 가 apoptosis

27 30) Krivenko 31) 9 Gy 24 7

48 2.3 TNF 가 TNF

cytokine 가 가

가 TNF가

31) TNF 1 3 cytokine 가

가 TNF

1 3 IL-1, IL-6 TNF가

가 apoptosis

cytokine apoptosis가

cytokine apoptosis

Kuida 32) Interleukin-1 converting enzyme (ICE) IL-6 TNF ICE

가 Fas Ab apoptosis가 ICE가

apoptosis가 IL-1 IL-6 TNF

apoptosis 32)

IL-1,

IL-6, TNF cytokine apoptosis

apoptosis cytokine 가

apoptosis cytokine 가

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Abstract

The Relationship between Radiation-Induced Apoptosis and the Expression of Cytokines in the Rat's Liver

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Purpose : To determine the role of cytokines in the apoptosis of rat's liver following irradiation.

Materials and Methods : Sprague-Dawley rats were irradiated to entire body with a single dose of 8 Gy. The rats were divided into 5 groups according to the sacrifice day after irradiation. The liver and blood after 1, 3, 5, 7, and 14 days irradiation were sampled for evaluation of mechanism of apoptosis and role of cytokine in relation to radiation-induced tissue damage. The study was composed of microscopic evaluation of liver tissue, in situ detection method for apoptosis, immunohistochemical stain of IL-1, IL-4, IL-6 and TNF, bioassay and radioimmunoassay of IL-6 in liver tissue and blood.

Results : Radiation-induced liver damage was noted from first day of radiation, and most severe parenchymal damage associated with infiltration of chronic inflammatory cells was seen in the groups of 5 days after radiation. A number of apoptosis were observed 1 day after radiation on both light microscope and in situ method. Afterwards, the number of apoptosis was gradually diminished. On immunohistochemical study, IL-1 and TNF were expressed 1, 3 days after radiation, but not expressed after that. IL-4 was not expressed in the entire groups. IL-6 was expressed with strong positivity in 1, 3 days after radiation. Bioassay and RIA of IL-6 in liver tissue and blood showed the highest value in 1 day after radiation, and the value is diminished after then.

Conclusion : Apoptosis seemed to be the important mechanism of radiation-induced liver damage, and is possibly induced by the release of cytokines, such as IL-1, IL-6, TNF in view the simultaneously increased appearance of apoptosis and cytokines.

Key Words : Radiation, Liver, Apoptosis, Cytokine