

하인두암 세포주에서 Cisplatin, 5-Fluorouracil, 방사선조사가 세포주기 및 세포고사에 미치는 영향

박홍석 · 고의경 · 이병주 · 왕수건

The Effects of Cisplatin, 5-fluorouracil, and Radiation on Cell Cycle Regulation and Apoptosis in Hypopharyngeal Carcinoma Cell Line

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—ABSTRACT—

Objectives and Background : In head and neck cancer including hypopharyngeal cancer, cisplatin and 5-fluorouracil (5-FU) usually have been used as neoadjuvant chemotherapeutic agents. We investigated the difference of the influences of cisplatin, 5-FU and radiation on p53 protein expression and cell responses (cell cycle arrest and/or apoptosis) in the hypopharyngeal carcinoma cell line (PNUH-12 ; mutant-type p53). **Method** : PNUH-12 cells were treated with cisplatin, 5-FU and radiation. The changes in the cells were assessed by cell cytotoxicity assay, Western blotting (p53 and p21^{WAF1/CIP1} proteins), DNA fragmentation assay, propidium iodide (PI) stain and DNA flow cytometry. **Results** : The expression of p53 protein was increased after treatment with cisplatin and 5-FU, but not radiation. The expression of p21^{WAF1/CIP1} protein was increased only after treatment with 5-FU, not cisplatin or radiation. With cisplatin and radiation, we observed apoptosis in both by DNA fragmentation and PI stain and increased the S phase in cisplatin and the G2 phase in radiation by DNA flow cytometry. But, with 5-FU, we couldn't observe apoptosis by DNA fragmentation and PI stain but only an increased G1 phase by DNA flow cytometry. **Conclusions** : In PNUH-12, radiation induced p53-independent apoptosis and p21^{WAF1/CIP1}-independent G2 phase cell-cycle arrest. Cisplatin induced p53-dependent apoptosis and p21^{WAF1/CIP1}-independent S phase cell-cycle arrest and 5-FU induced p53 and p21^{WAF1/CIP1}-dependent G1 phase cell-cycle arrest, not apoptosis. Cisplatin and 5-FU induced p53-dependent pathways, but radiation p53-independent pathway. The cell responses by cisplatin, 5-FU and radiation were all different pathways. Our results suggest that combined treatment with radiation and cisplatin or 5-FU may be effective because these have different molecular mechanisms of cell damage. (J Clinical Otolaryngol 2005;16:45-53)

KEY WORDS : Cisplatin · Fluorouracil · Hypopharyngeal neoplasms.

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서 론

가

방 법

가 가

세포배양

5 20~30%
 1) 가
 가 ,
 가 1)
 가 1)2)
 Cisplatin 5-fluorouracil(5-Fu)가
 platin DNA crosslinking DNA
 3)4) Cis-
 5-Fu DNA thymidy- PNUH - 12 12)
 late synthase(TS) 5)
 cisplatin alone PNUH - 12 100units/ml penicilin - streptomycin 37 ,
 20~30% , 5-Fu alone 10% FBS가 DMEM 37 ,
 15% 5-8) Cisplatin 5- cin 10% FBS가 DMEM 37 ,
 Fu 40% 5- CO2 .
 2~3 6~7 PBS
 0.05% trypsin - 0.02% EDTA
 , p53 ,
 DNA , 가 가 6~
 가 9-11) 7
 세포독성 검사
 p53- p53- 96 well plate well 1.8 x 10⁴
 cells/well , cisplatin
 p21 - p21 - p21 - 5-Fu 가 , 37 , 5% CO₂
 가 . IBL 437C irradiator(CIS Biointernational™, Paris, France)
 , p53 . 24 , 48 3 - (4,5 - dimethylthiazol -
 가 가 . 2 - yl) - 2,5 - diphenyltetrazolium bromide(MTT) 100
 p53 가 μl 가 4 for-
 PNUH - 12¹²⁾ cisplatin, 5-Fu

: Cisplatin, 5 - Fluorouracil, 가
 mazan DMSO 540 nm , 50 4
 protenage K(Gibco, Co. BRL) . Phe-
 nol extraction DNA 5.0M NaCl
Western Blot
 PNUH - 12 1 12,000rpm 10
 cisplatin, 5 - Fu, , pellet 2%
 24 lysis buffer 가 4 10 agarose gel 1 x TAE buffer 3~4 (50V)
 . 12,000rpm 10 ethidium bromide UV transillu-
 . (150 ul) 5 x sample loa- minator DNA .
 ding buffer , SDS - polyacrylamide gel
 15 V 30 gel nitro
 cellulose membrane(Amersham Corp.) .
 , nitro cellulose membrane 5% nonfat dry milk
 가 가 TNT buffer(0.5% Tween - 20, 100 mM
 Tris - cl pH 7.6 1.5M NaCl) 1
 . 1 p53(Oncogene, Ab -
 2) 500 1 . TNT buffer
 10 5 , 1000 2
 [Anti - mouse IgG, peroxidase - linked species -
 specific whole antibody(from sheep NA931 ECL™)]
 1 . TNT buffer 10
 5 , supersignal chemiluminescent
 substrate(Pierce) , radiogra-
 phic film . p21 (Bio-
 science, 610234) western blotting

Propidium iodide(PI) staining
 PNUH - 12 1 x 10⁶/ml 가 .
 48 .
 . PBS 70% ethanol 5
 , PI(propidium iodide Sigma, USA)

DNA electrophoresis
 PNUH - 12 1 x 10⁶ cell/ml seeding
 48 lysis buffer .
 4 30 4 10 12,000rpm

50 4
 . Phe-
 nol extraction DNA 5.0M NaCl
 100% isopropanol 가 - 70
 1 12,000rpm 10
 , pellet 2%
 agarose gel 1 x TAE buffer 3~4 (50V)
 ethidium bromide UV transillu-
 minator DNA .

DNA flow cytometry
 PNUH - 12 1 x 10⁵/ml
 48
 PBS . CycleTest™ PLUS DNA
 Reagent Kit(Becton Dickinson. San Jose. CA)
 DNA FACSsort flow cytometer(Bec-
 ton Dickinson. San Jose. CA)

결 과

세포독성 검사
 Cisplatin 가
 . 48 IC₅₀ 3 μM
 . 5 - Fu 가
 cell survival . 48 IC₅₀
 30 μM (Fig. 1). (1~2 Gy/min)

p53 단백질 과 p21 단백질
 Cisplatin(3 μM, 12hr) 5 - Fu(30 μM, 12hr)
 p53 가 . 5 -
 Fu p21 가가 cis-
 platin p21 가
 (Fig. 2). 4, 6, 8 Gy/min
 p53 p21 .

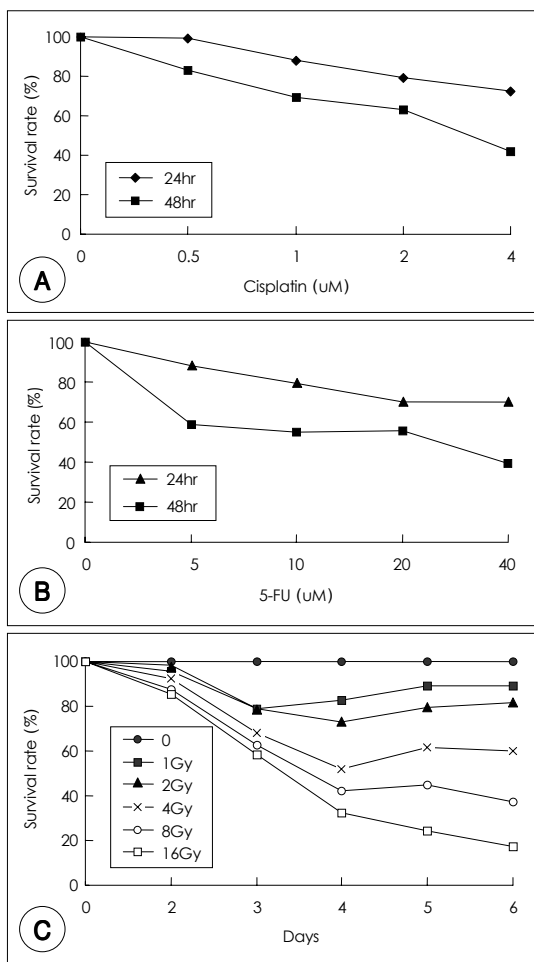


Fig. 1. Cytotoxicity of cisplatin, 5-FU, and irradiation in PNUH-12 cells with cell growth determined by MTT assay. Cells were treated with cisplatin (A) and 5-FU (B) for 24h, 48h and irradiation at 1 - 16 Gy/min for 2 - 6 days (C). Viability was expressed as percentage of cell growth relative to untreated controls (means SD). The results are derived from three different experiments.

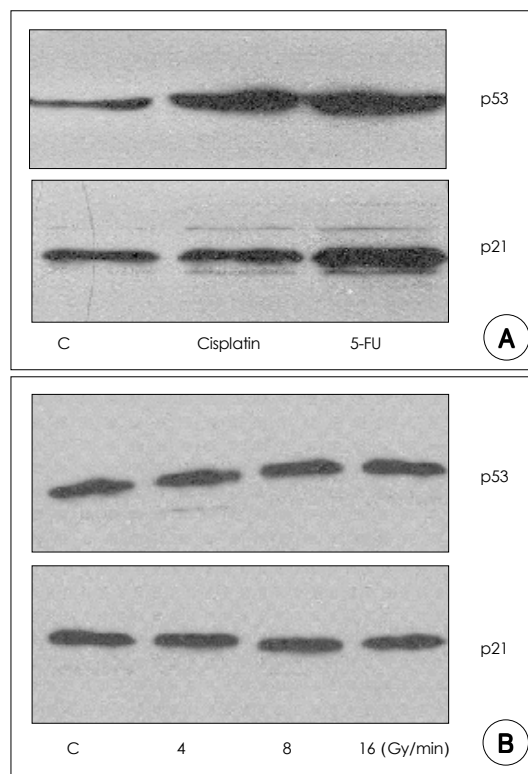


Fig. 2. Western blot analysis showing expression of p53 and p21^{WAF1/CIP1} proteins in response to cisplatin, 5-FU (A), or irradiation (B) in PNUH-12 cells. Cells were treated with cisplatin at 3 M or 5-FU at 30 M for 12h and irradiation at 4 - 16 Gy/min for 24h. The results of a single representative experiment are shown.

세포주기의 변화

Cisplatin(3 μM, 24hr)	14.2%	48.7%	가	S - phase가
5 - Fu(30 μM, 24hr)	가	가	가	5 - Fu(30 μM, 24hr)
가	(Fig. 5).	가	가	G1 phase가 64.4%
가	가	가	가	74.3%
가	가	가	가	G2 phase

세포고사

Cisplatin 48 , 8 μM DNA electrophoresis PI stain (Figs. 3 and 4). 5 - Fu 48 , 320 μM 가 가 . 32, 64, 128 Gy/min

고찰

p53 가 p53 . p53 p53

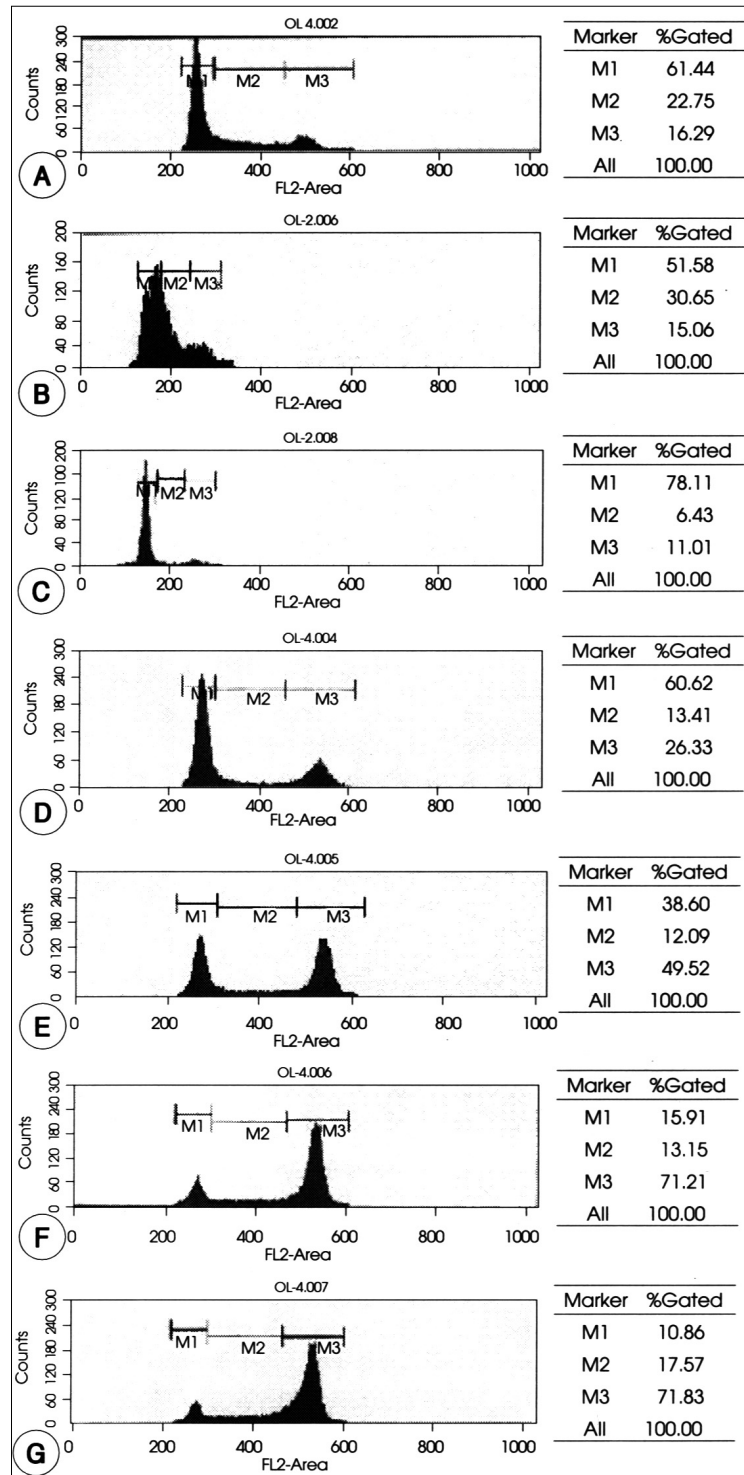


Fig. 3. Cell cycle analysis by flow cytometry. Characteristic DNA histograms of PNUH-12 cells, showing the progressive cell cycle changes observed after 24h of treatment with cisplatin at 3 M (B) or 5-FU at 30 M (C) and irradiation at 2 Gy/min (D), 4 Gy/min (E), 8 Gy/min (F), and 16 Gy/min (G), as compared with the untreated controls (A). Treated cells and controls were stained with propidium iodide, measured by FACS and cell phase distributions were determined using the CELLQuest. Marker : cell cycle. M1 : G1 phase, M2 : S phase, M3 : G2/ M phase.

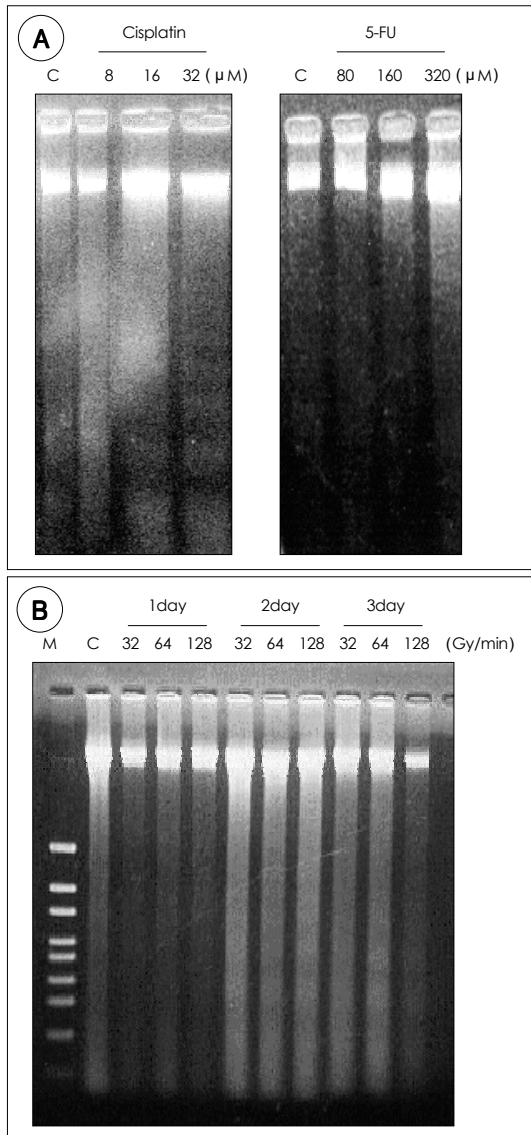


Fig. 4. DNA fragmentation analysis showing induction of apoptosis. PNUH-12 cells were treated with different concentrations of cisplatin, 5-FU as indicated for 48h (A), or with 32 - 128 Gy/min irradiation for different time intervals (B). M : 100 bp DNA marker, C : untreated control.

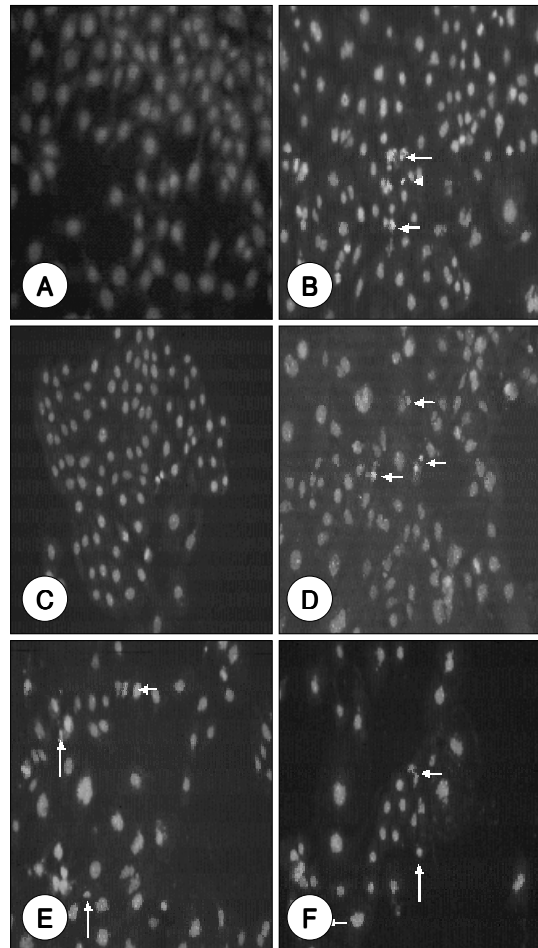
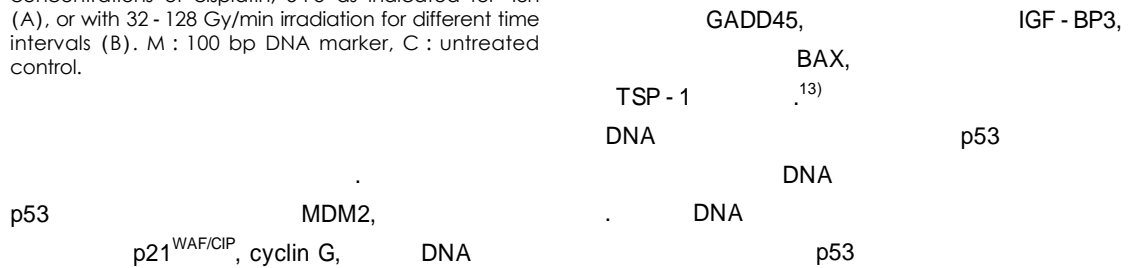
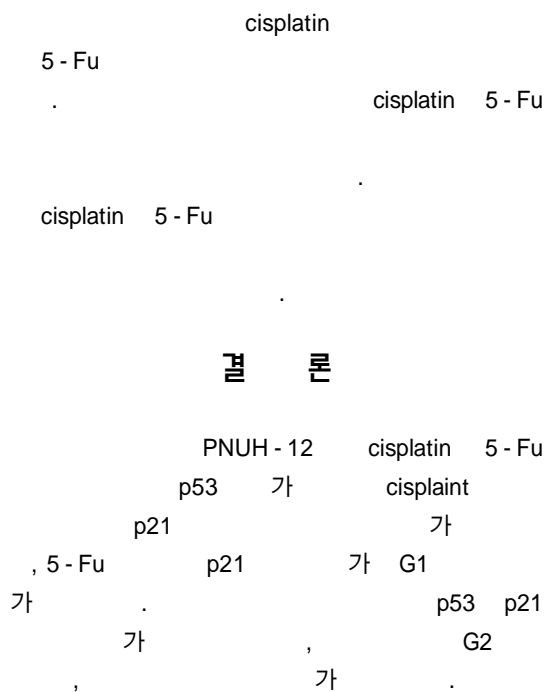


Fig. 5. Apoptosis in PNUH-12 cells treated with cisplatin, 5-FU, or irradiation by Propidium Iodide staining. The figure is a fluorescent microscopic view of nuclear staining with Propidium Iodide after the following treatments. A : Control. B : Cisplatin at 8 M, 48 h. C : 5-FU at 160 M, 48 h. D : Irradiation at 32 Gy/min, 48 h. E : Irradiation at 64 Gy/min, 48 h. F : Irradiation at 128 Gy/min, 48 h. The arrow indicates a condensed and fragmented apoptotic nucleus.



: Cisplatin, 5 - Fluorouracil, 가
¹⁴⁾ DNA p53 가 G2/M
 가 . 가 4)가 , PNUH - 12 cis-
 p53 , . platin S 가가 . Cis-
 p53 , . platin p53 가
 p53 가 가 가
¹⁵⁾ , , , 58 , 5 - Fu p53 가
 , , , , 58 p21 가 , G1
¹¹⁾ p53 가 5 - Fu p53 p21
 platin . cis- 가 p53 p21
 p53 가 가 21)가
¹⁶⁾ p53 5 -
 Fu 가 , Bcl -
 2/Bax , 17)
 p53 Bcl - 2 가가 . PNUH - 12 G2
 bleomycin p53 p21 . HeLa
 가 ¹⁸⁾ . G2 가 가 가
 p53 . 22)
 p53 - p53 - . PN- p53
 UH - 12 cisplatin 5 - Fu p53 . p53
 가 , p53 -
 PNUH - 12 cisplatin p53 p53 DNA
 가 p21 가 . p53
 , flow cytometry S 가 가 . p53
 p53 p21 가 가 ¹⁴⁾
¹⁹⁾ p53 2780CP cisplatin , , p53
 p53 p21 가 가
 , p53 가 ²⁰⁾ ²³⁾ , PNUH - 12 cisplatin 5 -
 가 . p53 가 Fu p53 가
 mRNA 가 cisplatin p53 cisplatin 가 , 5 - Fu
 p21 가 , cisplatin 가 p53
 G1/early S ,
 가 p21 p53 p53 가
¹⁶⁾ cisplatin .



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