

	(19) (12)	(KR) (A)	(11) (43)	10-2010-0040378 2010 04 20		
(51)	Int. Cl.		(71)			
	A61K 33/24 (2006.01) A61K 31/352 (2006.01)			253		
	A61P 35/00 (2006.01)					
(21)	10-2008-0099444		(72)			
(22)	2008 10 10					
	2008 10 10				502	902
				420	.	MN
				55455		
			(74)			
	:	5				
(54)	-					

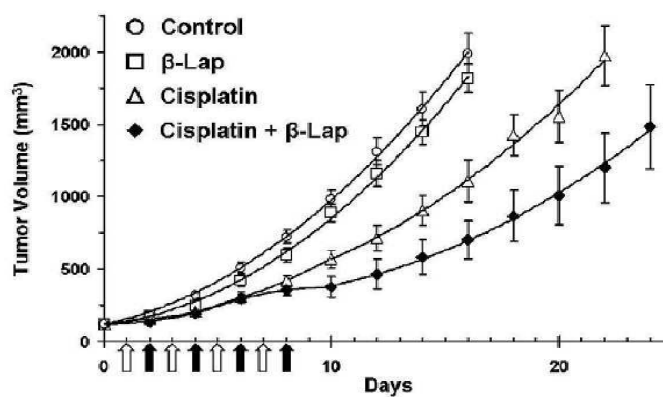
(57)

(cisplatin) - (β-lapachone)

NQ01

DNA

6



1

(cisplatin) - (β -lapachone) ,
 , - ,
 .

2

1 ,
 , 12 ~ 24 , - ,
 .

3

(cisplatin) - (β -lapachone) ,
 , - ,
 - 1 : 0.5 ~ 10 ,
 .

4

3 ,
 , 12 ~ 24 , - ,
 .

5

1 4 ,
 NQ01 , , -
 DNA ,
 .

[0001]

[0002]

- (β -lapachone) (quinone)

- [0003] - (β -lapachone) 1, 2 , (gemcitabine)
- [0004] death) (apoptosis) (clonogenic cell
- [0005] , - G1 S (apoptosis) .
- [0006] , -
- [0007] , - NQ01 NADH NADPH , DNA
- [0008] NADH NADPH Ca^{2+} , ATP , p53 , u- (calpain) , PARP
- [0009] NQ01 , - NQ01
- [0010] NQ01 16q22 , (ARE) .
- [0011] NQ01 , , (xenobiotics), 41 ~ 42 , (hypoxia) , (ionizing radiation) .
- [0012] , - NQ01
- [0013] , (cis-diamminedichloroplatinum) .
- [0014] (cis) DNA DNA 4
- [0015] .
- [0016] 10-0492016 (Ad-OA Ad-OA)
- [0017] 10-0147465 ()
- [0018] , -
- [0019] -
- [0020] NQ01 DNA
- [0021] -

[0022] (cisplatin) - (β-lapachone) ,

[0023] , -

[0024] , 12 ~ 24 -

[0025] -

[0026] , -

[0027] , FSaII , 12 ~ 24

[0028] 1 death) - , FSaII - (clonogenic

[0029] , - 12 ~ 24 - 5 μM (2B).

[0030] - DNA γ-H2AX foci .

[0031] 3 24 - γ-H2AX foci DNA

[0032] 24 NQ01 (4A) (4B)

[0033] NQ01 - NQ01 (5).

[0034] C3H FSaII 0.5 ~ 0.7 cm , -

[0035] , - 24 (6).

[0036] , - , NQ01

[0037] , -

[0038] , NQ01 , - DNA

- [0039] , , .
- [0040] < 1> 1
- [0041] - (β -Lapachone, 3,4-dihydro-22,2-dimethyl-2H-naphthol[1,22b]pyran-5,6-dione)
(cisplatin ; *cis*-diamminedichloroplatinum) (St. Louis, MO) .
- [0042] - (dimethyl sulfoxide,DMSO) 5 μ M -20 .
- [0043] - , HP- β -
CD(β -hydroxypropyl- β -cyclodextrin) .
- [0044] , .
- [0045] C3H (fibrosarcoma) FSaII , .
- [0046] , RPMI 1640 (Gibco BRI, Grand Island, NY) 10 % (Hyclone
Laboratories Inc., Logan, UT), (50 units/mL), (50 /mL) 37
(), 95 %, CO₂ 5%() .
- [0047] FSaII 23 $\pm$ 3 h .
- [0048] FSaII 5 μ M 1 , PBS 12 ,
- 2.5 μ M 4 .
- [0049] < 2> 2
- [0050] 1 , - 5.0 μ M .
- [0051] < 3> 3
- [0052] 1 , - 7.5 μ M .
- [0053] < 4> 4
- [0054] 1 , - 10 μ M .
- [0055] < 5> 5
- [0056] 1 , FSaII 5 μ M 1 , PBS
24 , - 2.5 μ M 4 .
- [0057] < 6> 6
- [0058] 5 , - 5 μ M .
- [0059] < 7> 7
- [0060] 5 , - 7.5 μ M .

- [0061] < 8> 8
- [0062] 5 , - 10 μM .
- [0063] < 1 ~ 4>
- [0064] (2.5, 5, 7.5, 10 μM) , 1 .
- [0065] < 5 ~ 8>
- [0066] - (2.5, 5, 7.5, 10 μM) , 4 .
- [0067] < 1>
- [0068] 1.
- [0069] C3H (fibrosarcoma) FSaII ,
- [0070] , FSaII 23 $\pm$ 3 h .
- [0071] (exponential growth phase) EDTA 1 mmol/L (0.25 %)
- 10 .
- [0072] , 10 % RPMI 1640 , RPMI 1640 5 mL T-25 25 cm^3 .
- [0073] 95%, CO₂ 5% 37 ,
- (1 8) , .
- [0074] (10:1 v/v) , , 1 %
- (crystal violet) .
- [0075] 50 , .
- [0076] 2.
- [0077] 1) - FSaII
- [0078] , 1 .
- [0079] , - (1 4) - 2.5 μM ,
- [0080] (5 8) .
- [0081] 2) - FSaII
- [0082] FSaII - 2A .
- [0083] 5 μM (2) 14.0 $\pm$ 1.5 % (2A-a), - 5 μM (6) 18.1 $\pm$ 2.9 % (2A-b) .
- [0084] 5 μM , - 5 μM 2.5 % (2A-c) .
- [0085] 5 μM , - 5 μM 2.1

$\pm 0.5 \%$ (2A-d).

- [0086] 5 μM , 12 - 5 μM (2)
1.4 $\pm 0.7 \%$ (2A-e).
- [0087] 5 μM , 24 - 5 μM (6)
0.24 $\pm 0.5 \%$ (2A-f).
- [0088] , - , .
, 12 ~ 24 - , .
- [0089] , - FSaII
(2B).
- [0090] , 5 8 5 8 ,
2B .
- [0091] 2B , 24 , - (5
8) .
- [0092] , 24 NQ01 - .
- [0093] < 2> γ -H2AX foci
- [0094] 1.
- [0095] FSaII DNA H2AX (γ -H2AX) foci
.
- [0096] , 37 , 5 μM , -
5 μM - .
- [0097] PBS 2 , 1:1 20 , 1 anti- γ -
H2AX (1:300 dilution in 1% bovine serum albumin) , 1 FITC (Invitrogen Co.)
2 .
- [0098] PBS 3 , 4',6'-diamidine-2-phenylindole(DAPI; Invitrogen Co)
antifade .
- [0099] γ -H2AX foci .
- [0100] 2.
- [0101] 3(A,B) , , γ -H2AX foci 2 ~ 7
, 7 foci γ -H2AX .
- [0102] γ -H2AX , DAPI γ -H2AX %
.
- [0103] 5 μM 0 24 43.8 % 66.7 % γ -H2AX
.
- [0104] - 5 μM 1 , 73.3 % γ -H2AX .
- [0105] 5 μM , 0 24 - 5 μM ,
77.7 % 92.2 % γ -H2AX .
- [0106] , γ -H2AX % 24 , - 24
DNA γ -H2AX foci

- [0107] < 3> NQ01
- [0108] 1.
- [0109] NQ01 (immunofluorescence microscopy metho
d)
- [0110] (exponential growth phase) FSaII 2 μ M 1 ,
, 37 , PBS .
- [0111] 1 : 1 20 , 1 % (bovine serum albumin)
, -NQ01 (PBS 1:100 ; Santa Cruz Biotechnology, Inc.) 2 ,
FITC (Invitrogen Co) 2 1 .
- [0112] DAPI (Invitrogen Co) Prolong gold antifade 10 ,
NQ01 .
- [0113] 2.
- [0114] 4A FSaII 2 μ M 1 0, 12, 24 NQ01
.
- [0115] DAPI .
- [0116] NQ01 ,
NQ01 .
- [0117] , NQ01 12 24 .
- [0118] < 4> NQ01
- [0119] 1.
- [0120] 3 NQ01 .
- [0121] FSaII 1 , 2 RPMI 1640 37
(0, 4, 8, 12, 24) .
- [0122] EDTA 1 mmol/L 0.25 % 10 , PBS 2
, (pH 7.4, 1% Triton X-100, 0.1% sodium dodecyl sulfate, 20 mM Tris-HCl, 150 mM
sodium fluoride, 2 mM phenylmethyl sulfonyl fluoride, 10 mM iodoacetamide, 10 /mL aprotinin and 10
/mL leupeptin) .
- [0123] 60 sodium dodecyl sulfate-polyacrylamide gel 12% .
- [0124] (transfer) (192 mM glycine, 25 mM sodium dodecyl
sulfate, and 10% methanol) Hybond-P (Amersham Life Sciences, Inc., Arlington Heights, IL)
- (Trans-Blot) .
- [0125] nonfat dry milk pH 7.4 TBST , anti-NQ01 (1:1000 ; Santa
Cruz Biotechnology, Inc.) , anti-rabbit G 2 (1:1000 ; Amersham Life
Sciences, Inc.) HRP(horseradish peroxides) .
- [0126] .
- [0127] Image J computer program (National Institutes of Health, USA) .
- [0128] - .

- [0129] 2.
- [0130] 4B 5 μ M 1 FSaII NQ01 .
- [0131] .
- [0132] FSaII NQ01 , 24 NQ01 .
- [0133] < 5> (dicoumarol)
- [0134] 1.
- [0135] NQ01 .
- [0136] NQ01 .
- [0137] FSaII 50 μ M(Sigma Chemical Co.) - 5 μ M 4 .
- [0138] , - 5 μ M 50 μ M .
- [0139] , , 5 μ M 1 PBS 2 , 24 37 .
- [0140] , , 5 μ M 1 PBS 2 , 24 37 .
- [0141] , - 5 μ M 50 μ M 4 , .
- [0141] 2.
- [0142] , 5 , FSaII .
- [0143] - 32.9 $\pm$ 0.38 % .
- [0144] - (32.9 $\pm$ 0.38 %) 62.0 $\pm$ 5.03 % , - NQ01 .
- [0145] - 5 μ M 5 μ M 1 24 37 .
- [0146] 6.05 $\pm$ 1.17 % 0.52 $\pm$ 0.18 % , NQ01 .
- [0147] < 6>
- [0148] 1.
- [0149] (exponential growth phase) FSaII ,
- [0150] 0.05 mL 2×10^5 cells C3H (20g) .
- [0151] 7 ~ 9 90 ~ 110 4 .
- [0152] HP- β -CD(β -hydroxypropyl- β -cyclodextrin) .
- [0153] 1, 3, 5, 7 2 / .

[0154] 2, 4, 6, 8 - 40 / .

[0155] - 4 (1,3,5,7 /2,4,6,8).

[0156] , ($V=a^2b/2$; a b) .

[0157] (Protocol number:0412A66350, approved by the University of Minnesota Institutional Animal Care Use Committee).

[0158] 2.

[0159] 6 C3H FSaII - ,

[0160] 5 6.8 ± 0.4 .

[0161] - 40 mg/kg 4 , 5 7.7 ± 0.4 .

[0162] 2 mg/kg 4 5 10.8 ± 0.8 , 4.0 .

[0163] , - 5 , 15.4 ± 1.4 .

[0164] 8.6 .

[0165] , - .

[0166] 1 - FSaII .

[0167] 2 FSaII

[0168] A : FSaII (%) .

[0169] a: b: -

[0170] c: -

[0171] d: , 0 h -

[0172] e: , 12 h -

[0173] f: , 24 h -

[0174] B : FSaII .

[0175] ○ : -

[0176] ● : 5 μ M 24 -

[0177] 3 FSaII γ -H2X foci

[0178] A : γ -H2X foci() DAPI ()

[0179] :

[0180] Cis-0 h: 1 h

[0181] Cis-24 h: 1 h 24

[0182] β -Lap: - 4 h

[0183] Cis(0h) β -Lap: 1 h - 4 h

[0184] Cis(24h) β -Lap: 1 h , 24 - 4 h

[0185] B : γ -H2AX foci (%)

[0186] Cis-0 h: 1 h

[0187] Cis-24 h: 1 h 24

[0188] β -Lap: - 4 h

[0189] Cis.0h. β -Lap.0 h : 1 h - 4 h

[0190] Cis.24 h. β -Lap.0 h : 1 h , 24 - 4 h

[0191] 4 FSaII NQ01

[0192] A : NQ01

[0193] B : NQ01

[0194] 5 FSaII

[0195] Dic:

[0196] β -Lap: -

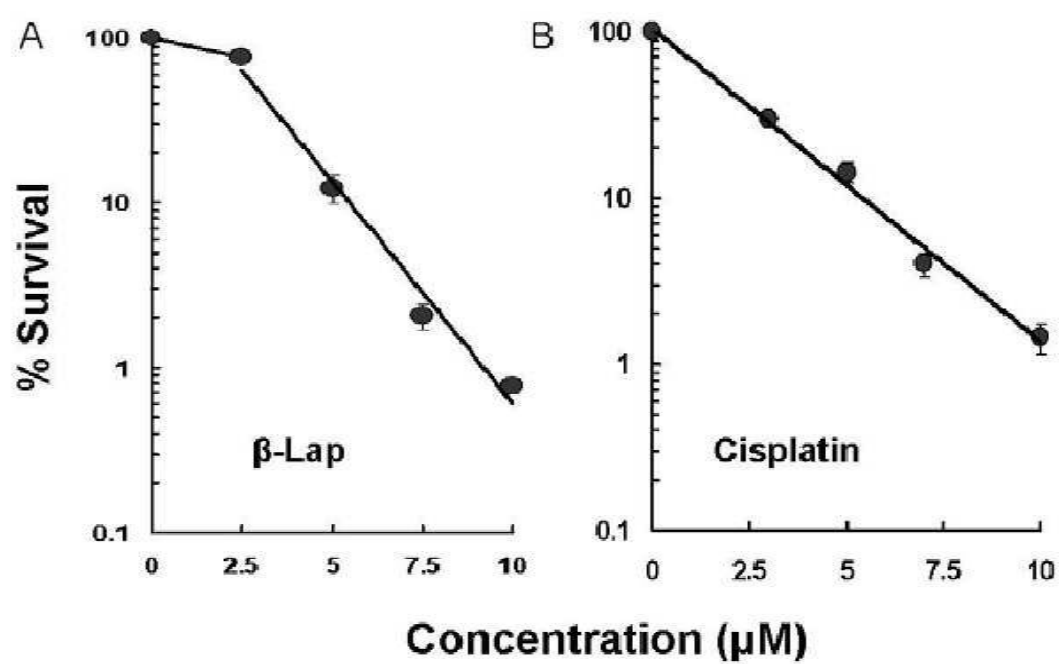
[0197] Dic+ β -Lap: + -

[0198] Cis+ β -Lap: 1 , 24 ,
- 4

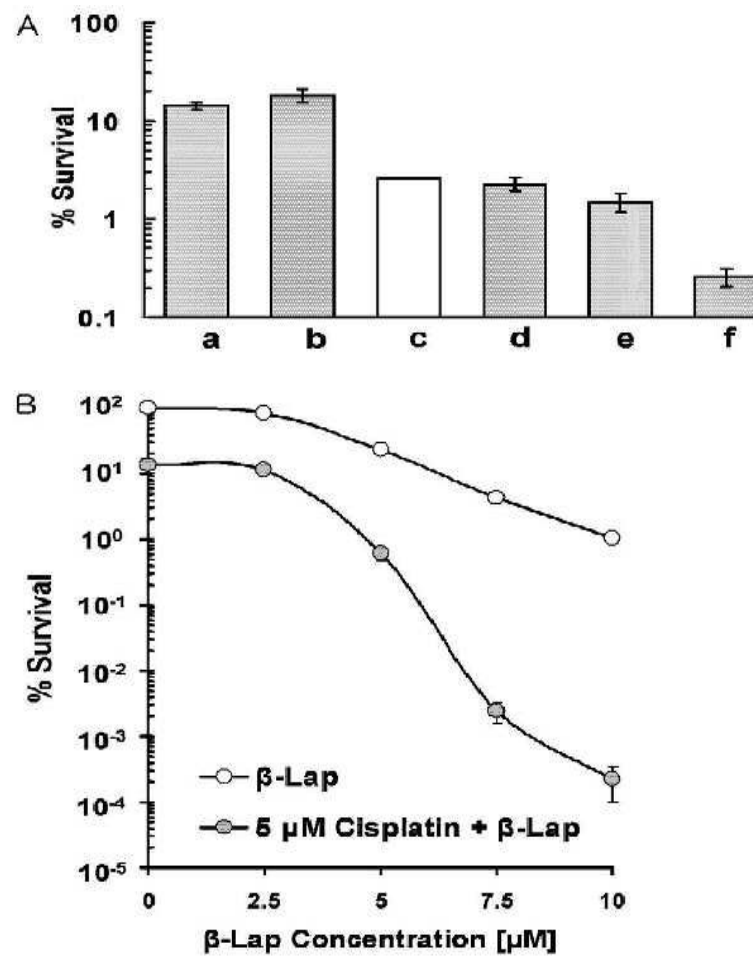
[0199] Cis+Dic+ β -Lap: 1 , 24 ,
- 4

[0200] 6 FSaII

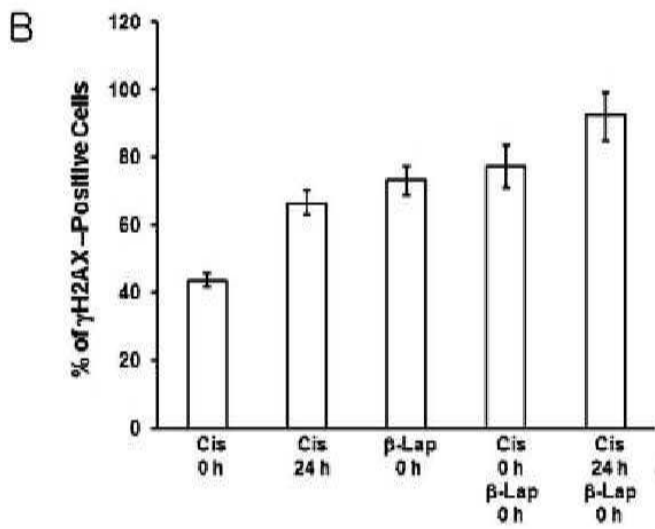
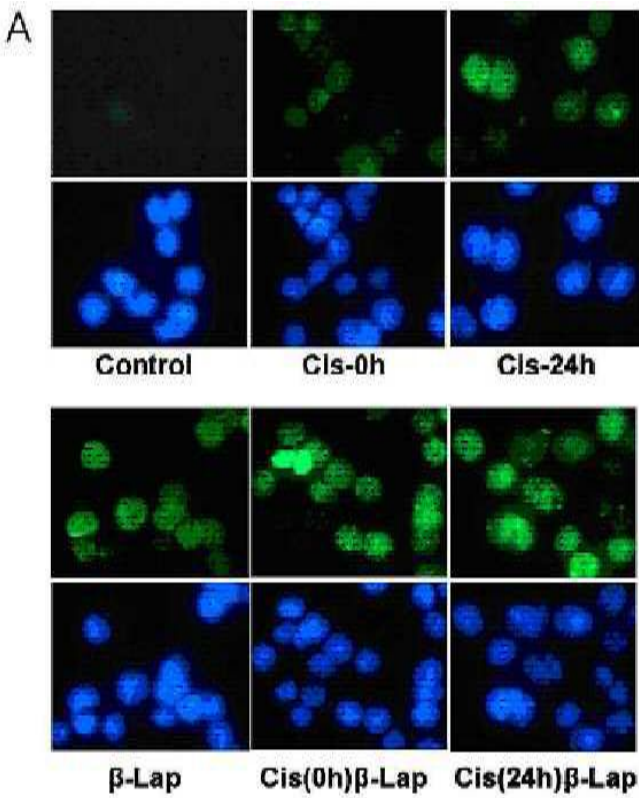
1



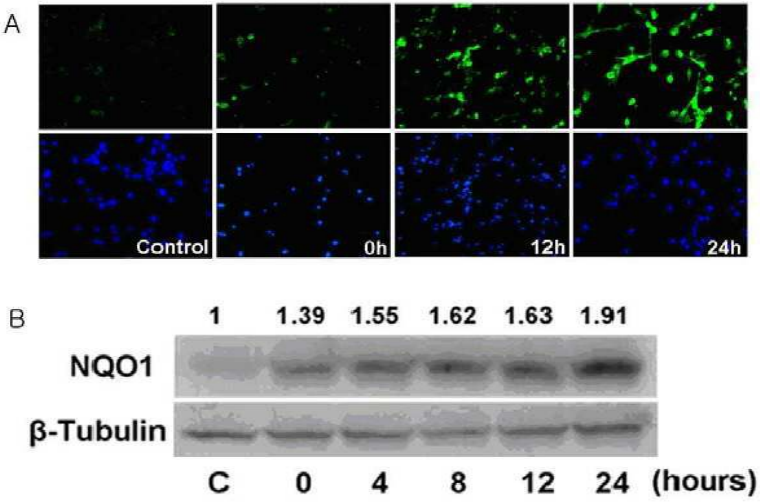
2



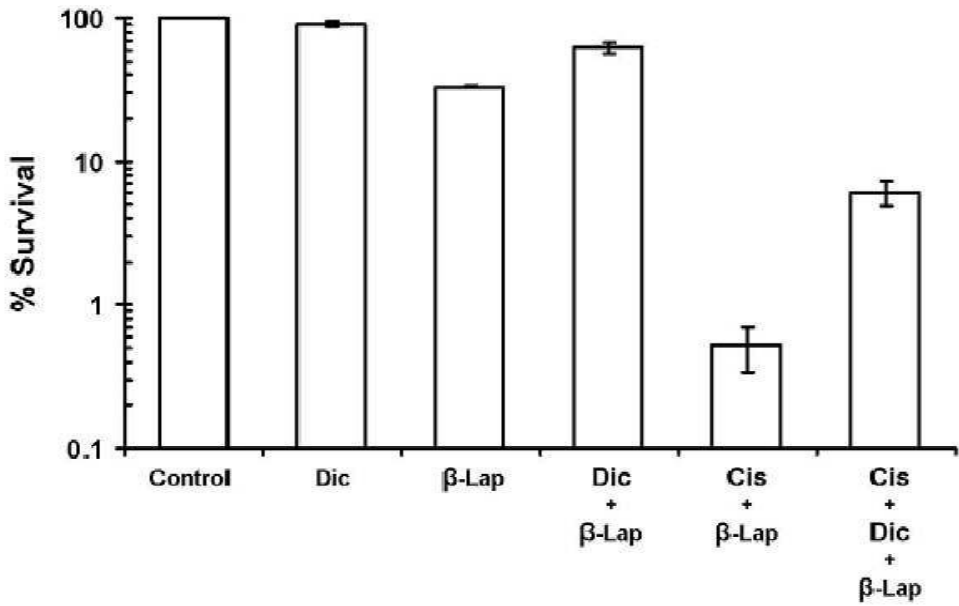
3



4



5



6

