

리도카인에 의한 p815 세포의 고사

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Apoptosis of p815 Cells by Lidocaine

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-ABSTRACT-

Background and Objectives : Mast cells play a major role in pathogenesis of allergic rhinitis. Lidocaine is widely used as a short-acting local anesthetic and an anti-arrhythmic agent. Lidocaine also has anti-inflammatory properties by inhibiting the effect of inflammatory cytokines. Although lidocaine is reported to suppress allergic reactions, there have been no report about lidocaine's direct inhibitory effect on mast cells. The purposes of this study are to identify whether lidocaine directly induces apoptosis of mast cells *in vitro* and know the pathway of apoptosis.

Materials and Methods : After culture of p815 cells, mouse mastocytoma cells, the cells were treated with 2.5 μ M, 5 μ M lidocaine for 24, 48 and 72 hours and then LD₅₀ of p815 cells was calculated by MTT (β 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay. For identification of apoptosis of p815 cells, flow cytometric analysis in monoclonal antibody to annexin V and cell cycle was done. We also estimated the expression of procaspase-3, procaspase-8, procaspase-9, AIF (apoptosis inducing factors) to know the pathway of apoptosis.

Results : The LD₅₀ of p815 cells is 3.70 μ M after lidocaine treatment for 24 hours, 1.82 μ M for 48 hours and 1.72 μ M for 72 hours. Lidocaine induced apoptosis in time and dose dependent manner. Procaspase-3 expression was significantly decreased while procaspase-8, procaspase-9 and AIF were not changed. **Conclusion** : Our results suggest lidocaine can induce apoptosis in mast cells via caspase-3 dependent pathway. Further studies on the pathway of apoptosis and clinical use will be needed. (J Clinical Otolaryngol 2005;16:247-252)

KEY WORDS : Mast cell · Apoptosis · Lidocaine · Allergic rhinitis.

서 론

(cytokine)

: 2005 8 15

: 2005 9 16

: , 602 - 739

17가 10

가

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1)2)

channel Na⁺ Ca²⁺ (Dimethyl sulfoxide; Sigma, St. Louis, MO)
 amide 10 ELISA 570 nm
 650 nm .⁷⁾
 ,³⁾⁴⁾
 가 LD₅₀ .
 .⁶⁾ 가
 유세포 분석을 통한 DNA 함량과 세포고사의 측정
 2.5 μM 5 μM 48
 ,³⁾⁴⁾⁶⁾ p815 PBS 2.5 × 10⁶ cells/ml
 . 250 μL trypsin/PBS 가
 . 10 . Trypsin inhi-
 bitor RNase 200 μL 가
 10 propidium iodide 200 μL
 . DNA CellFIT
 . p815 program FACSsort flow cytometer (Becton
 Dickinson, San Jose, CA) DNA .⁷⁾
 . G0, G1 sub G0, G1

재료 및 방법

비만세포배양

Mouse mastocytoma cell p815 (ATCC TIB 64) 10% fetal bovine serum, 100 unit/ml penicillin (Gibco, Gaithersburg, MD) 100 μg/ml streptomycin (Gibco, Gaithersburg, MD) DMEM (Dulbecco's modified Eagle's medium) 37 °C, 5% CO₂, 95% humidity 2~3

리도카인 처리 후 비만세포고사의 분석

4 × 10³ cells/ml 96 well plate
 200 μL 2.5, 5 μM
 24, 48, 72 MTT
 100 μL 0.5 mg/ml MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide; Sigma, St. Louis, MO) 가 37 °C 4
 . 100 μL DMSO

annexin V - FITC staining kit (Trevigen, Inc., Gaithersburg, MD, USA) propidium iodide (PI) annexin V - FITC flow cytometer
 . (late apoptotic cells) Annexin V+/PI+ (early apoptotic cells) Annexin V+/PI-
 .
 3 Mann-Whitney test p<0.05

Caspase general 억제제인 Z-VAD-fmk를 이용한 세포고사 경로의 분석

가 caspase caspase Z - VAD - fmk procaspase - 3, procaspase - 8, procaspase - 9, AIF (apoptosis inducing factor) fluorescence activated cell

scan(FACS) . p815 0.2% FBS가 . 405 nm caspase ELISA reader
 DMEM 24 0.2%
 DMEM Z - VAD - fmk 2
 . Trypsin
 4 450 × g 10
 (lysis buffer)
 15 4 , 15,000 ×
 g 20 Ac - Asp -
 Glu - Val - Asp - pNA 37 4

결 과

리도카인에 의한 비만세포의 생존율

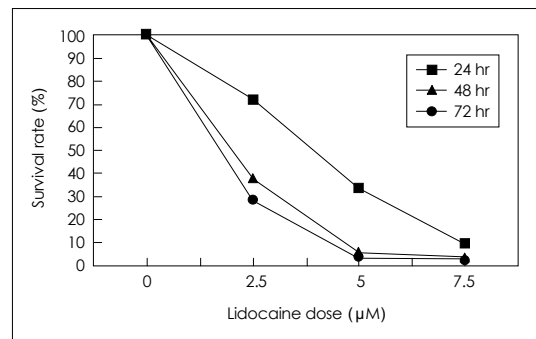


Fig. 1. Effect of lidocaine on the mast cell survival. Cells were treated with various concentrations of lidocaine and survival rate was accessed by MTT assay for 24, 48 and 72 hours.

Table 1. Survival rate of p815 cells determined by MTT assay according to concentration and treatment time of lidocaine (%)

Lidocaine	Time	24 hours	48 hours	72 hours
2.5 μM		71.70 ± 0.34*	37.85 ± 1.32*	27.75 ± 1.32*
5.0 μM		33.40 ± 0.14†	5.80 ± 0.05†	2.60 ± 0.03†

* : p < 0.05, † : p < 0.05

MTT
 가 가
 (p < 0.05).

24, 48, 72
 100% 가 2.5, 5 μM
 (p < 0.05). 7.5 μM 24
 10% 가
 (p > 0.05). 24
 p815 LD₅₀ 3.70 μM, 48
 1.82 μM, 72 1.72 μM

(Table 1, Fig. 1).

유세포 분석을 통한 DNA 성분 및 세포고사의 측정

48 p815
 PI annexin V
 0.20%, 2.5 μM, 5 μM
 7.32%, 26.60%
 PI annexin V
 0.13%, 2.5 μM, 5 μM
 0.10% 0.02%

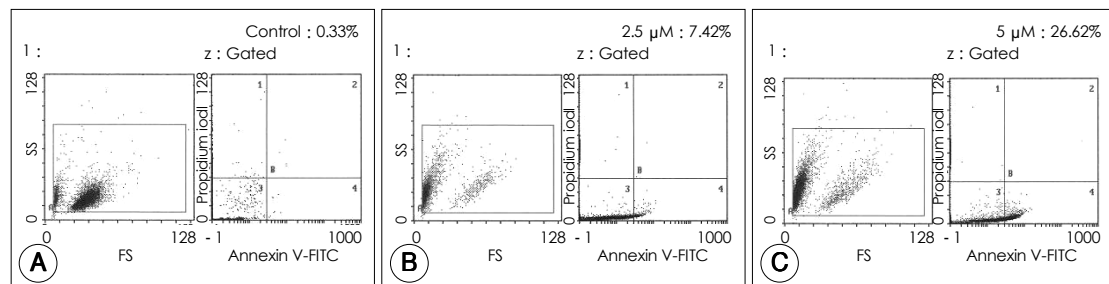


Fig. 2. Flow cytometry with monoclonal antibody to Annexin V. A : Untreated (control). B and C : 48 hours after treatment with 2.5 μM, 5 μM lidocaine, respectively. The proportion of annexin V (+) cells according to lidocaine concentration was shown 0.33% in control, 7.42% in 2.5 μM and 26.62% in 5 μM.

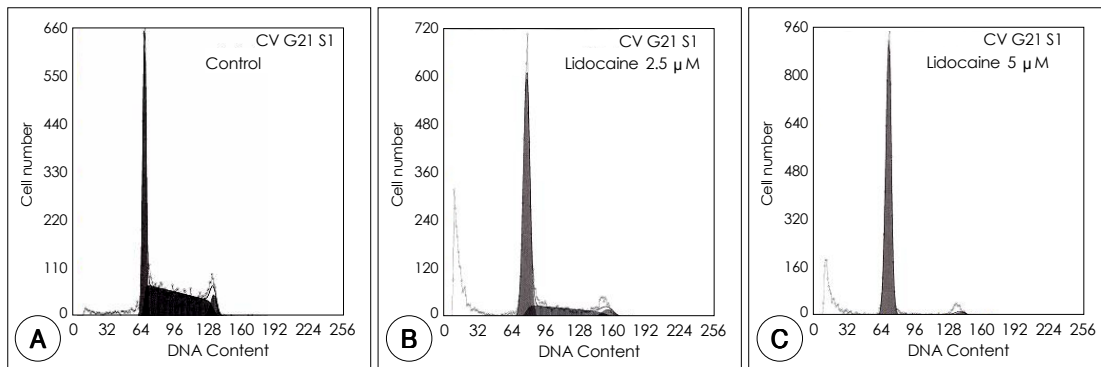


Fig. 3. Flow cytometry of cell cycle according to lidocaine concentration. A : Untreated (control). B and C : 48 hours after treatment with 2.5 μ M, 5 μ M lidocaine, respectively. The proportion of sub G0, G1 cells is increased with increment in lidocaine concentration.

Table 2. DNA content of lidocaine treated cells (%)

Lidocaine (μ M)	G0/G1 (%)	S (%)	G2/M (%)
0	38.7 \pm 0.4	55.2 \pm 0.4	6.06 \pm 0.3
2.5	69.9 \pm 0.5	25.5 \pm 0.4	4.60 \pm 0.4
5	91.7 \pm 0.4	5.42 \pm 0.3	2.86 \pm 0.3

Value : mean \pm standard deviation

annexin V
0.33% , 2.5 μ M, 5 μ M
7.42%, 26.62%
가 annexin V 가
(Fig. 2).

2.5 μ M 50%
가 가 .
sub G0, G1 38.7%, 가
2.5 μ M, 5 μ M 69.9%, 91.7% , 1962
가 가 (Table 2), S, G2, M
가

(Fig. 3).
2.5 μ M 48 procas-
pase - 3 , procaspase -
8, procaspase - 9, AIF

가 caspase (effector) caspase
caspase 3 (Fig. 4).

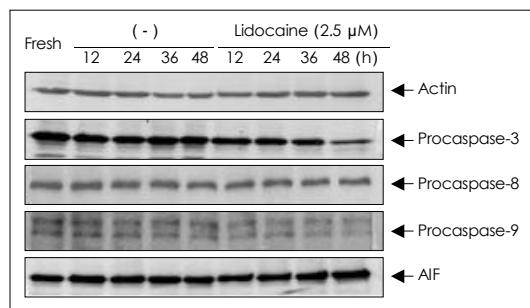


Fig. 4. Effect of lidocaine on the expression of procaspases and AIF (apoptosis inducing factor). Procaspase-3 expression decreases in 2.5 μ M of concentrated lidocaine after 48 hours. However, there is no change in procaspase-8, procaspase-9 and AIF.

고 찰

.⁸⁾⁹⁾ Hunt 가

.¹⁰⁾ Decco 가

p815

6 가

5 ,

5) , caspase caspase (cascade) caspase - 2, 8 9 caspase caspase - 3, 6 7 caspase 15)

Ca²⁺ ,

6) FasL tumor necrosis factor(TNF) (death ligand)가

Ca²⁺ .¹¹⁾ Ohnishi (death receptor) (death signal) caspase - 8 caspase - 10 가

Okada ,¹²⁾¹³⁾ Tanaka . cytochrome c가 caspase - 9가 .

T Ca²⁺ channel caspase - 3 Cas- pase - 3 , caspase - 8 caspase - 9

T (leukotrienes, LTC₄, (prostaglandins, PGD₂) caspase .¹⁶⁾¹⁷⁾ p815 2.5, 5 μM 24, 48, 72

LTD₄), , TH₂ , annexin V flow cytometer

p815 (in vitro) 가 Procaspase - 3, procaspase - 8, procaspase - 9, AIF procas- pase - 3 가 cas- pase - 3 procaspase - 8 procaspase - 9 , caspase - 3 가 가

가 .¹⁴⁾ (proteo- lytic system) caspase(cysteine aspartate proteases) 가

. Caspase procaspase , caspase aspartic acid caspase - 3 가 가

결 론

가

중심 단어 :

2005 (2005 - 34)

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