Polyamine Ibuprofen

Effect of Ibuprofen on the Changes of Polyamine Level and Neuronal Cell Damage after Transient Global Ischemia in Gerbil

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Background : In brain ischemia, increased arachidonic acid metabolism can play important roles in neuronal damage. Ibuprofen was reported to have a protective role against neuronal damage in focal brain ischemia and reperfusion. The present study was designed to investigate whether ibuprofen can inhibit the global ischemia-induced neuronal damage and changes of polyamine (PA) level which is known to related to the neuronal damage, breakdown of blood brain barrier, and brain edema. Methods : Male Mongolian gerbils were used in this study. Transient global ischemia was induced by occlusion of bilateral common carotid arteries for 3 min with microclips. Ibuprofen was administered immediately after ischemia. The animals were sacrificed one day after ischemia for PA measurement and sacrificed 5 days after ischemia for histological evaluation. Histological examination was performed by counting surviving neuronal cells in one mm of CA1 area in dorsal hippocampus. Results : Cerebral cortex and hippocampal putrescine(PU) levels in vehicle-treated ischemic group significantly increased comparing to sham-operated animals and the increase of PU was attenuated by ibuprofen administration (50 mg/kg). Hippocampal spermine level decreased significantly after ischemia. Hippocampal neuronal cell damage in CA1 area was markedly observed in vehicle-treated animals compared to sham operated animals. Ibuprofen administration at the dose of 50 mg/kg significantly inhibited hippocampal CA1 neuronal damage compared to vehicle-treated animals. Conclusions : Ibuprofen attenuates PA response following transient global ischemia and may have putative neuroprotective effect against neuronal damage induced by global ischemia. J Korean Neurol Assoc 20(3):265~272, 2002

,^{1,2} COX-2 가가 cyclooxygenase(COX) .³ COX-2 arachidonic acid prostaglandin (PG) thromboxane(TX) 가 . COX .⁴⁻⁶ COX-2 type 1(COX-1) type 2(COX-2) 가 가 eicosanoids (free radical) . COX-1 7-8 COX-2 Manuscript received December 19, 2001. COX Accepted in final form March 13, 2002. 9-11 Address for correspondence Sang-Doe Yi, M.D. (non-steroidal anti-inflammatory Department of Neurology, Keimyung University drugs, NSAIDs) COX-2 School of Medicine. 194 Dongsan-dong, Daegu, 700-310, Korea 12-14 Tel: +82-53-250-7832 Fax: +82-53-252-1605 COX-2 E-mail : sdlee@dsmc.or.kr

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. NSAIDs ibuprofen

. Ibuprofen 가 , ,¹⁵⁻¹⁶, COX , , glutamate . polyamine(PA) putrescine

(PU), spermidine(SD) spermine(SM) PA , .¹⁸⁻¹⁹ PA

, PA , (blood brain barrier) . PA 7ト ,²¹⁻²² .²³ Paschen ²⁴ PA PU

가 . PA PA . .25-26 gerbil , NSAIDs ibuprofen PA 가

1. 60~80 g Mongolian gerbils(Meriones ungiculatus) .

2. Chloral hydrate(Sigma Chemical Co., St. Luise, MO, USA) 400 mg/kg 2 cm

(CMA, Stockholm, Sweden) 37±0.5 ibuprofen(Sigma Chemical Co., St. Luise, MO, USA) 10, 25 50 mg/kg 100 g 0.2 ml7ł

(microclip)

3

3. 5 (1) Sham : (PA n=5; , n=6) (2) Vehicle : (PA , n=5; n=6) (3) Ibuprofen 10 mg/kg : 10 mg/kg ibuprofen (PA , n=4; , n=6) (4) Ibuprofen 25 mg/kg 25 mg/kg ibuprofen (PA , n=4; , n=6)

(5) Ibuprofen 50 mg/kg : 50 mg/kg ibuprofen (PA , n=5; , n=8)

4. PA PA Spragg Hutchings ²⁷ . 24

Eppendorf -70 0.4 M perchloric . (2 mM disodium EDTA 4×10^{-5} M acid 1,8-diaminooctane 10) 가 homogenizer (12,000 g, 4 , 15 min) . 1 M sodium bicarbonate 100 µl 300 µl 4-fluoro-3-nitrobenzotrifluride(FNBT) 20 60 40 µl 1 M histidine 가 5 가 2 ml 2-methylbutane 2 PA . 5 가

methanol(500 µ) 20 µ Hamilton (mobile phase) acetonitrile 86 14() 10 7F (degassing) 1 mL , column Rainin ODS C18 column [250 mm()×4 mm()] . UV(ultraviolet) 242 nm

.

5. 5 chloral hydrate (2 IU/mI)phosphate-buffered saline(PBS, pH 7.2) 10% formalin 가 10% formalin (24~48). rotary microtome 6 µm hematoxylin eosin CA1 .

CA1 1 mm

Eke 28

6.

PU, SD, SM 1,8-diaminooctane Sigma (Sigma Chemical Co., St. Luise, MO, USA)

, FNBT Aldrich (Sigma Chemical Co., St. Luise, MO, USA) , acetonitrile J.T. Baker (J.T.Baker Co., Phillips- burg, NJ, USA) .



7.						
	(PA) ANOVA			4		
Scheffe test						
±			, p	0.05		
	가					
1.		PA				
ibuprofen						
PU	sl	sham (9.5±0.8		5±0.8		
nmol/g tissue)	ve	vehicle (27.5±3.5		. 5± 3 . 5		
nmol/g tissue)		가 (p<0.01,		<0.01,		
Fig. 1-A),	-A), 가 ibuprofen 10 mg/kg					
(29.2±4.3 nmol/g tissue) 25 mg/kg						
(27.4±3.9 nmol/g tissue)						
, 50 mg/k	g					
(19.7±1.5 nmol/g tissue, p<0.05, Fig. 1-A).						
SD	sh	sham		(168.2±2.5		
nmol/g tissue)	ve	vehicle (168.4±8.8		.4±8.8		
nmol/g tissue)						
(Fig. 1-B), ibuprofen 10, 25 50 mg/kg						
(158.2±5.2 nmol/g tissue, 158.8±5.2						
nmol/g tissue	153.6±1	1.1 nm	ol/g tiss	ue)		



Figure 1. Changes of putrescine (A), spermidine (B), and spermine (C) levels in the gerbil cortex after global ischemia and effects of ibuprofen administration. Putrescine levels are given nmol/g wet tissue. *p<0.05 in comparison with vehicle-treated gerbils (VEH); **p<0.01 in comparison with shamoperated gerbils. n = 4-5. Sham: sham-operated; VEH: vehicle-treated; IB10: ibuprofen 10 mg/kg administered; IB25: ibuprofen 25 mg/kg administered; IB50: ibuprofen 50 mg/kg administered gerbils. Data expressed as mean ± SEM.

Sham

VEH

IB10

IB25

IB50

vehicle (Fig. 1-B). SM sham (636 ±17.2 nmol/g tissue) (610 vehicle ±17.2 nmol/g tissue) (Fig. 1-B), ibuprofen 10, 25 50 mg/kg580±40 nmol/g tissue, 607.9±26.6 (nmol/g tissue 555.4±32.8 nmol/g tissue) vehicle (Fig. 1-B).

2. ΡA ibupro fen ΡU (9.9 ± 0.4) sham vehicle nmol/g tissue) (22.1±2.1 nmol/g tissue) 가 가 (p<0.01, Fig. 2-A), ibuprofen (22.4±2.2 nmol/g tissue) 10 mg/kg 25 (20.5±3.6 nmol/g tissue) mg/kg , 50 mg/kg (15.9±1.4 nmol/g tissue, p<0.05, Fig. 2-A). (144.7 ± 2.7) SD sham nmol/g tissue) (150.2 ± 6.3) vehicle





ibuprofen 10, 25	50 mg/kg	(136.3±		
1.8 nmol/g tissue,	136.7 ± 2.8	nmol/g	tissue		
134.7±7.2 nmol/g	tissue)	vehicle	e		
	(Fig. 2-B).				
SM	sham	(450).6±11.4		
nmol/g tissue)	vehicle	(40)	2.8±11.7		
nmol/g tissue)		(F	ig. 2-C),		
ibuprofen 10, 25	50 mg/kg	(412.0±		
39.4 nmol/g tissue, 402.2 ± 25.9 nmol/g tissue					
385±16.2 nmol/	g tissue)	vehicl	е		
		(Fig.	2-C).		

Gerbil		
CA1	가 sham	294.5±
5.4	vehicle	13.3
±3.7		(p<0.01,
Fig. 3 4).		ibuprofen
	10 mg/kg	12.2±5.5
	, 25 mg/kg	51.7
±25.3		
. Ibuprofe	97.9±28.5	
		(p<0.05, Fig.
3,4).		

3.



Figure 2. Changes of putrescine (A), spermidine (B), and spermine (C) levels in the gerbil hippocampus after global ischemia and effects of ibuprofen administration. Putrescine levels are given nmol/g wet tissue. p<0.05 in comparison with vehicle-treated gerbils (VEH); **p<0.01 in comparison with sham-operated gerbils. n = 4-8. Sham: sham-operated; VEH: vehicle-treated; IB10: ibuprofen 10 mg/kg administered; IB25: ibuprofen 25 mg/kg administered; IB50: ibuprofen 50 mg/kg administered gerbils. Data expressed as mean \pm SEM.



Figure 3. Microphotographs of the hippocampal CA1 area in the gerbil 5 days after transient global ischemia (hematoxylin and eosin staining, \times 200). Effect of ibuprofen administration on the number of surviving cells in the CA1 area of hippocampus five days after transient global ischemia in gerbils. CA1 area in sham-operated (A), in vehicle-treated (B), in ibuprofen 10 mg/kg administered gerbils (C), in ibuprofen 50 mg/kg administered gerbils (D). Bar = 50 µm.

			. ³³ SSAT	ODC 7	ŀ
				가	가
	gerbil		. ³⁴ ODC	; 가	PU 가가
PA	, PU 💈	가	가	SSA	AT 가 가
ibuprofei	n		PU	가	
			PA PU		가
1.	polyam	line	, ³⁵ PU	가	
ibupr	ofen				
	ibuprofen	PA			
			35-36		32,37
PA	, ,		PA		
18,25	PA	, kainate	. Paschen	35	ODC
			alph	a-difluoromet	hylornithin₽U
	,				, Baskaya ²⁶
	. ²⁹⁻³² PU PA		ODC		
ornithine	decarboxylase(O	DC) 가	. Doga	an ³⁶ PU	가
가	, SD SM di	iamine	PA	oxidase	PU
	가	가	3		
	. SD	D SM SD/SM N1-	PU	sham	가
acetyltra	nsferase(SSAT) 가	, 가	50 mg/kg ib	uprofen
	PA oxidase	e PU	. 1	0 mg/kg 25	mg/kg ibuprofen
F	PU 가		PU	가	



Figure 4. Effect of ibuprofen administration on the number of surviving cells in the CA1 area of hippocampus 5 days after transient global ischemia in gerbils. *p<0.05 in comparison with vehicle treated-gerbils. Sham, sham-operated (n = 6); VEH, vehicle-treated (n = 6); IB10: ibuprofen 10 mg/kg administered(n = 6); IB25: ibuprofen 25 mg/kg administered (n = 6); IB50: ibuprofen 50 mg/kg administered gerbils (n = 8). Data are expressed as mean ± SEM.







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