

= 빨제강연(11/2(화)) =

Dietary Supplementation Protects Cells from Damage in Animal Models

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Which cells specifically & Why?

Neuronal Cells
Skin Cells

- 뇌졸중**
- 단일질환으로 사망원인 1위(2002, 통계청)
 - 삶의 질 저하
 편마비, 감각장애, 감정 조절 장애,
 인지기능 저하 등 합병증 발생
 - 암세포와 달리 예방이 가능함.

뇌졸중 환자의 혈관



그림 2-1

동맥 경화가 심한 뇌혈관의 단면도.
외설로 가리킨 부분(원 부분)은 지
방질이 동맥의 벽 안에 끼어 동맥의
벽이 두꺼워진 모습. 외설쪽으로 기
리킨 것은 혈관 안에 생긴 혈전.

To prevent Stroke, we should
know the risk factors

**Risk factors reported from
Western Countries**

- | | |
|------------------------|-----|
| ● Smoking | + |
| ● Hypercholesterolemia | + |
| ● Hypertension | + |
| ● Lack of exercise | + |
| ● Diabetes Mellitus | + |
| ● Obesity | + |
| ● High sodium | ??? |

Risk factors for stroke: Adjusted OR from multiple logistic regression model (1)

	Men				Women			
	CI OR(95%CI)	P	CI OR(95%CI)	P	CI OR(95%CI)	P	CI OR(95%CI)	P
HT	6.0 (2.6-14.1)	.01	18.9 (3.9-91.0)	.01	12.7 (4.2-37.7)	.01	20.0 (3.8-15.4)	.01
DM	5.4 (1.7-17.5)	.01	—	—	—	—	—	—
Heavy drinker	—	—	10.4 (0.9-115.9)	.05	—	—	—	—
Sodium intake	1.4 (1.2-1.6)	.01	1.4 (1.1-1.7)	.01	1.5 (1.3-1.7)	.01	1.5 (1.2-1.7)	.01

CI, confidence interval; OR, odds ratio

Risk factors for stroke: Adjusted OR from multiple logistic regression model (2)

	Men				Women			
	CI OR(95%CI)	P	CI OR(95%CI)	P	CI OR(95%CI)	P	CI OR(95%CI)	P
Vegetable consumption	0.8 (0.7-1.0)	.05	—	—	—	—	—	—
Fat consumption	—	—	0.3 (0.1-0.8)	.025	—	—	—	—
Abd. ST	1.1 (1.0-1.1)	.05	1.1 (1.0-1.3)	.025	1.2 (1.1-1.3)	.01	—	—
Triceps ST	—	—	—	—	0.8 (0.7-0.9)	.01	—	—
Recent exercise	—	—	—	—	0.2 (0.1-0.5)	.01	0.2 (0.1-1.0)	.05

CI, confidence interval; OR, odds ratio; ST, skinfold thickness

Risk factors for Stroke

Smoking	+	—
Hypercholesterolemia	+	—
Hypertension	+	+
Lack of exercise	+	+
Diabetes Mellitus	+	+
Obesity	+	±
High sodium??	??	+

+ Previously Known Risk factors from Western Countries

+ Our study with Koreans (Choi-Kwon & Kim, 1998)

영양 상태와 뇌출혈 발생

● 영양 과다 또는 비만: 뇌출증의 위험인자

● 영양 부족: 뇌출증의 위험인자?

알려져 있지 않음.

관찰내용:

- 뇌출혈患者는 영양 부족 상태가 많음.
- 지방의 섭취가 부족

Risk factors for stroke: Adjusted OR from multiple logistic regression model (2)

	Men				Women			
	CI OR(95%CI)	P	CI OR(95%CI)	P	CI OR(95%CI)	P	CI OR(95%CI)	P
Vegetable consumption	0.8 (0.7-1.0)	.05	—	—	—	—	—	—
Fat consumption	—	—	0.3 (0.1-0.8)	.025	—	—	—	—
Abd. ST	1.1 (1.0-1.1)	.05	1.1 (1.0-1.3)	.025	1.2 (1.1-1.3)	.01	—	—
Triceps ST	—	—	—	—	0.8 (0.7-0.9)	.01	—	—
Recent exercise	—	—	—	—	0.2 (0.1-0.5)	.01	0.2 (0.1-1.0)	.05

CI, confidence interval; OR, odds ratio; ST, skinfold thickness

Undernutrition as a possible cause of intracerebral hemorrhage

● Measurement of nutritional status

- 3 biochemical parameters
- 5 anthropometric parameters

● (Choi et al, Acta Neuro Scan, 1998)

Undernutrition as a possible cause of intracerebral hemorrhage

- Measurement of nutritional status
 - 3 biochemical parameters
 - 5 anthropometric parameters
- (Choi-Kwon et al, *Acta Neuro Scan*, 1998)

Nutritional status of stroke patients

	ICH (n=21)		CI (n=67)		Control (n=122)		χ^2
	n	%	n	%	n	%	
Obesity	2	9.6	16	23.9	20	16.6	1.54
Undernutrition	13	61.9	17	25.4	16	13.2	25.4***
Normal	6	28.5	34	50.7	86	70.2	

Demographic data, nutritional indicators(1)

	ICH (n=21)	CI (n=67)	Control (n=122)	F value
Age(y)	54(10)	66(10)	66(8)	1.6
Education(y)	6.5(4.4)	7.6(4.7)	6.6(3.5)	0.48
HT(%)	95***	70**	25	
DM(%)	5	25***	10	
Ischemic heart disease	0	4	2	
Cigarette smoking	0	9**	2	
Alcohol drinking	5	0	1	

*P<.05, **P<.025, ***P<.01

Demographic data, nutritional indicators(2)

	ICH (n=21)	CI (n=67)	Control (n=122)	F value
WBC(mm^3)	8510(4738)◦	6961(2569)	6846(1389)	4.6**
Lymphocytes(mm^3)	1426 (617)◦	2043(625)◦	2589(586)	31.2***
Hemoglobin(g/dl)	12.6(1.5)	12.5(1.6)	12.8(1.0)	1.3
Hematocrit(%)	36.3(6.8)◦	37.7(4.9)◦	42.2(3.1)	33.8***
Albumin(g/dl)	4.1(0.6)	4.0(0.3)◦	4.3(0.3)	18.0***
Cholesterol(mg/dl)	196(45)	210(41)	2.7(42)	10.6

*P<.05, **P<.025, ***P<.01 ◦ significant compared to control, ◚ significant compared to CI

Demographic data, nutritional indicators(3)

	ICH (n=21)	CI (n=67)	Control (n=122)	F value
TSF(mm)	16.4(7.4)◦	19.6(7.2)◦	22.2(4.9)	10.0***
SSF(mm)	15.7(7.7)◦	20.2(8.7)	20.9(7.2)	4.0**
ASF(mm)	28.9(11.2)◦	34.3(10.2)◦	26.8(6.5)	17.3***
LBM(kg)	36.7(5.9)	39.2(5.8)	38.1(4.8)	2.0
Body fat(%)	31.2(5.3)	32.6(5.9)	33.3(4.9)	1.5
BMI(kg/m^2)	22.8(4.2)◦	24.1(3.1)◦	24.5(3.1)	5.7***

*P<.05, **P<.025, ***P<.01 ◦ significant compared to control, ◚ significant compared to CI

TSF,triceps skinfolds thickness; SSF,subscapular skinfolds thickness; ASF,abdominal skinfolds thickness;
LBM,lean body mass; BMI,body mass index

뇌졸중 예방인자는?

● 체조 및 고기질 섭취

● 운동

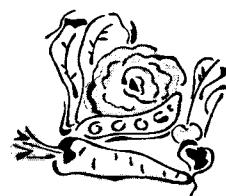
● 생선 섭취

뇌졸증은 원시 시대에도 있었나?

- 뉴기니아령 기타바지역: 인구 2,250명 중 뇌졸증 또는 심장질환 환자 없음.
 - 주식: 감자, 고구마, 콩코넛, 야채, 물고기
- 우간다 카밀라 지역의 뇌졸증 환자 수
 - 1940년대: 뇌졸증 환자 없음.
 - 1950년대: 11%(전체 사망과 환자의)
 - 1960년대: 34%

채소, 과일

Vit C와 E가 항산화제로 작용 (Gillman, 1995):



Don't know which one to believe

- Zutphen study: Flavonoids의 뇌졸증 보호효과 보고 (Keli et al., 1996)
- 역학 조사 연구: Carotenoids, Vit C, Vit E가 뇌졸증 보호 효과 없음 (Ascherio, 1999)
- 우리나라에서는 채소 섭취가 노경색 발생 보호효과를 보임 (Choi-Kwon, 1999)

Then, how about Fish ??

- Zutphen study : 생선 20g/day, 뇌졸증 발생 보호효과 (Keli, 1994)
- Zhang 등 (1999)은 WHO의 생전성취에 관한 자료에서 생선의 섭취가 많은 일본과 아일랜드, 또 다른 국가에서 생선의 섭취와 뇌졸증으로 인한 사망률과의 흥미로운 상관성을 보고.



What we really need is Evidence

Why animal models?

- Not too many variables
- Not that time consuming
- Little variable interaction
- Cost effective



Questions

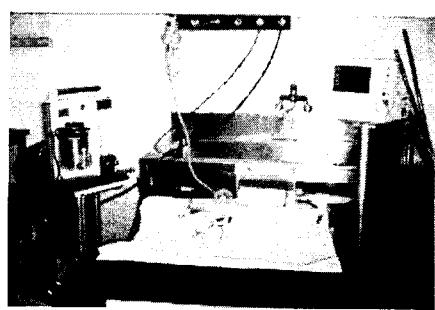
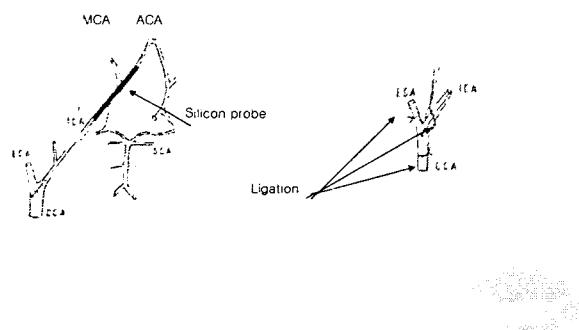
- Will the fish-oil supplementation prevent neuronal cell from the injury?
- What will be the mechanism of prevention of cell damage?

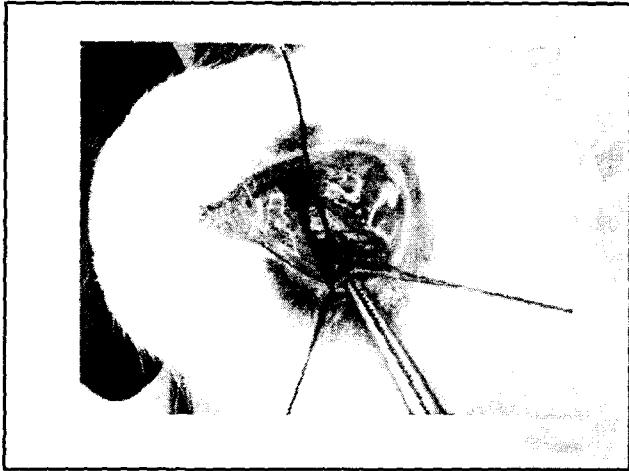
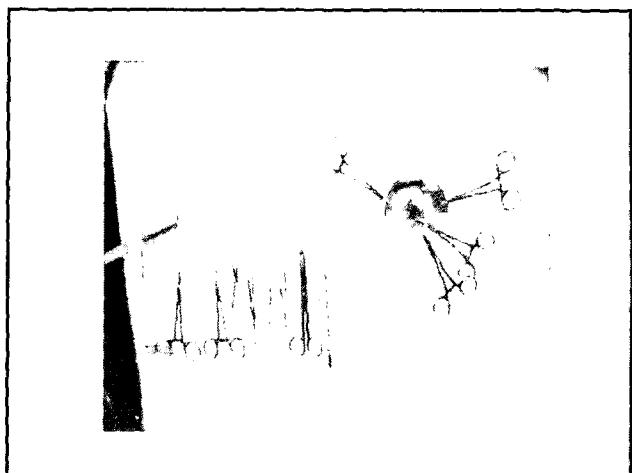
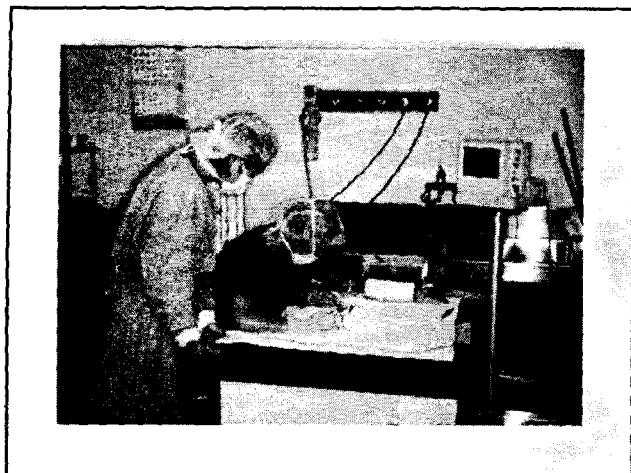
Biotechnology

- Spectrophotometer for antioxidant enzyme measurement
- Gas chromatography for lipid measurement in the membrane
- Image analysis for brain infarction volume
- H & E Staining for cellular changes
- EM for cellular morphological changes

Temporal changes in cerebral antioxidant enzyme activities after ischemia and reperfusion in a rat focal brain ischemia model: effect of dietary fish oil
(Choi-Kwon et al, Developmental brain research, 2004)

(Middle Cerabral Artery Occlusion Model (Nagasawa, 1989)





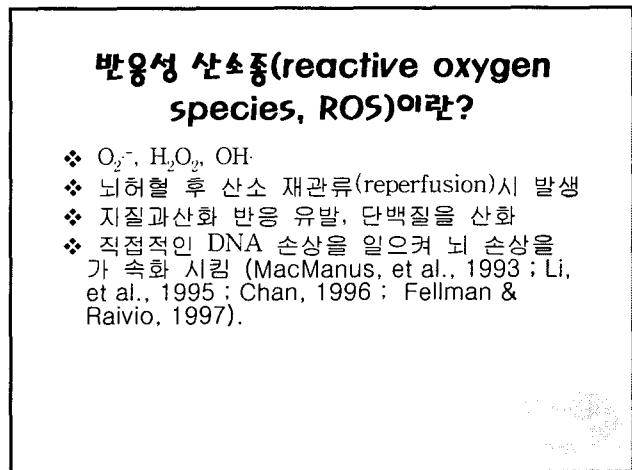
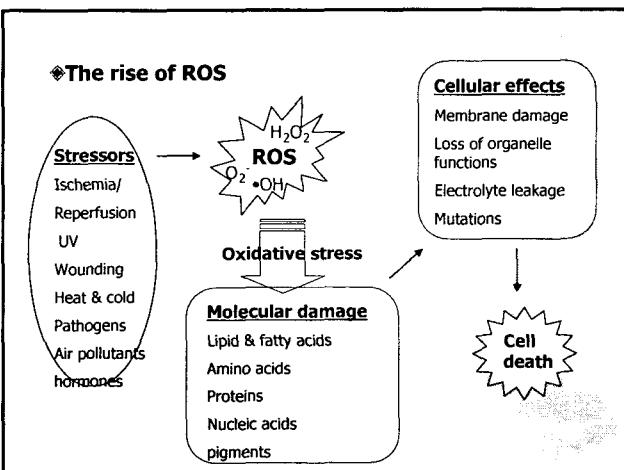
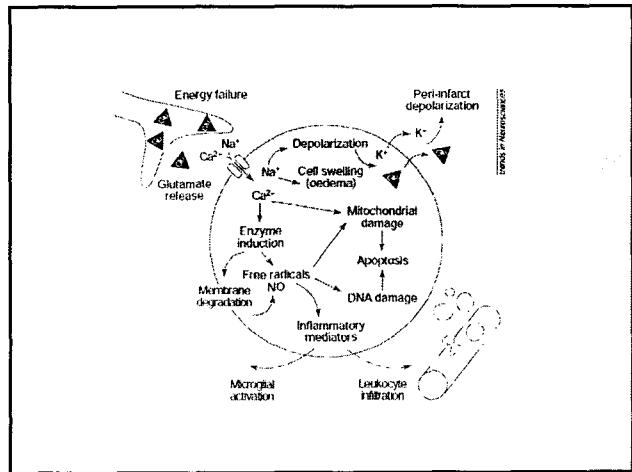
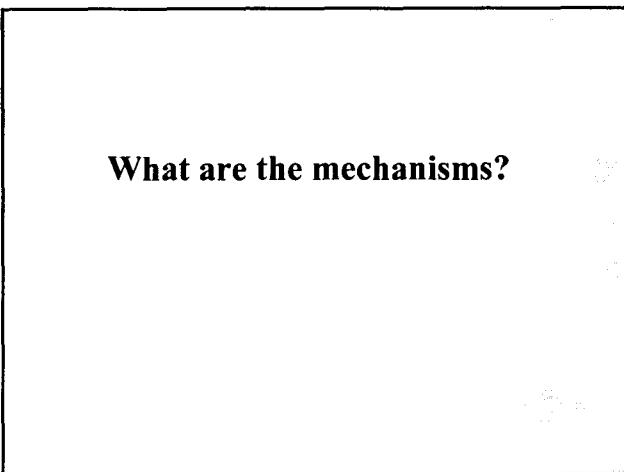
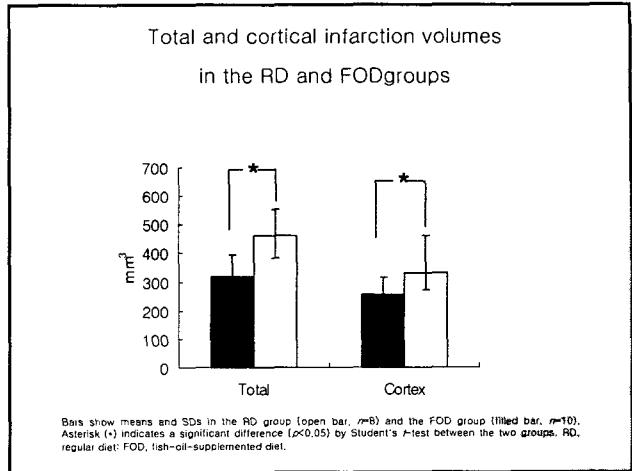
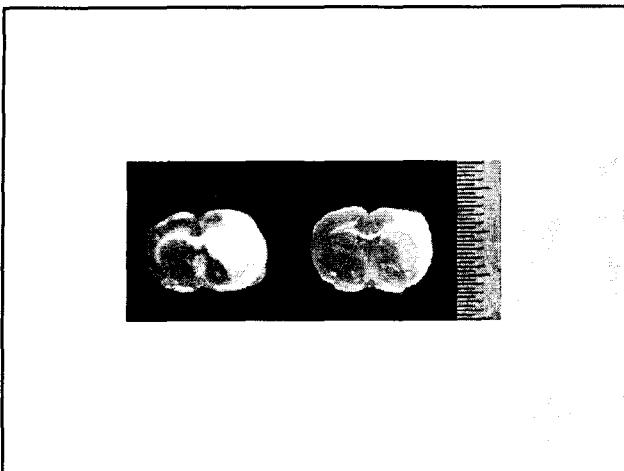
How do you know that DHA contained in fish oil have reached to the membrane of the brain?

Fatty acid composition of the right cerebral cortex
(percentages of total fatty acids)

Fatty acid	RD (n=5)	FOD (n=5)
Myristic acid, C _{14:0}	0.23 ± 0.13	0.04 ± 0.09
Palmitic acid, C _{16:0}	19.94 ± 1.20	17.58 ± 0.44
Palmitoleic acid, C _{16:1n-7}	0.17 ± 0.13	0.46 ± 0.05
Stearic acid, C _{18:0}	22.60 ± 4.96	26.79 ± 0.70
Oleic acid, C _{18:1n-9}	13.45 ± 2.43	16.31 ± 0.22
Linoleic acid, C _{18:2n-6}	1.83 ± 0.37	2.00 ± 0.77
Arachidic acid, C _{20:0}	1.56 ± 1.24	0.20 ± 0.02
AA, C _{22:4n-6}	12.82 ± 3.13	10.33 ± 0.34
Eicosapentaenoic acid, C _{20:5n-3}	0.21 ± 0.17	0.71 ± 0.16
Eruic acid, C _{22:1}	1.54 ± 1.80	2.49 ± 0.21
DHA, C _{22:6n-3}	23.17 ± 2.33	25.33 ± 0.47
DHA/AA, C _{22:6n-3} / C _{22:4n-6}	1.85 ± 0.29	2.46 ± 0.10

Data are means ± SDs. Asterisk indicates a significant difference ($p<0.05$) by Student's *t*-test between the two groups. RD, regular diet; FOD, fish-oil-supplemented diet; DHA, docosahexaenoic acid; AA, arachidonic acid.

Fish oil supplementation will decrease infarction size?

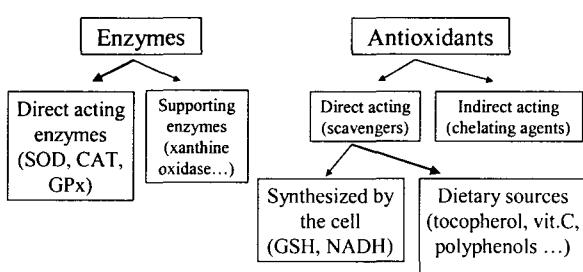


뇌조직은 ROS에 특히 취약한가?

The answer is Yes, and the Reasons are:

- ❖ 체내 다른 조직에 비해 지방조직을 많이 암모.
- ❖ SOD, CAT, GPx 등의 암산화 효소의 활성도와 GSH 양 량이 상대적으로 낮음 (Mizuno & Ohta, 1986 ; Cohen, 1988 ; Chan, 1996).
- ❖ 높은 비율로 산소를 소비하여 산화적 손상과 어혈에 특히 취약함 (Agranoff, 1984 ; Hill & Swizer, 1984 ; Cham, 1996).

● Antioxidant defense system



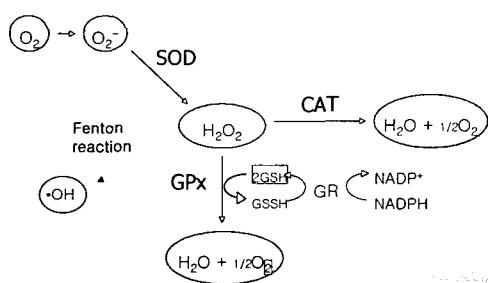
- Kohen R (1999). *Biomed & Pharmacother* 53

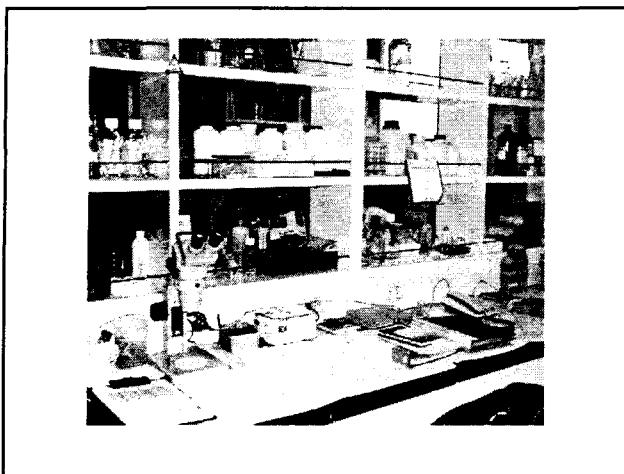
항산화 효소

(Antioxidant enzymes)

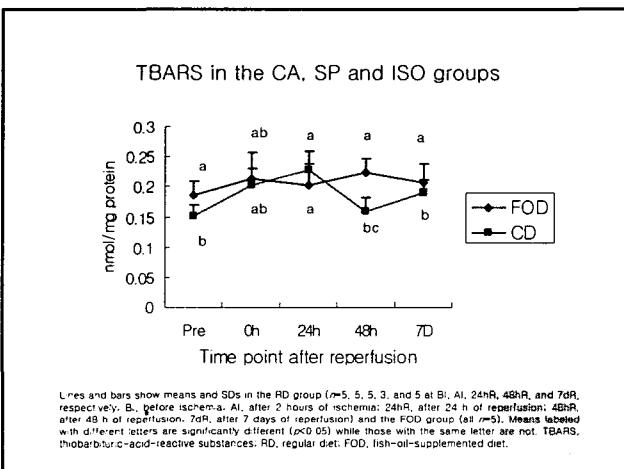
- ❖ Superoxide dismutase(SOD)
- ❖ Catalase(CAT)
- ❖ Glutathione Peroxidase(GPx)

Detoxification of ROS by antioxidant enzymes

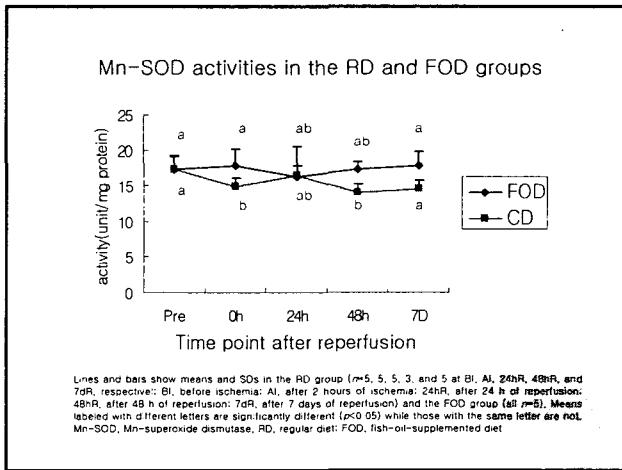
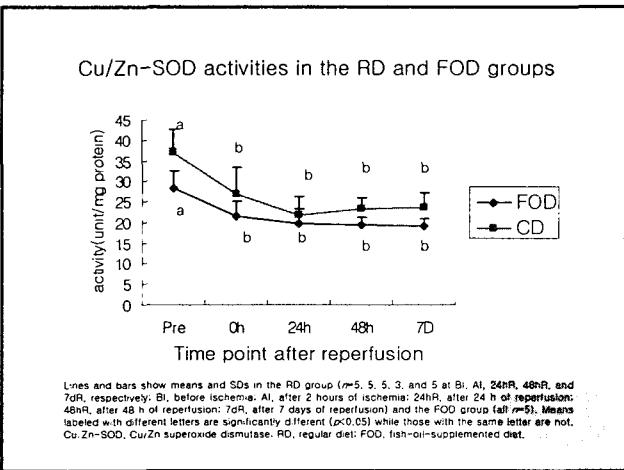


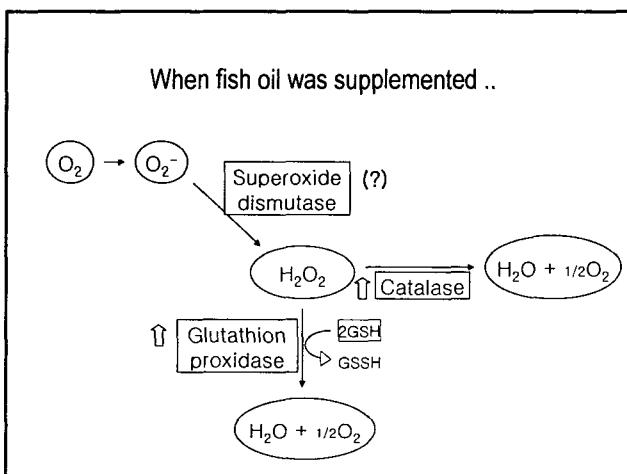
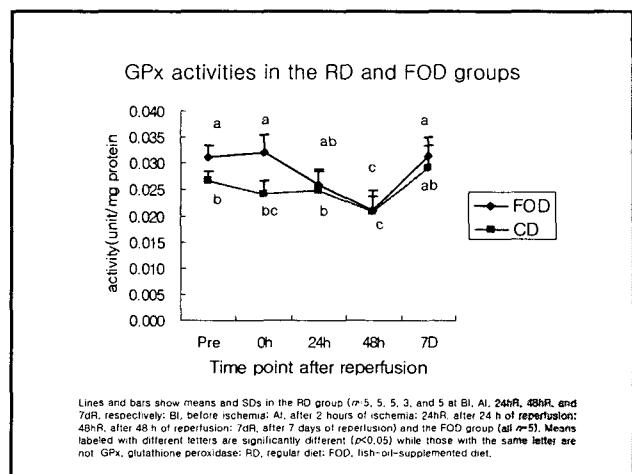
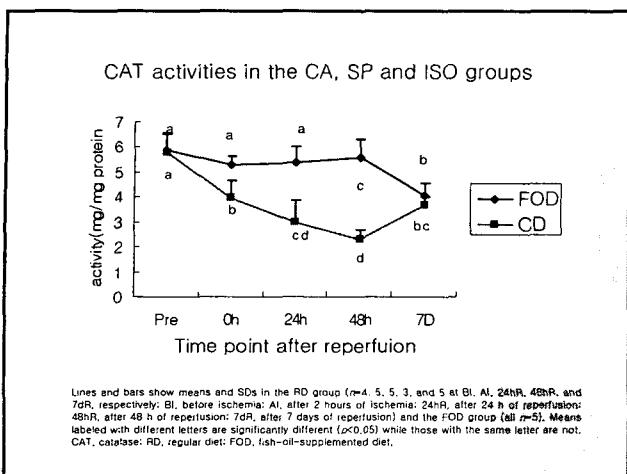


Fish oil에 함유된 DHA가 지질과 산화 형성을 낮출 수 있나?

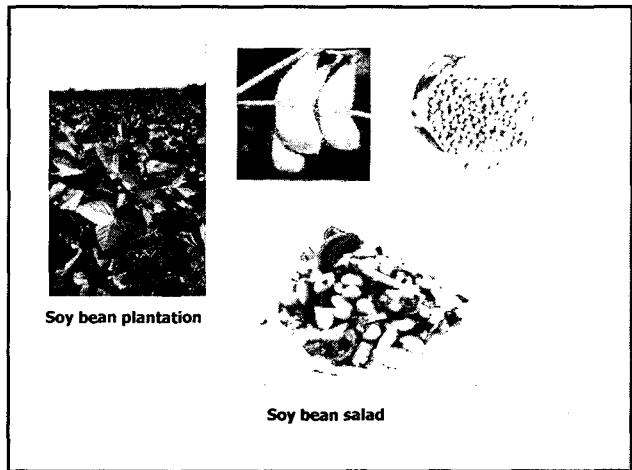
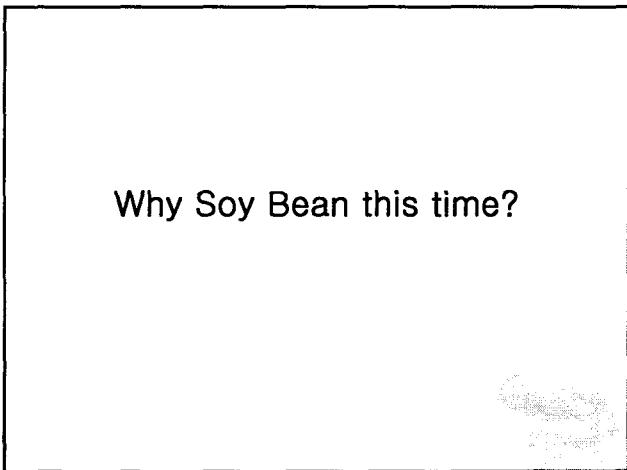


Fish oil 보충이 항산화요소의 활성도에 미치는 효과는?





- ### Next Questions
- 1. Will the Soy Bean supplementation prevent neuronal cells from the injury?
 - 2. If yes, then what will be the mechanism of prevention from cell damage?



❖ 대두 단백질의 항산화 효과

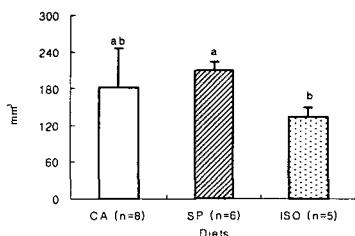
- LDL의 산화를 감소시키는 항산화 물질을 포함(Riikka, 2000)
- 지질 과산화 형성 지표가 되는 TBARS 측정치를 낮추어 산화적 스트레스가 적음 (Sekizaki, et al.)

Isoflavone

- ❖ 항산화 효과 및 면역성 강화 보고(genistein and diadzein)(Hawrylewicz et al., 1995 ; Kennedy, 1995 ; Herman, et al., 1995).

대두 단백질과 isoflavone 식이 섭취가 뇌하혈 유발 후 재관류 시 뇌경색 크기에 미치는 영향은?

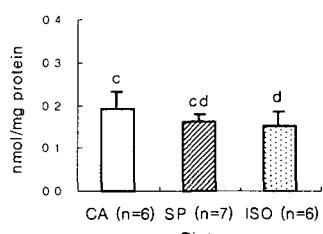
Cortex infarction volumes
in the CA, SP and ISO groups



Bars show means \pm SDs in the CA, SP and ISO groups. Means labeled with different letters are significantly different ($p<0.05$) by Duncan's multiple range test, while those with the same letter are not. CA, 20% casein-based diet; SOY, 20% soy protein-based diet; ISO, 0.2% isoflavone-supplemented diet.

대두 단백질과 isoflavone 식이 섭취가 뇌하혈 유발 후 재관류 시 대뇌피질의 지질과산화물 함량에 어떤 영향을 미치나?

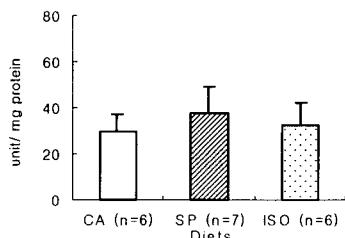
TBARS in the CA, SP and ISO groups



Bars show means \pm SDs in the CA, SP and ISO groups. Means labeled with different letters are significantly different ($p<0.05$) by Duncan's multiple range test, while those with the same letter are not. CA, 20% casein-based diet; SOY, 20% soy protein-based diet; ISO, 0.2% isoflavone-supplemented diet; TBARS, thiobarbituric-acid-reactive substances.

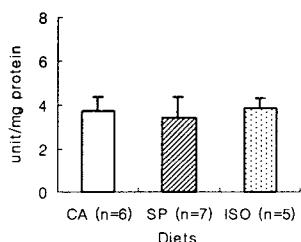
대두 단백질과 isoflavone 식이
섭취가 뇌허혈 유발 후 재관류 시
대뇌피질의 항산화 효소 활성도
에 미치는 영향은?

SOD activities in the CA, SP and ISO groups



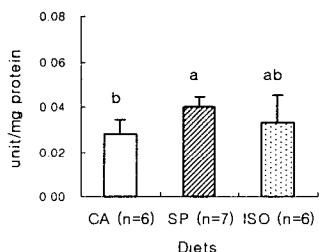
Bars show means \pm SDs in the CA, SP and ISO groups. Means labeled with different letters are significantly different ($p<0.05$) by Duncan's multiple range test, while those with the same letter are not. CA, 20% casein-based diet; SOY, 20% soy protein-based diet; ISO, 0.2% isoflavone-supplemented diet; SOD: superoxide dismutase.

CAT activities in the CA, SP and ISO groups



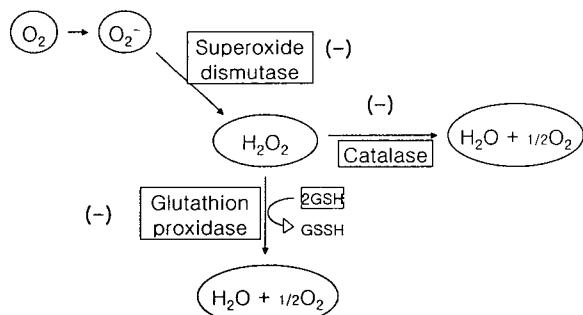
Bars show means \pm SDs in the CA, SP and ISO groups. Means labeled with different letters are significantly different ($p<0.05$) by Duncan's multiple range test, while those with the same letter are not. CA, 20% casein-based diet; SOY, 20% soy protein-based diet; ISO, 0.2% isoflavone-supplemented diet; CAT, catalase.

GPx activities in the CA, SP and ISO groups



Bars show means \pm SDs in the CA, SP and ISO groups. Means labeled with different letters are significantly different ($p<0.05$) by Duncan's multiple range test, while those with the same letter are not. CA, 20% casein-based diet; SOY, 20% soy protein-based diet; ISO, 0.2% isoflavone-supplemented diet; GPx: glutathione peroxidase.

When Soy protein/isoflavone was supplemented ..



대두 단백질 식이가 LDL의 양을 감소시킬 수 있나?

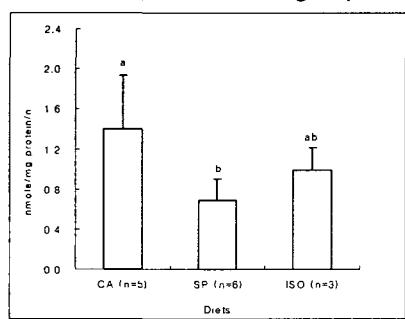
Plasma lipid concentrations
in the CA, SP, and ISO groups

	CA (n=14)	SP (n=13)	ISO (n=14)
TC (mmol/l)	76.14 ± 11.35	77.08 ± 13.74	71.57 ± 11.8
HDL-C (mmol/l)	29.86 ± 5.32	29.23 ± 6.22	28.36 ± 4.38
LDL-C (mmol/l)	10.29 ± 2.5 ^a	9.46 ± 2.5 ^a	6.86 ± 2.93 ^b
LDL-C/HDL-C	0.35 ± 0.12 ^a	0.33 ± 0.08 ^a	0.25 ± 0.11 ^b
TC/HDL-C	2.58 ± 0.33	2.68 ± 0.33	2.54 ± 0.3

Data are means ± SDs in the CA, SP, and ISO groups. Means with different superscript letters are significantly different ($p<0.05$) by Duncan's multiple range test, while those with the same letter are not. TC, total cholesterol; CA, 20% casein-based diet; SP, soy protein isolate-based diet; ISO, 0.2% isoflavone-supplemented diet.

대두 단백질 식이가 뇌내 아세틸 콜린의 양에 영향을 미칠 수 있나?

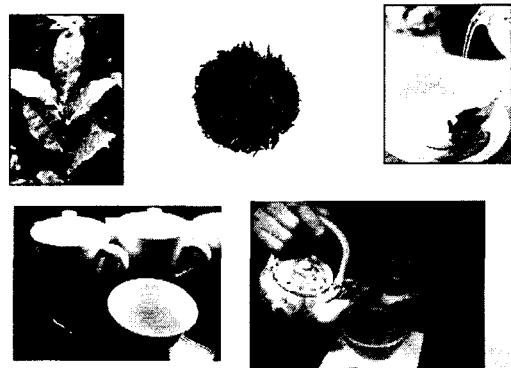
Acetylcholinesterase activities
in the CA, SP and ISO groups



Bars show means ± SDs in the CA, SP and ISO groups. Means labeled with different letters are significantly different ($p<0.05$) by Duncan's multiple range test, while those with the same letter are not. CA, 20% casein-based diet; SP, 20% soy protein-based diet; ISO, 0.2% isoflavone-supplemented diet.

Catechin and Skin cells

S.E. Jeon, et al.
The Photomedicine Society, 2003.

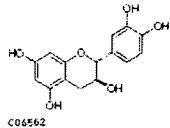


Flavonoids

- 그리스어로 황색을 의미하는 flavus에서 유래 식물색소
- Flavon
- Isoflavon : genistein
- Favonol : quercetin
- Favanone
- Flavan-3-ol
- Catechin : (+)-catechin, 녹차 폴리페놀성분
- Anthrocyanidin

● (+)-catechin

- 차, 과일, 야채, 적포도주, 코코아 등에 풍부함



- Functions
 - 프리라디칼 소거
 - Fe과 Cu와 같은 금속이온의 칼레이트
- 이러한 작용은 aromatic ring의 2개의 인접한 hydroxy group에 의한 oxidation-reduction에 의한 것으로 추측됨

What is Ultra Violet light?

● Ultra violet light

UVA (320-400nm)

: photoaging, pigmentation

UVB (290-320nm)

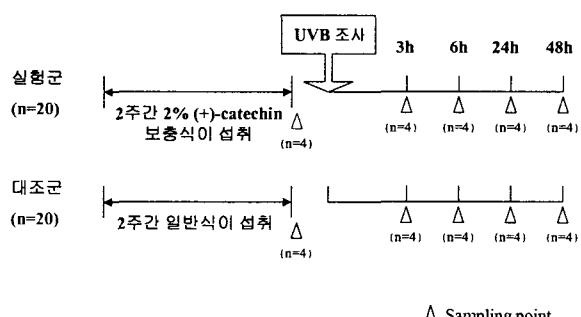
: erythema, inflammation, hyperplasia, DNA damage & mutation, skin cancer

UVC (200-290nm)

: skin cancer



Experimental design



Materials & Methods

● **Animal :** male BALB/c mice, 4-6weeks old

● **Diets**

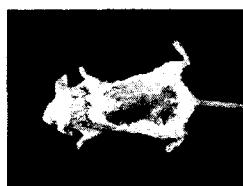
Regular diet (AIN93G rodent diet base)
2% (+)-catechin supplemented diet

● **UVB irradiation :** BLE-IT158 lamp
200mJ/cm²

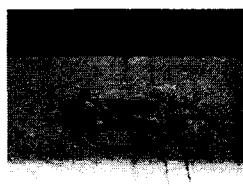
◆ UVB Irradiation



BALB/c mouse



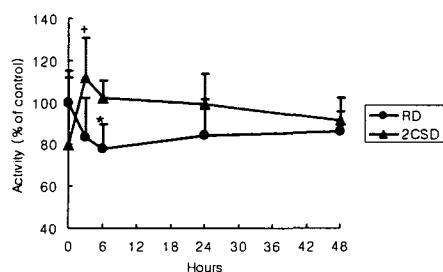
After shaved with electric clipper



UVB irradiation in the UV chamber (200 mJ/cm²)

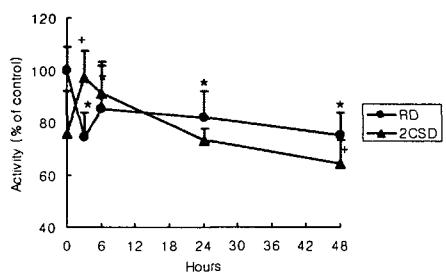
UVB 조사 전 (+)-catechin 보충식이 섭취가 UVB 조사 후 피부 항산화 효소의 활성도에 미치는 영향은?

SOD activity



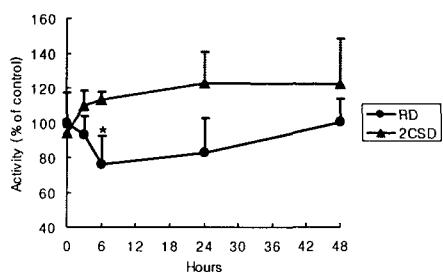
*+, + p<.05 compared with non-irradiated group within the same treated group
* for RD, + for 2CSD

Catalase activity



*+, + p<.05 compared with non-irradiated group within the same treated group
* for RD, + for 2CSD

GPx activity

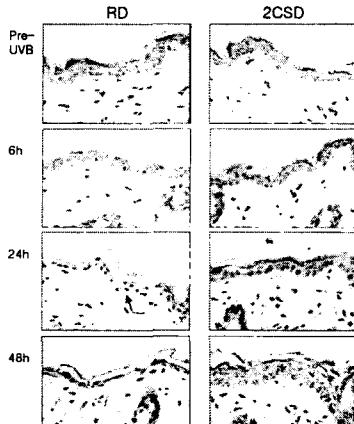


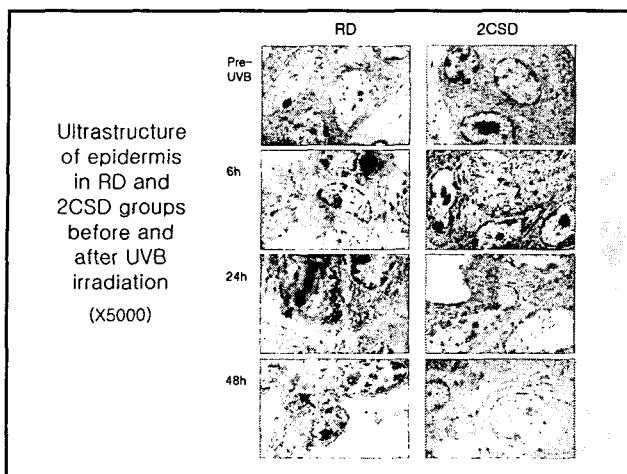
*+, + p<.05 compared with non-irradiated group within the same treated group
* for RD, + for 2CSD

UVB 조사 전 (+)-catechin 보충식이 섭취가 UVB 조사 후 조직손상에 미치는 영향은?

H & E staining
Electron microscopic examination

Effects of (+)-catechin supplemented diet on the BALB/c mice skin after UVB irradiation (H & E, 400x)





Conclusions

- RD군에서 SOD, CAT, GPx 활성도의 감소
- RD군은 Catechin군과 달리 항산화효소 활성도 감소 후 조직학적 변화 관찰됨
 - 항산화효소의 활성도가 산화적 손상에 대한 주요 방어기제로 작용함을 간접적으로 시사함.
 - catechin 섭취가 항산화효소의 활성도를 보존하여 산화적 손상을 감소시키는 것으로 생각됨.

Evidence Searchers

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- Sang-Eun Jeon,
- Myoung-Ae Choe,
- Kyoung-Chan Park



감사합니다

