16th Interntional Conference on Ulcer Research
March 23, 2018, Seoul, Korea

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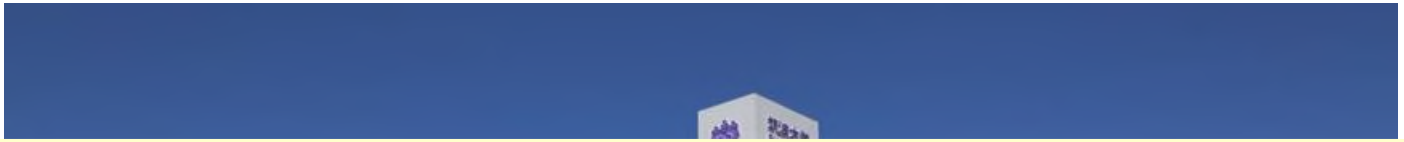

Role of NRF2 in Protection of GI Tract against Oxidative Stress

Akinori Yanaka, MD & PhD

Dept. of Gastroenterology
Hitachi Medical Education and Research Center
University of Tsukuba

Oxidative Stress and GI Diseases

1. GERD
2. *H.pylori*-related GI Diseases
3. NSAIDs-induced GI Injury
4. IBD
5. Cancers
6. Functional Disorders

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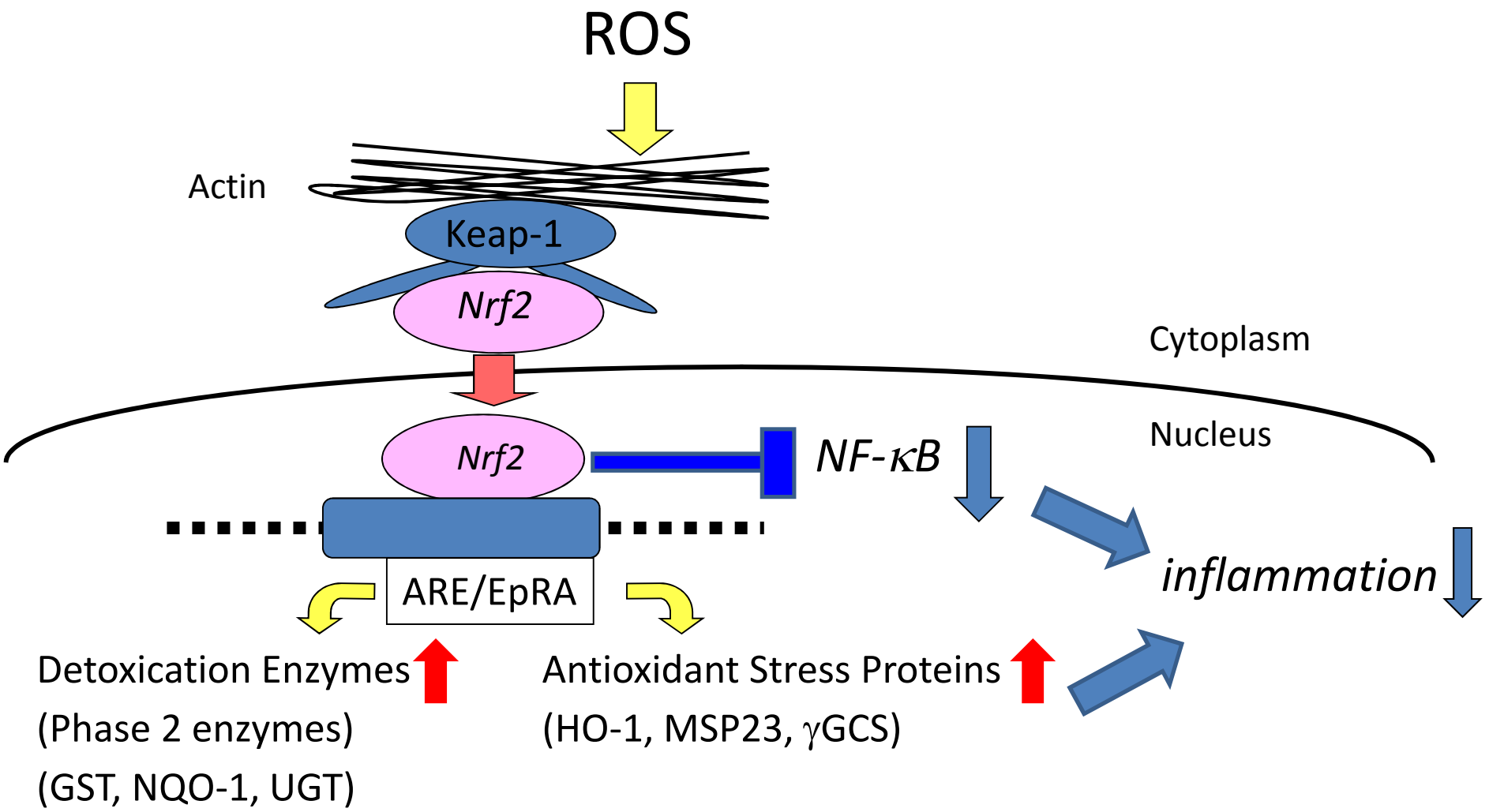
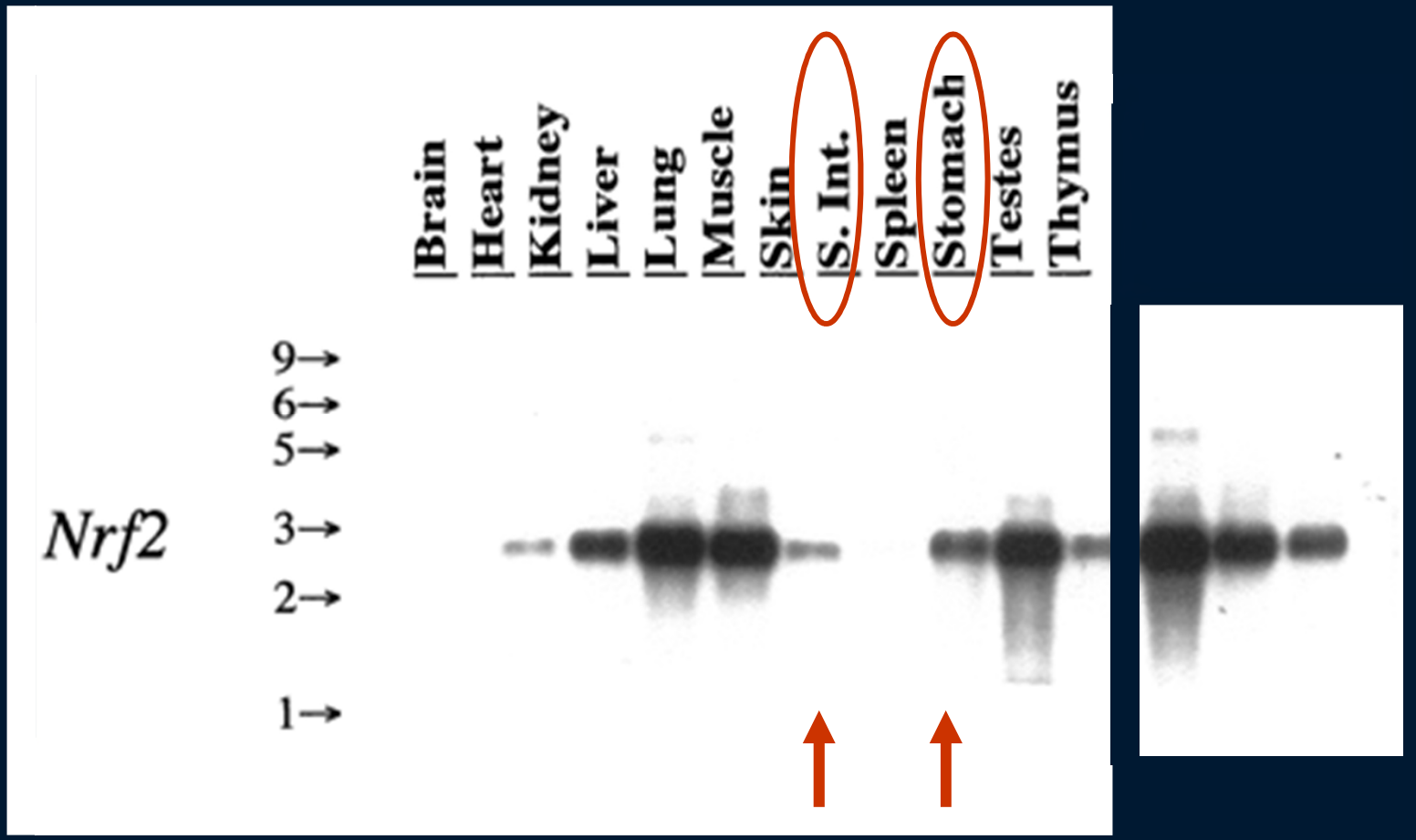
Cells withstand oxidative stress by *nrf2-keap 1 System*



Nrf2 is Strongly Expressed in Gastrointestinal Mucosae



McMahon M, et al. Cancer Res. 2001;61:3299-3307.

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Role of Sulforaphane in Protection of GI Tract against *H.pylori*- and NSAID-Induced Oxidative Stress

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University of Tsukuba

Today's Talk

1. General Concept of Sulforaphane

2. *H.pylori* and Sulforaphane

3. NSAIDs and Sulforaphane

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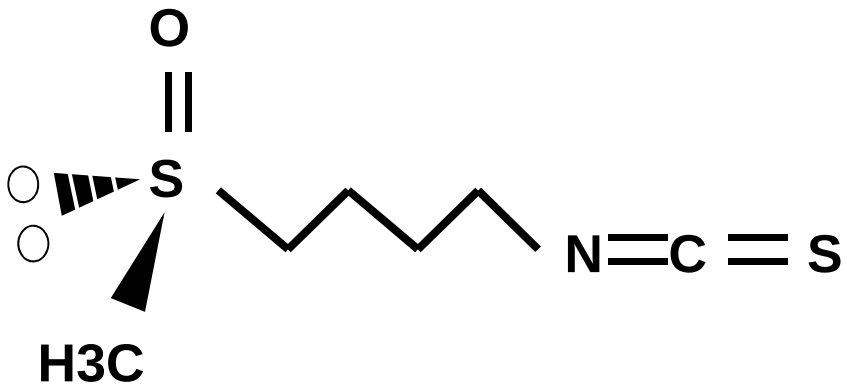
Sulforaphane as an Anti-Oxidant Enhancer

1. SFN, a member of an isothiocyanate family, is abundantly present in broccoli sprouts

2. SFN affords chemoprevention against various types of cancer via activation of antioxidant enzymes.

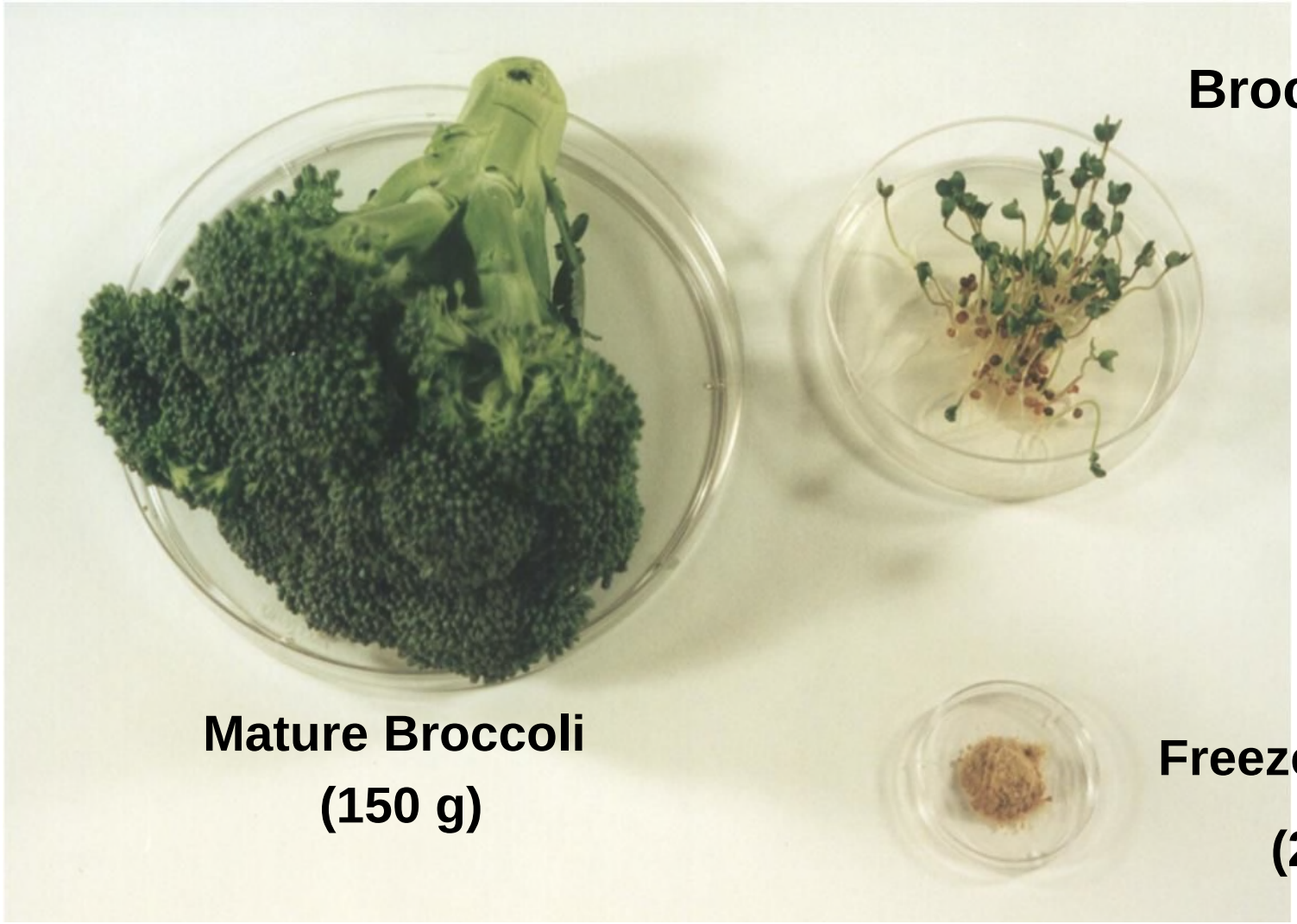
SULFORAPHANE



BROCCOLI SPROUTS

Sulforaphane is Rich in Broccoli Sprouts

Sulforaphane (24 mg) is included in



Broccoli Sprouts

(5 g)

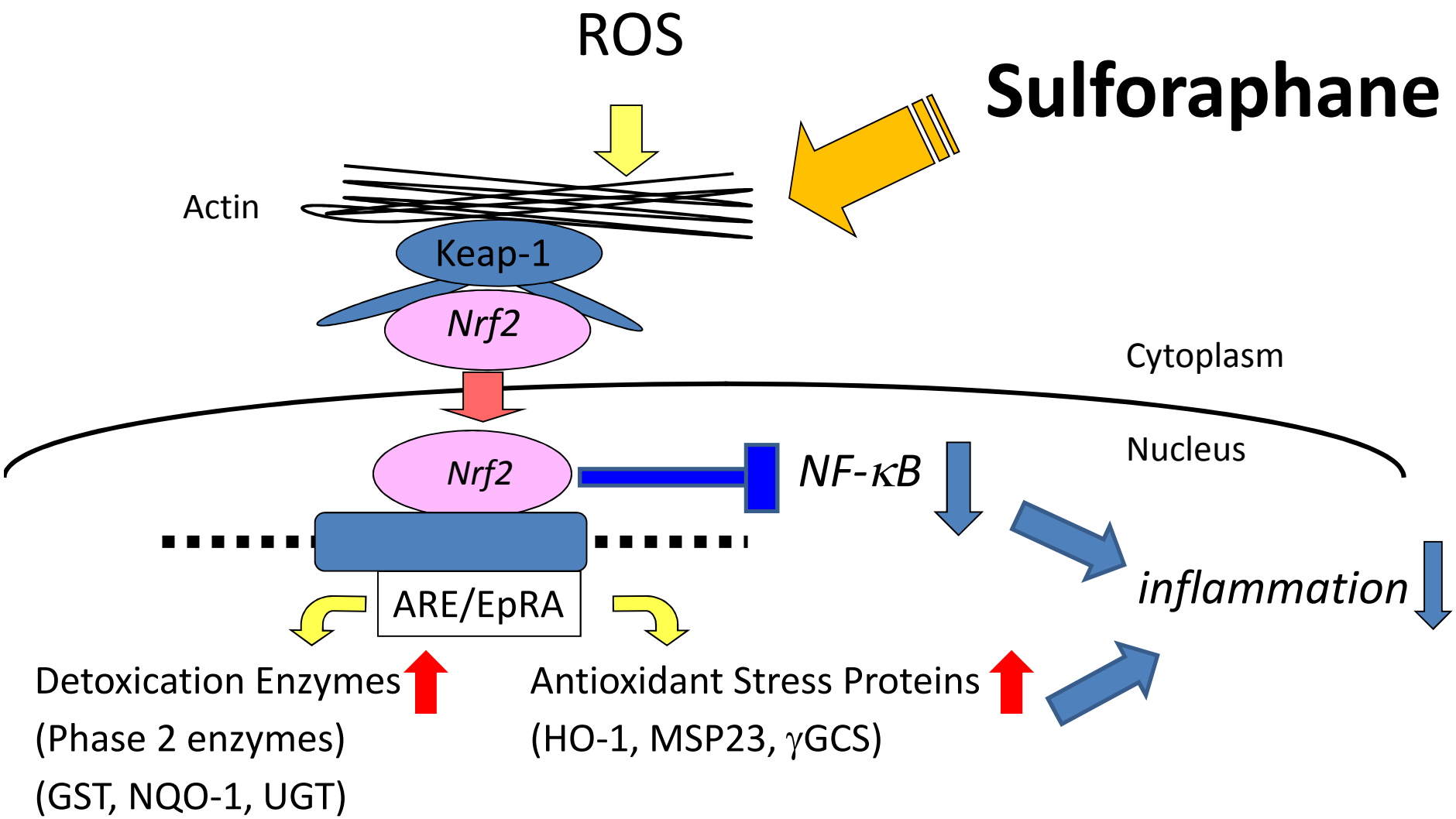
Mature Broccoli

(150 g)

Freeze Dried BS

(250 mg)

Sulforaphane Enhances Anti-Oxidant Activity via *nrf2-keap 1 System*



Sulforaphane Inhibits *H.pylori* Activity and Ameliorates *H.pylori*-induced Gastritis In Mice and Humans

Yanaka A, et al. Cancer Prevention Research 2:353-360,2009.

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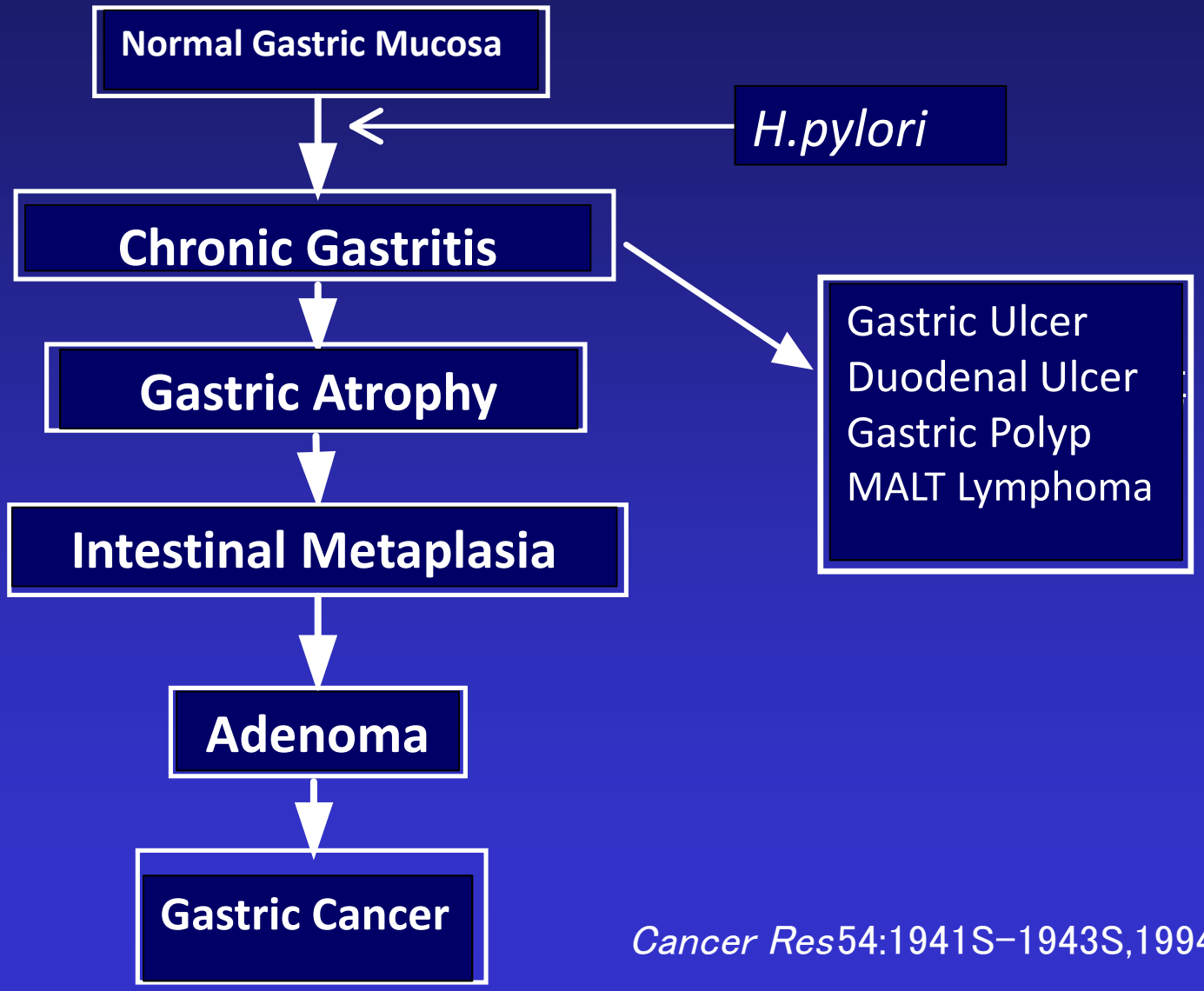
H.pylori and Gastro-Duodenal Diseases

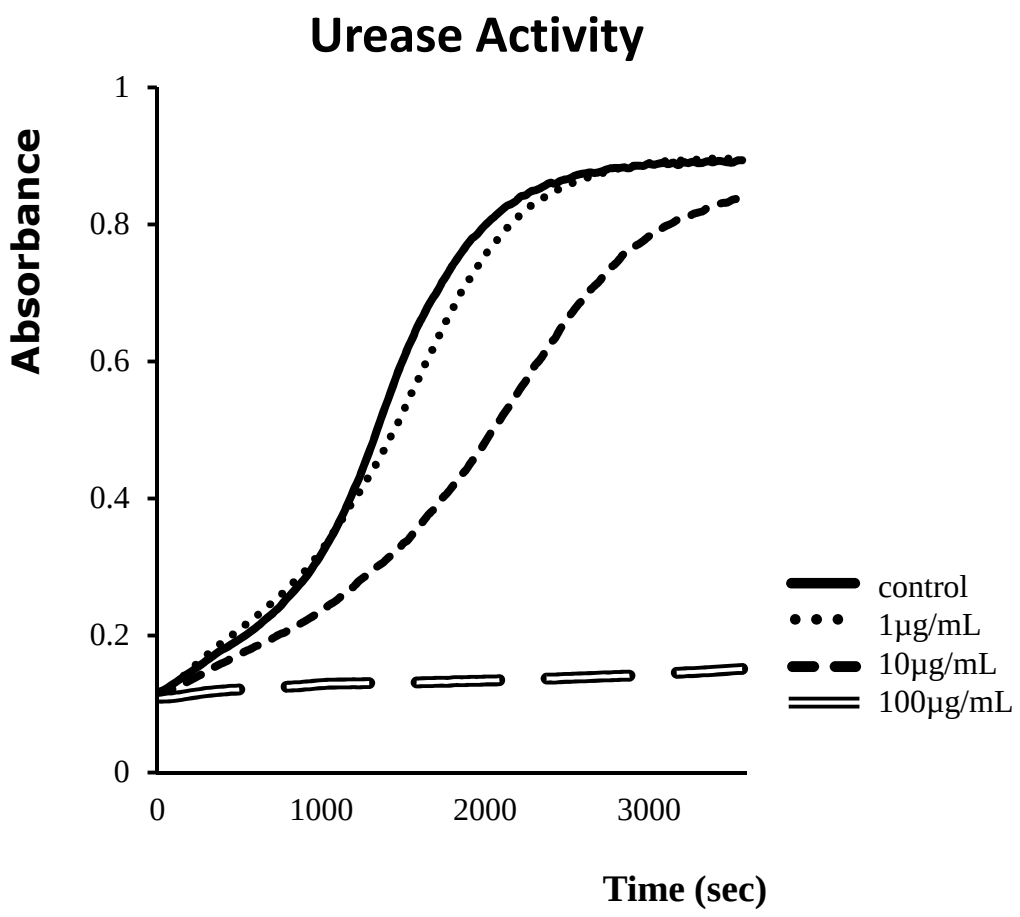


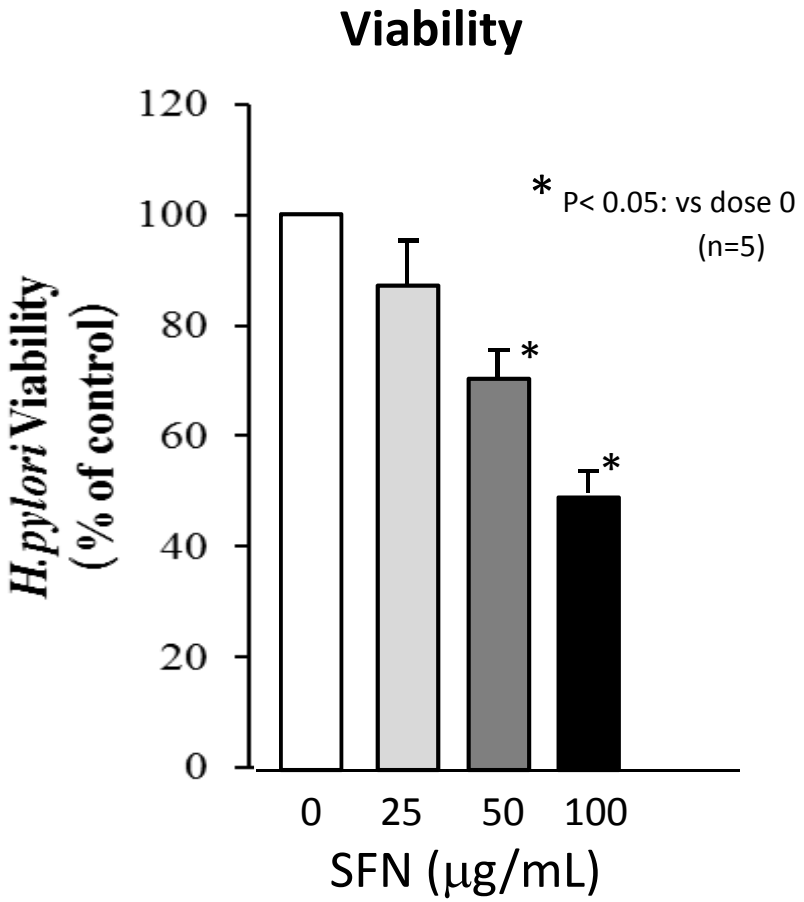
Cancer Res 54:1941S–1943S,1994

Sulforaphane has strong Bactericidal Activity against *Helicobacter pylori in vitro*

Fahey JW, et al. PNAS 99:7610, 2002

SFN Markedly Inhibits Urease Activity and Viability of H.pylori





H. pylori -induced Chronic Gastritis Model in Mice

Nrf2+/+ vs Nrf2-/-

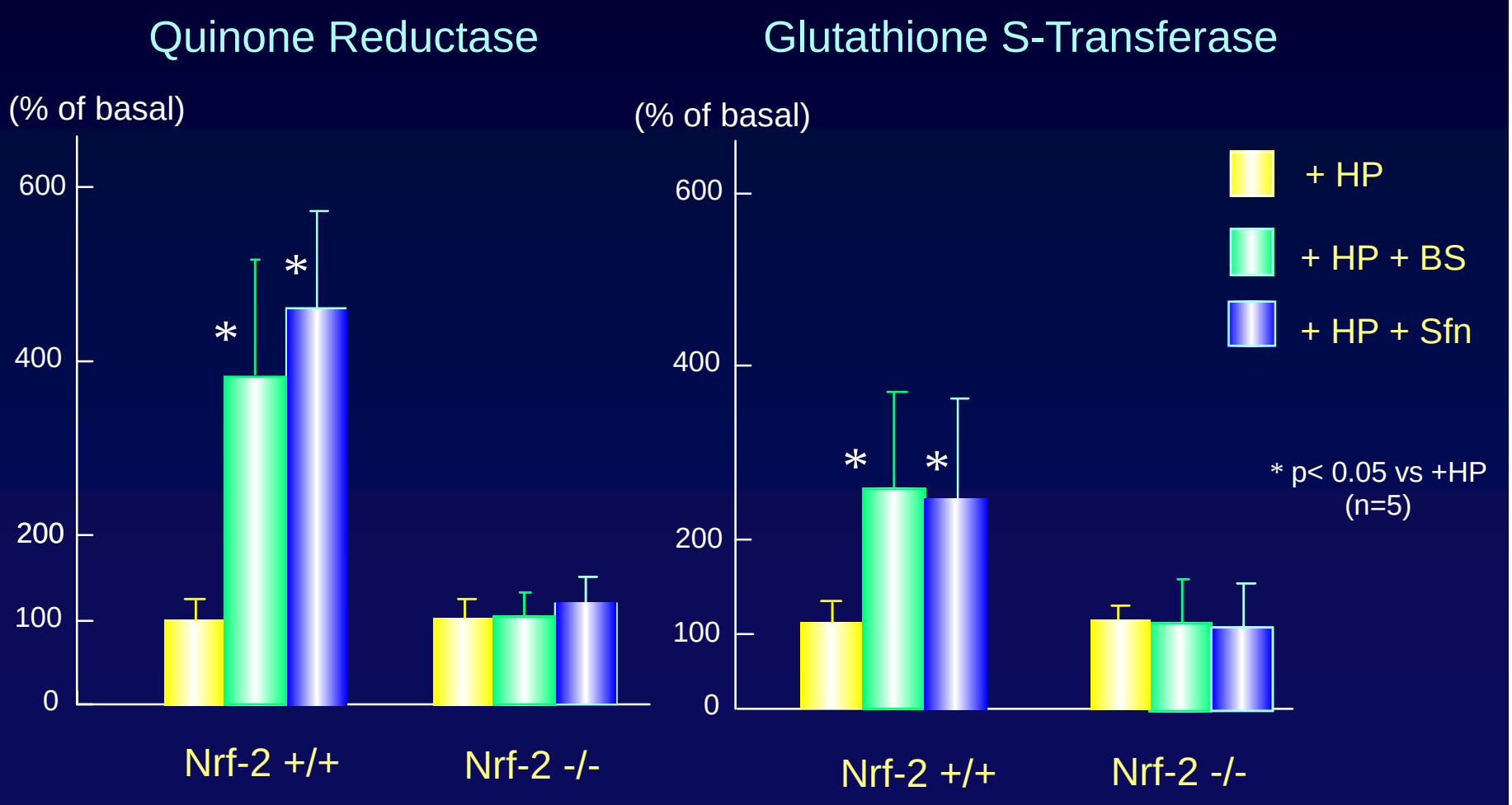
C57/BL6 Mice

+

H.pylori (SS-1)

Lee A, et al. Gastroenterology 1997.

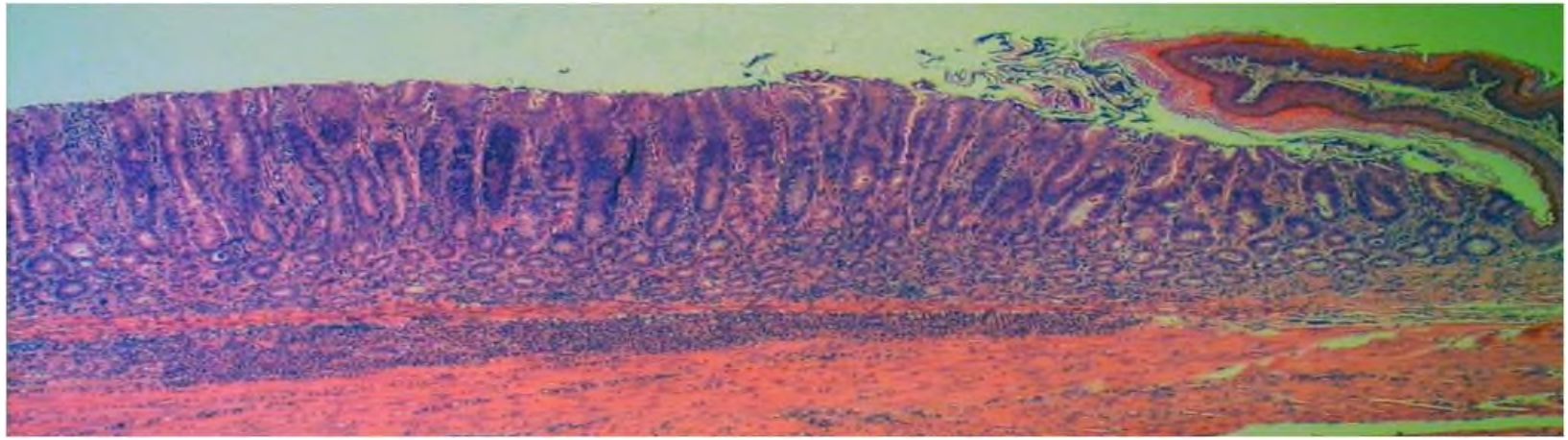
Broccoli Sprouts Enhance Anti-Oxidant Enzymes *Via Nrf2-dependent Mechanisms*



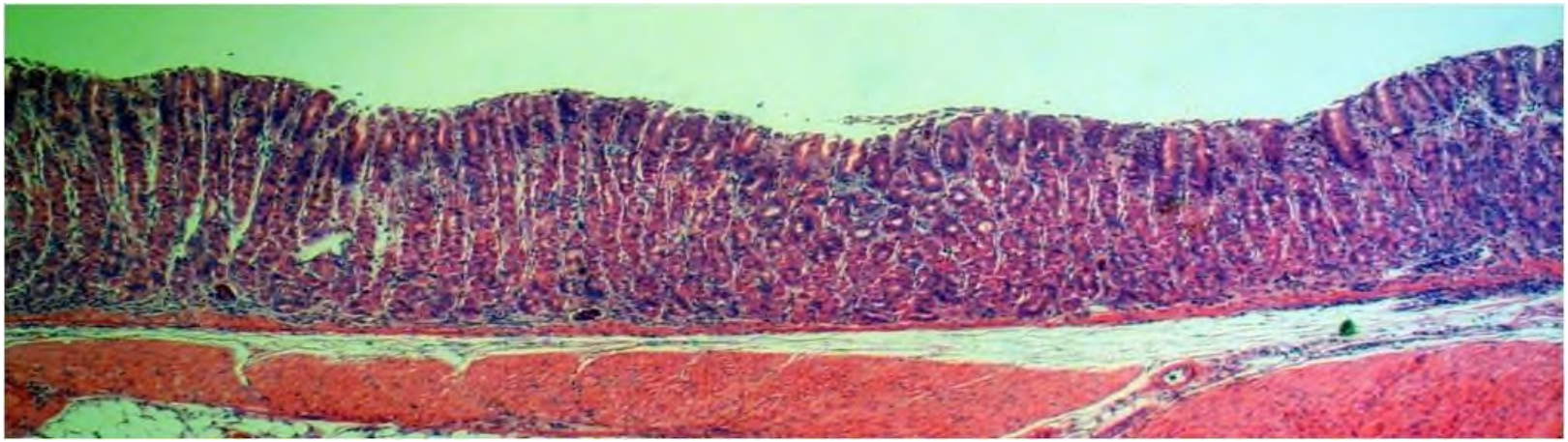
Yanaka A, et al. Cancer Prevention Research 2: 353-360,2009.

Broccoli Sprouts Markedly Attenuate Corpus Gastritis in *H.pylori*-infected Nrf2+/+ Mice fed with High Salt Diet

- BS

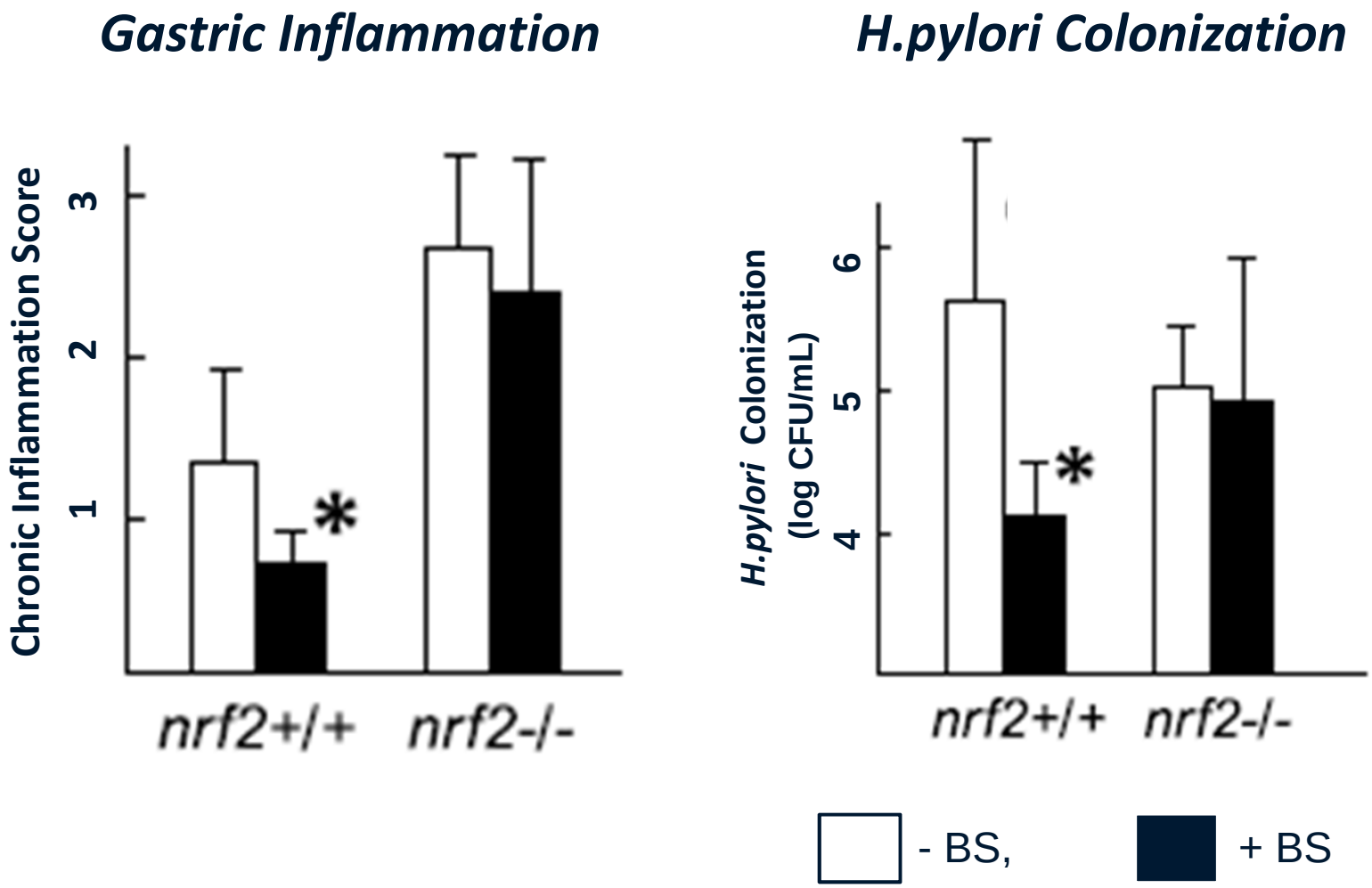


+ BS



Yanaka A, et al. Cancer Prevention Research 2: 353-360,2009.

Broccoli Sprouts Attenuates Corpus Gastritis and Inhibits H.pylori Colonization in Nrf2+/+, but not in Nrf2-/- mice



Sulforaphane and *Helicobacter pylori*

Results from Basic Study

1. Sulforaphane suppresses *Helicobacter pylori (Hp)* urease activity and reduces *viability in vitro.*

2. Sulforaphane mitigates Hp-induced corpus gastritis *in vivo.*

3. These effects of Sulforaphane appear to be mediated by nrf2-depdendent upregulation of anti-oxidant enzyme activity, and by direct inhibition of Hp activity.

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Clinical Trial for Sulforaphane

Enroll
50 subjects

Broccoli Sprouts
or Alfalfa Sprouts

8 weeks

Broccoli SuperSprouts®
250 mg Sulforaphane/100g
(Murakami Noen Co. Ltd.)

BS Group
25 subjects

Urine
Hp-Ab

AS Group
25 subjects

0 28 56 112 days

Blood, Urine Stool, UBT

Blood, Urine Stool, UBT

Blood, Urine Stool, UBT

Blood, Urine Stool, UBT

Approved by IRB in University of Tsukuba

Dietary SGS (30 – 60 mg) enhances anti-oxidant enzymes in humans

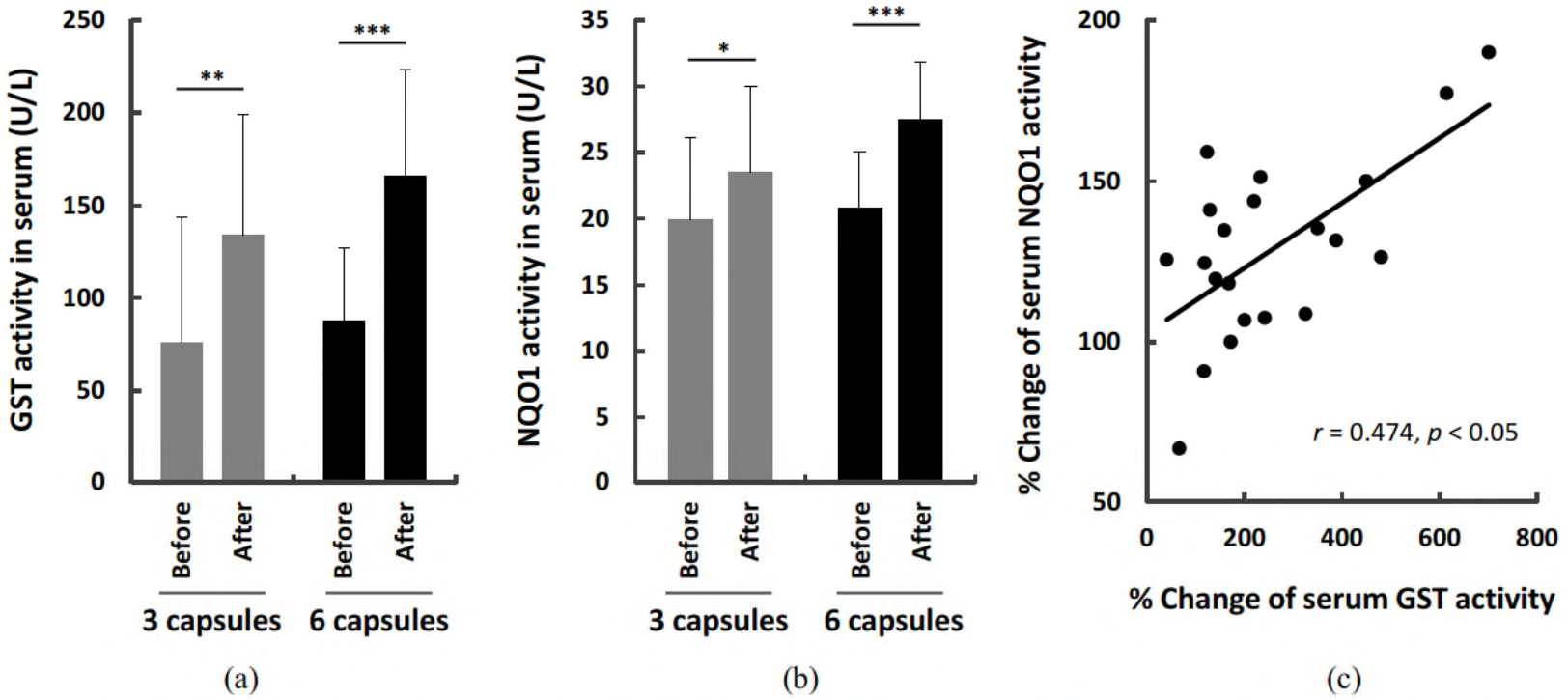


Figure 3. Serum activities of phase 2 enzymes, GST (A) and NQO1 (B) in subjects before and 24 after single administration of 3 and 6 capsules of BS supplement (n = 11 and 10, respectively), and the relationship between the percentage changes of GST and NQO1 activities in individual subjects (C). *: $p < 0.05$, and ***: $p < 0.001$ (paired t-test). Correlation coefficient (r) and significance (p) were analyzed by the Spearman correlation test.

Ushida Y, Yanaka A,et al. Food and Nutirition Sciece 2015; 6: 1603-1612.

Measurements

Degree of Gastritis

Serum Pepsinogen

Eiken Pepsinogen I & II (ELISA)

H.pylori Colonization

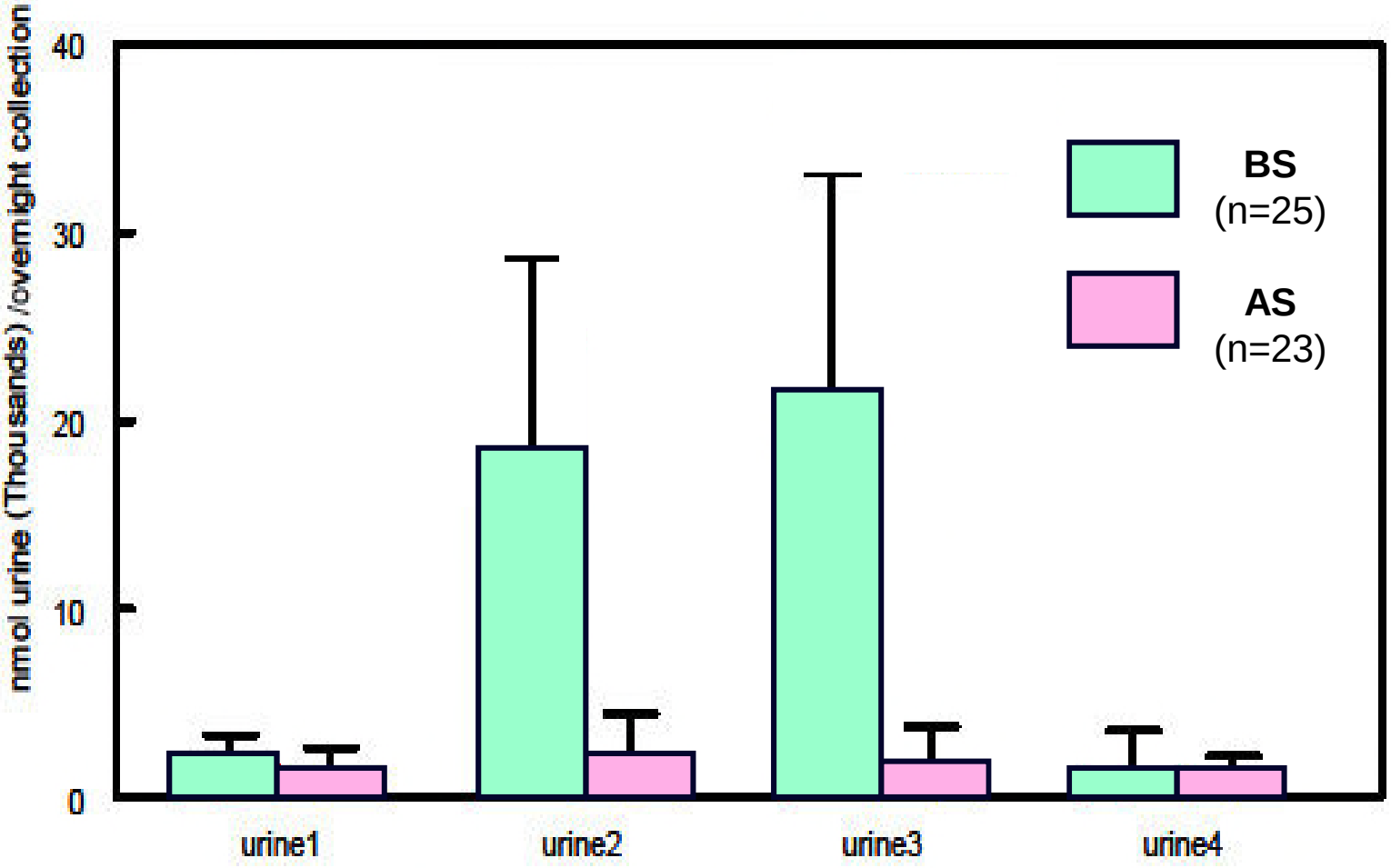
H.pylori Specific Stool Antigen (HpSA)

Meridian HpSA ELISA®

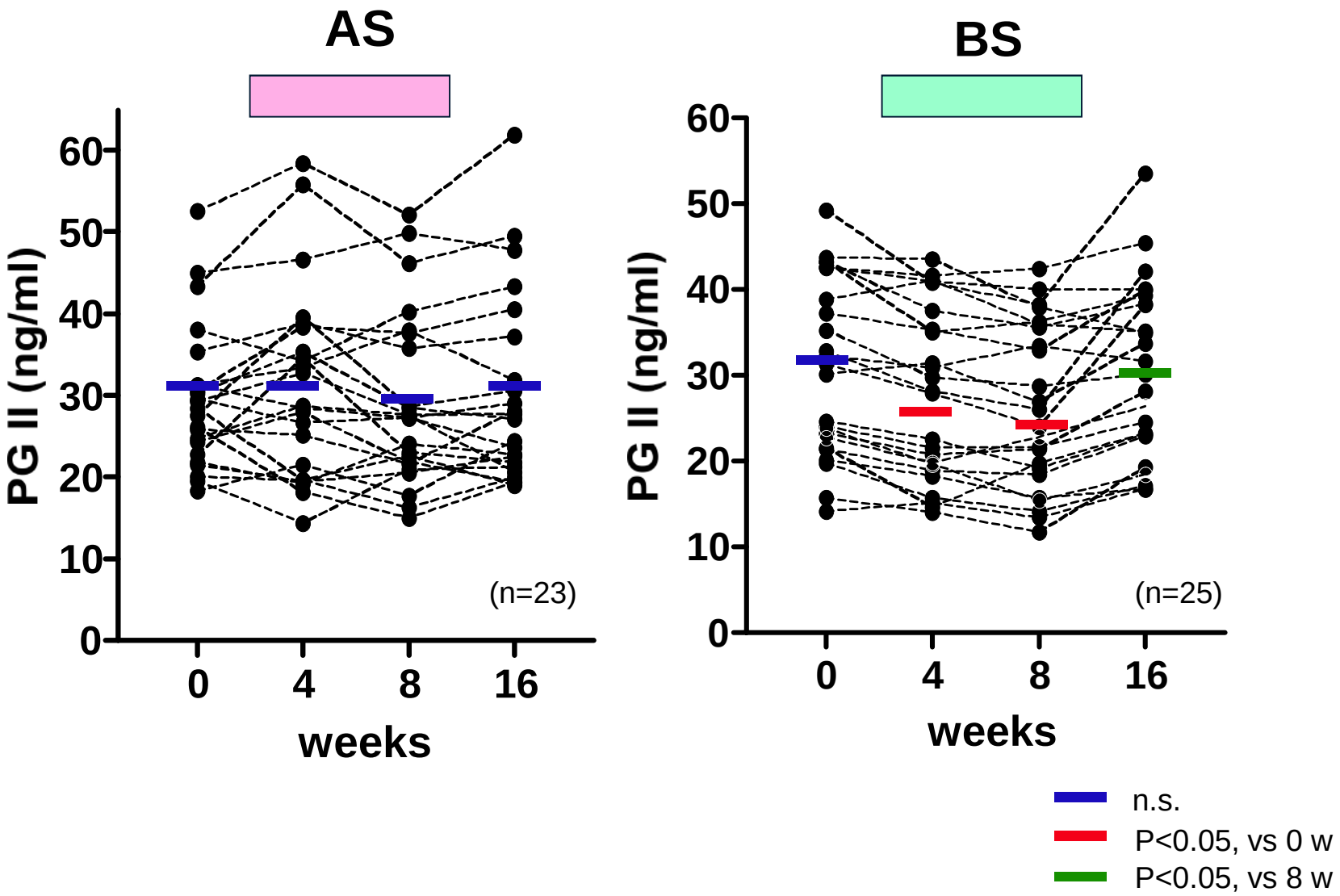
Urea Breath Test (UBT)

In vivo Assessment for *H.pylori* Urease Activity

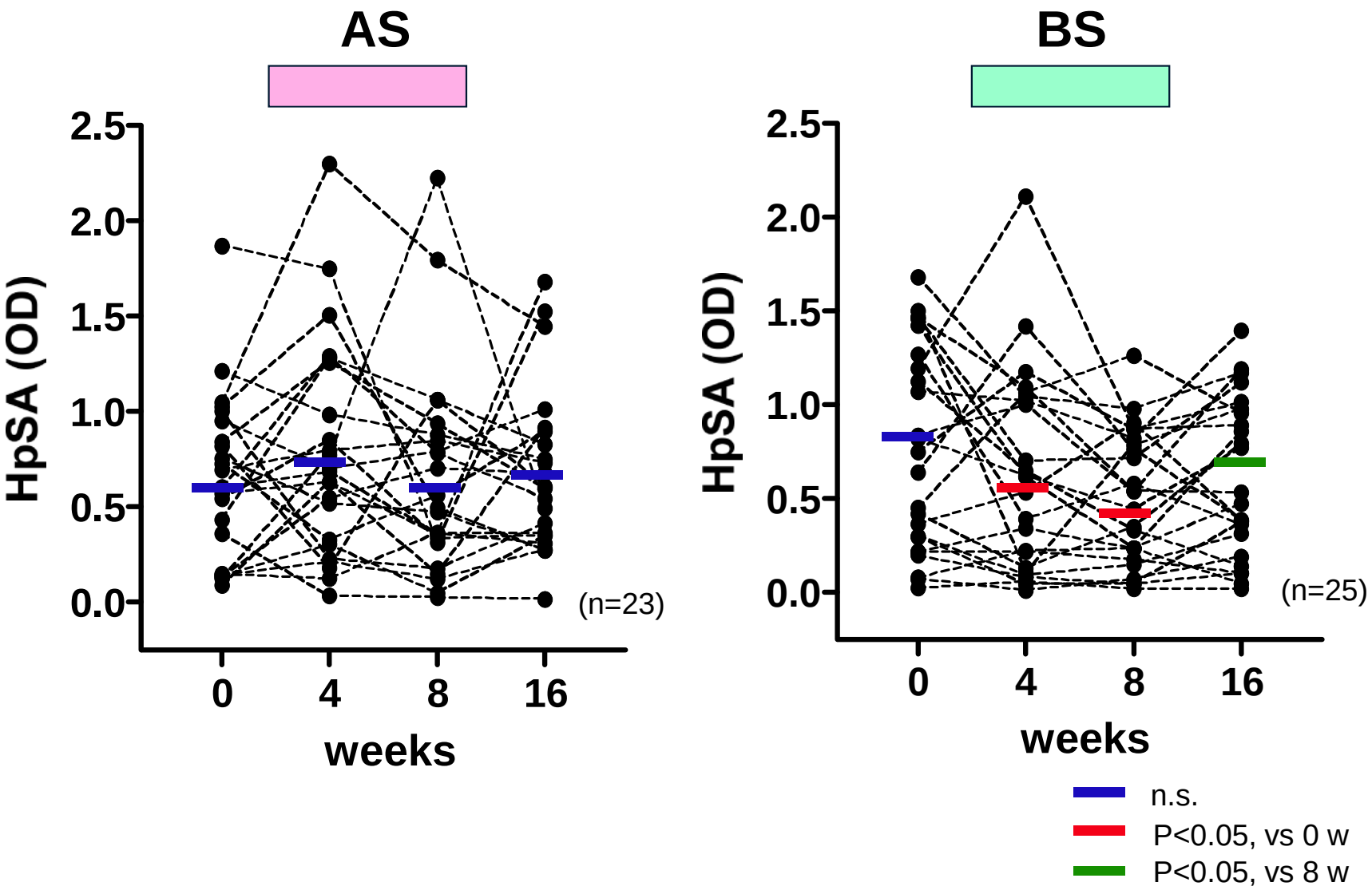
Changes in Urinary DTC Output during BS/AS Treatment



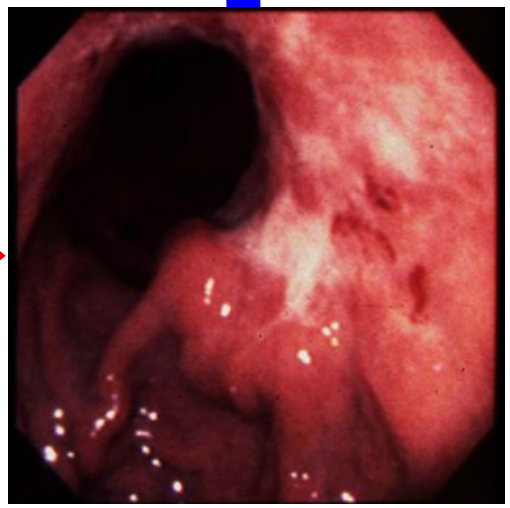
Effects of Broccoli or Alfala Sprouts on Serum PG II Level



Effects of Broccoli or Alfala Sprouts on *H pylori-specific* Stool Antigen



Broccoli Sprouts (Sulforaphane)



H. pylori **Gastric Cancer**

Sulforaphane and *Helicobacter pylori*
- Results from Clinical Trial -

1. In *H.pylori*-infected human subjects,

   Daily intake of Sulforaphane-rich Broccoli Sprouts for 8 wks

   1) decreased *Helicobacter pylori (Hp)* colonization.

   2) improved chronic gastritis activity.

2. Daily intake of Sulforaphane-rich Broccoli Sprouts
   may be useful for chemoprevention against gastric cancer.

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Chemoprotection against Gastric Cancer by Sulforaphane

H.pylori Eradication

+

Nrf2 Stimulation

Sulforaphane Protects Small Intestine From Aspirin/NSAIDs-Induced Injury

Yanaka A, et al. Current Pharmaceutical Design 19:157-162, 2013.

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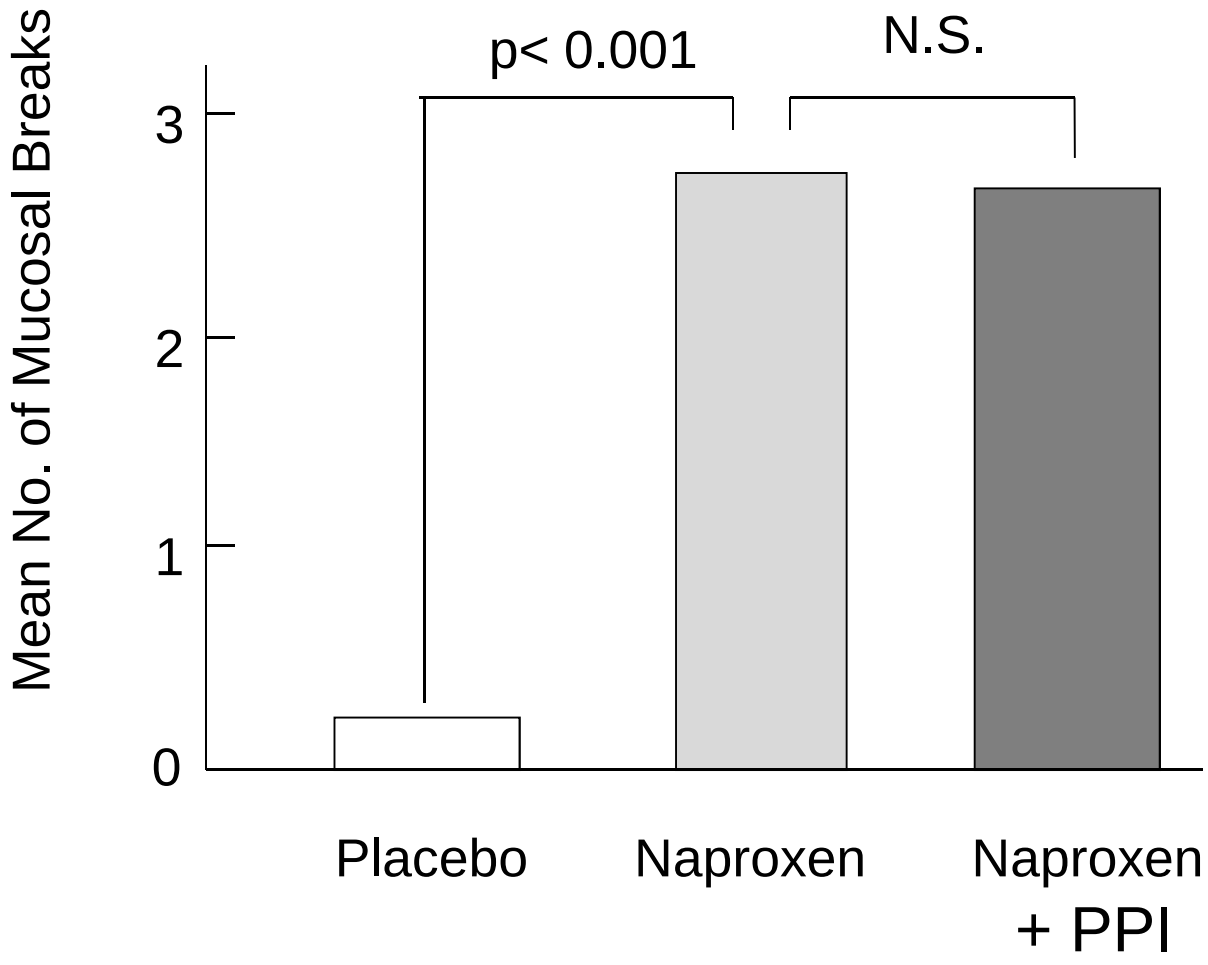
NSAIDs frequently induces ulcers in small intestine

		Incidence of ulcer
NSAIDs users	(n=21)	71 %
NSAIDs non-users	(n=20)	10 %

Graham DY, et al. Clin Gastroenterol Hepatol. 2005;3:55-59.

Acid Inhibitors are not effective for small intestinal ulcers



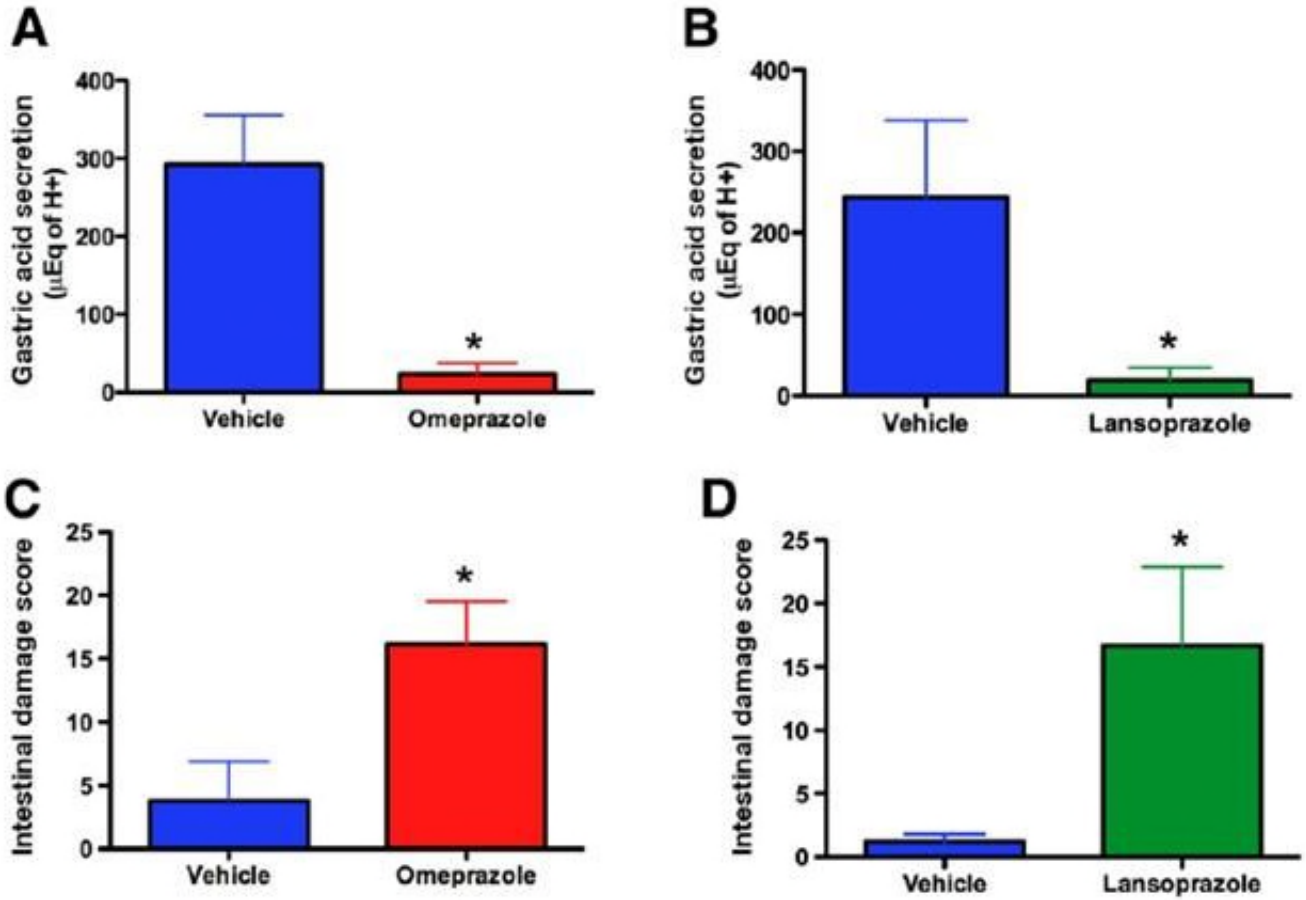
Goldstein JL,, et al. Clin Gastroenterol Hepatol. 2005;3:133-141.

GASTROENTEROLOGY 2011;141:1314–1322

Dysbiosis induced by PPI Tx exacerbates NSAID-induced ulcers in small intestine

JOHN L. WALLACE,* STEPHANIE SYER,* EMMANUEL DENOU,* GIADA DE PALMA,* LINDA VONG,* WEBB McKNIGHT,* JENNIFER JURY,* MANLIO BOLLA,‡ PREMYSL BERCIK,* STEPHEN M. COLLINS,* ELENA VERDU,* and ENNIO ONGINI‡

*Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, Ontario, Canada; and ‡NicOx Research Institute, Bresso, Italy



Background

Putative Mechanisms for NSAIDs/Aspirin-Induced Small Intestinal Injury

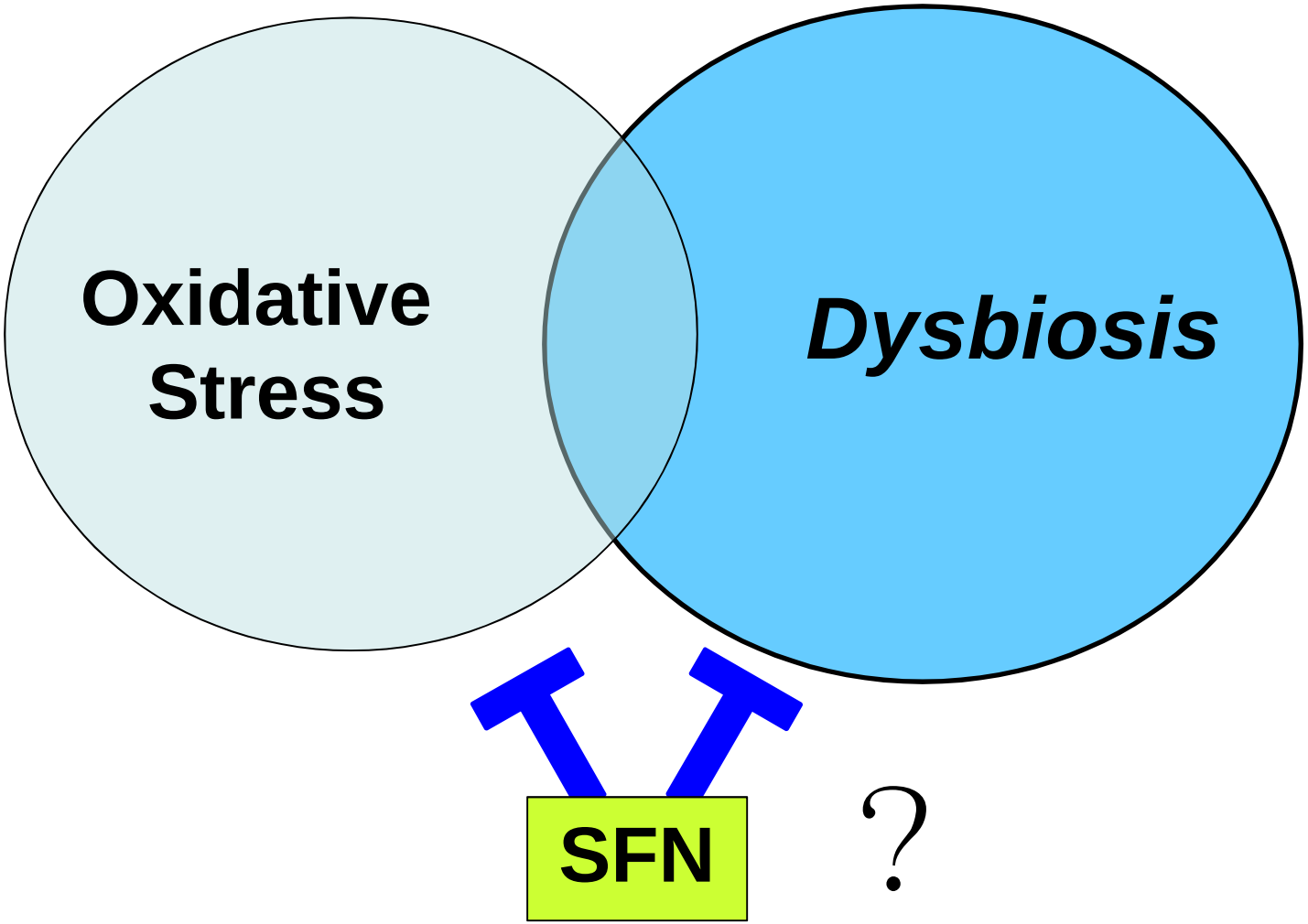
1) Role of Oxidative Stress

- Lipid Peroxidation of Cell Membrane
- Functional Disturbance of Mitochondria
- Ischemia-Reperfusion Injury
- Activation of Neutrophils

2) Role of Intestinal Microbiota

Whittle BJ. Eur J Pharmacol. 2004;500(1-3):427-39.

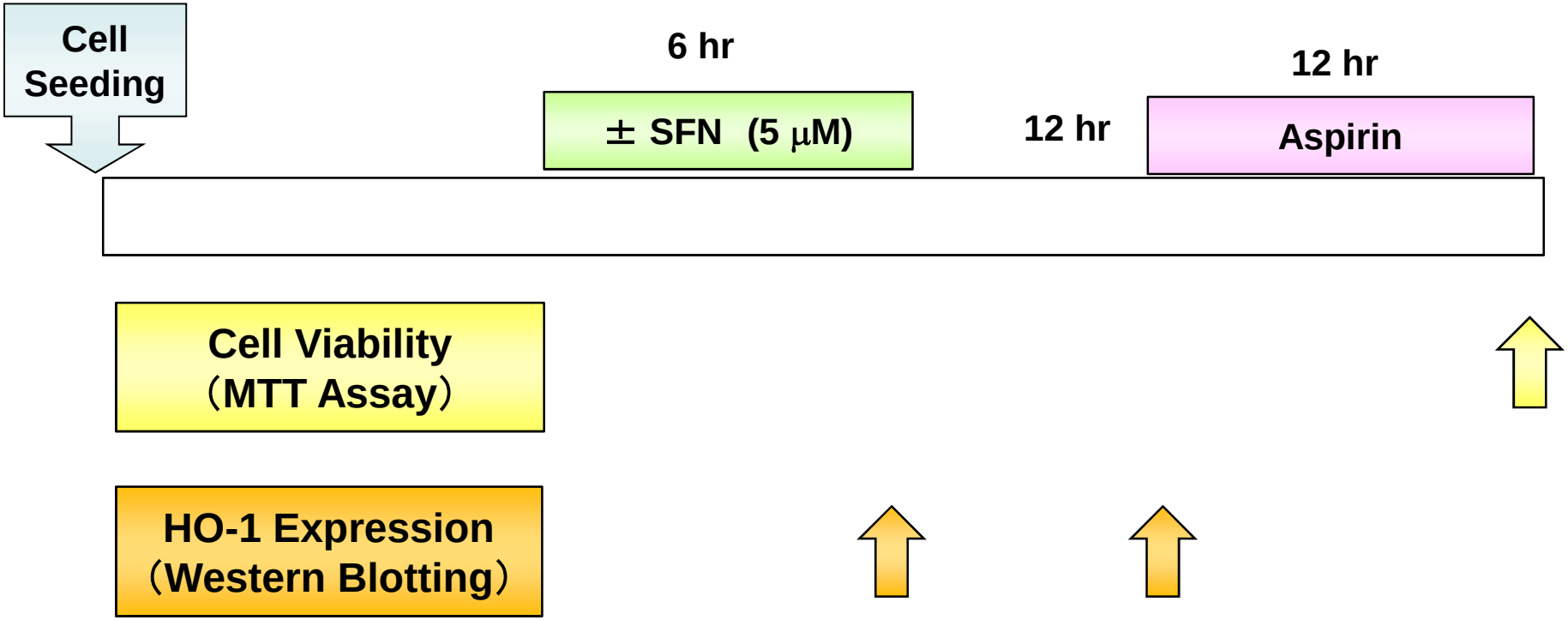
NSAID-induced Small Intestinal Ulcers



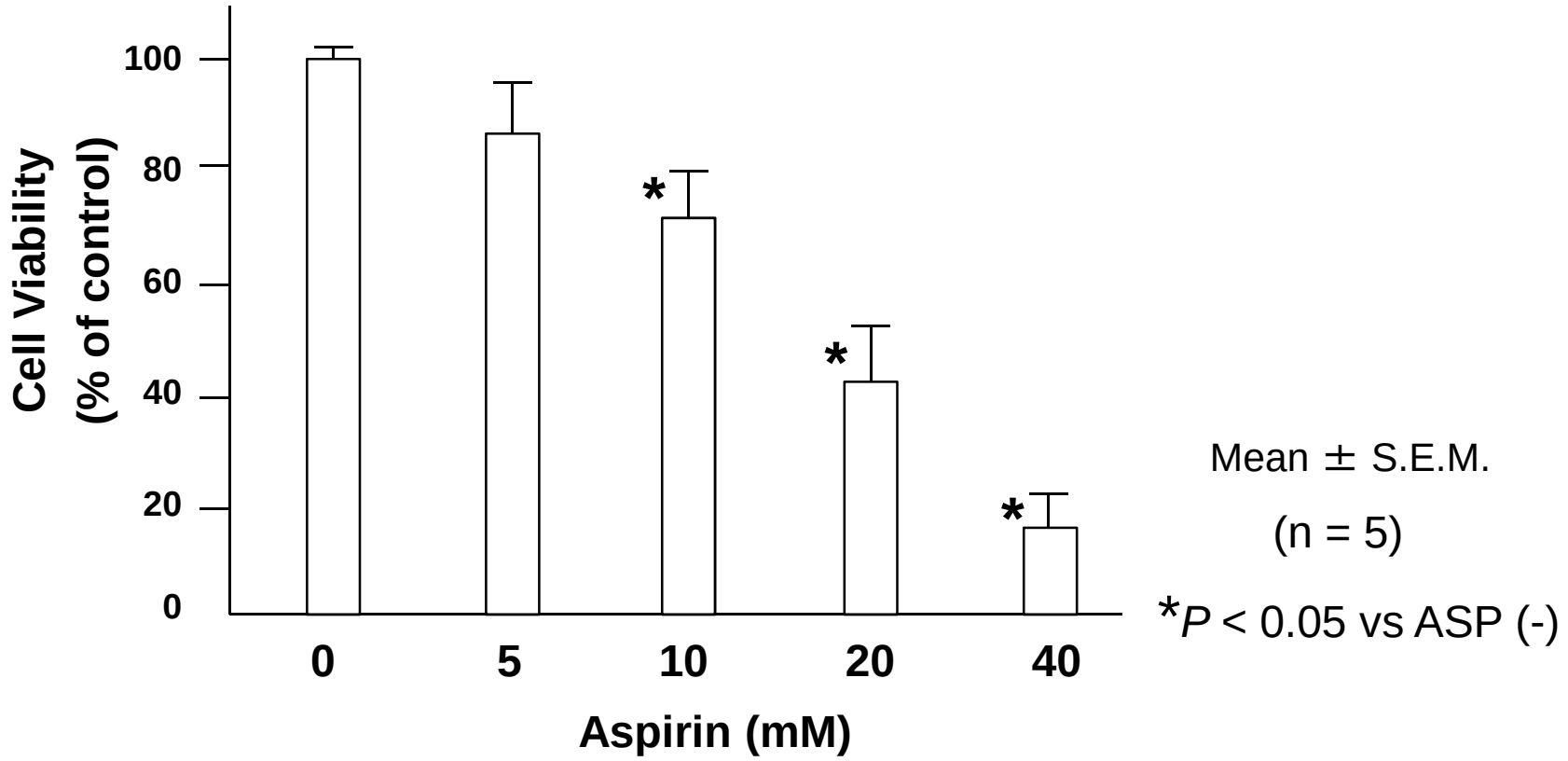
In Vitro Study

- Cell Line
  - IEC6 (rat small intestinal epithelial cell)
- Reagents
  - Aspirin
  - Sulforaphane (SFN)
  - Zinc Protoporphyrin IX (HO-1 inhibitor)
- Cell Viability : MTT Assay
- HO-1 Expression : Western Blotting

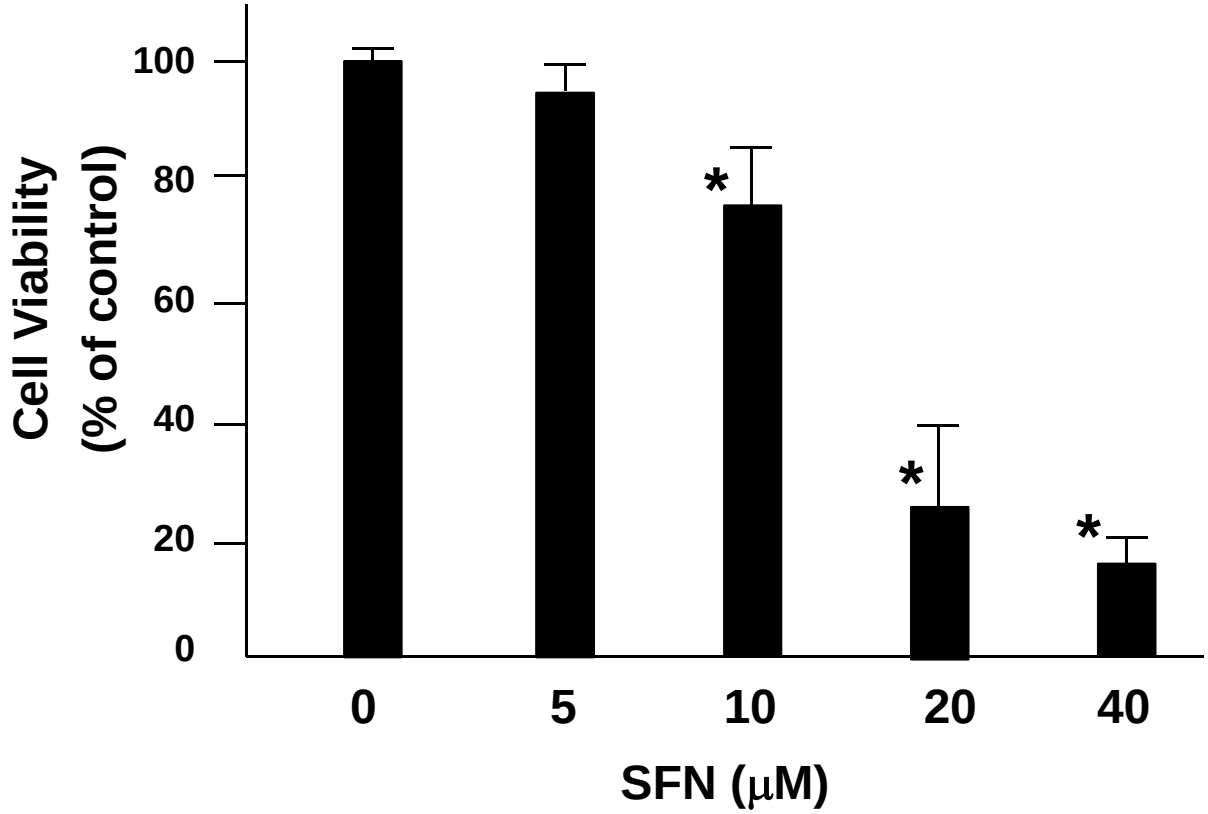
Experimental Protocol (1)



Aspirin Dose-Dependently Induces Injury in Small Intestinal Cells (IEC6) *in vitro*



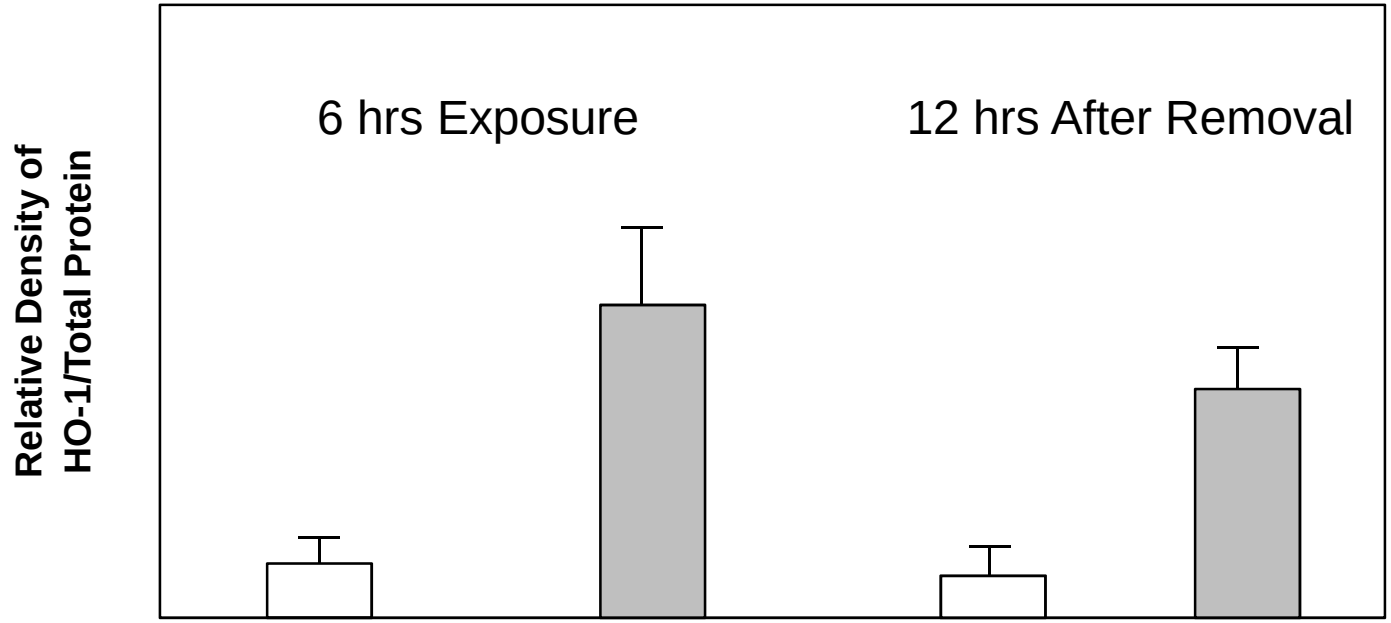
Effects of Sulforaphane on Viability of IEC6 Cells

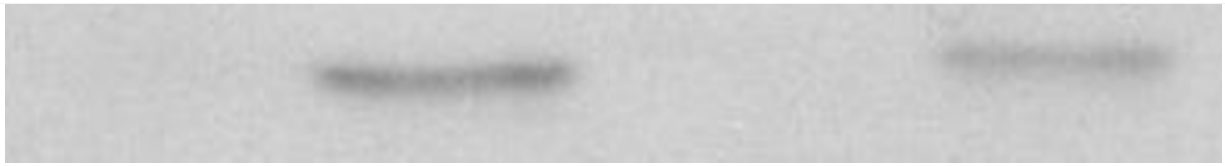


Mean ± S.E.M.

(n = 5)

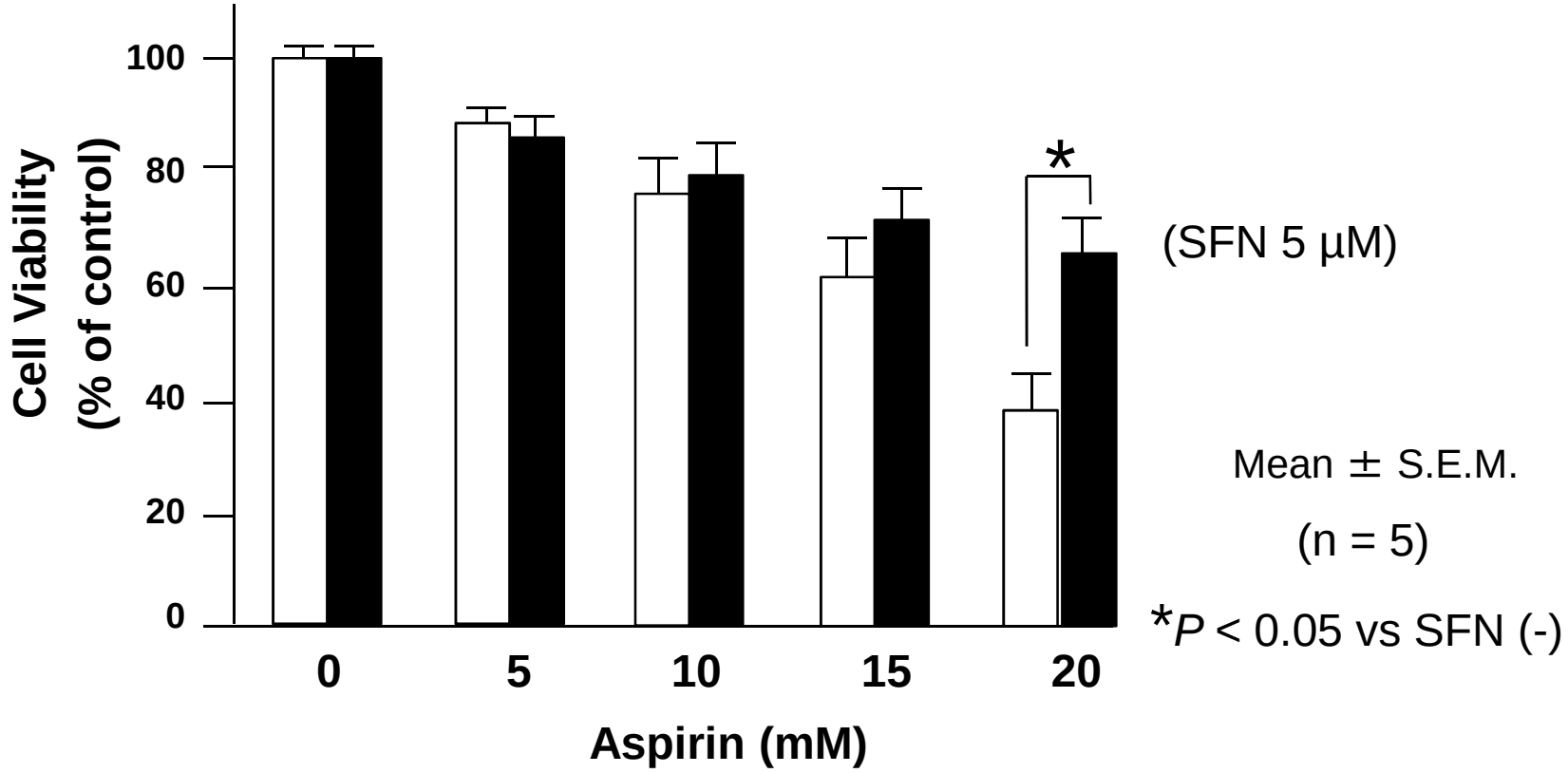
Low Dose of Sulforaphane Enhanced HO-1 Expression in IEC6 Cells



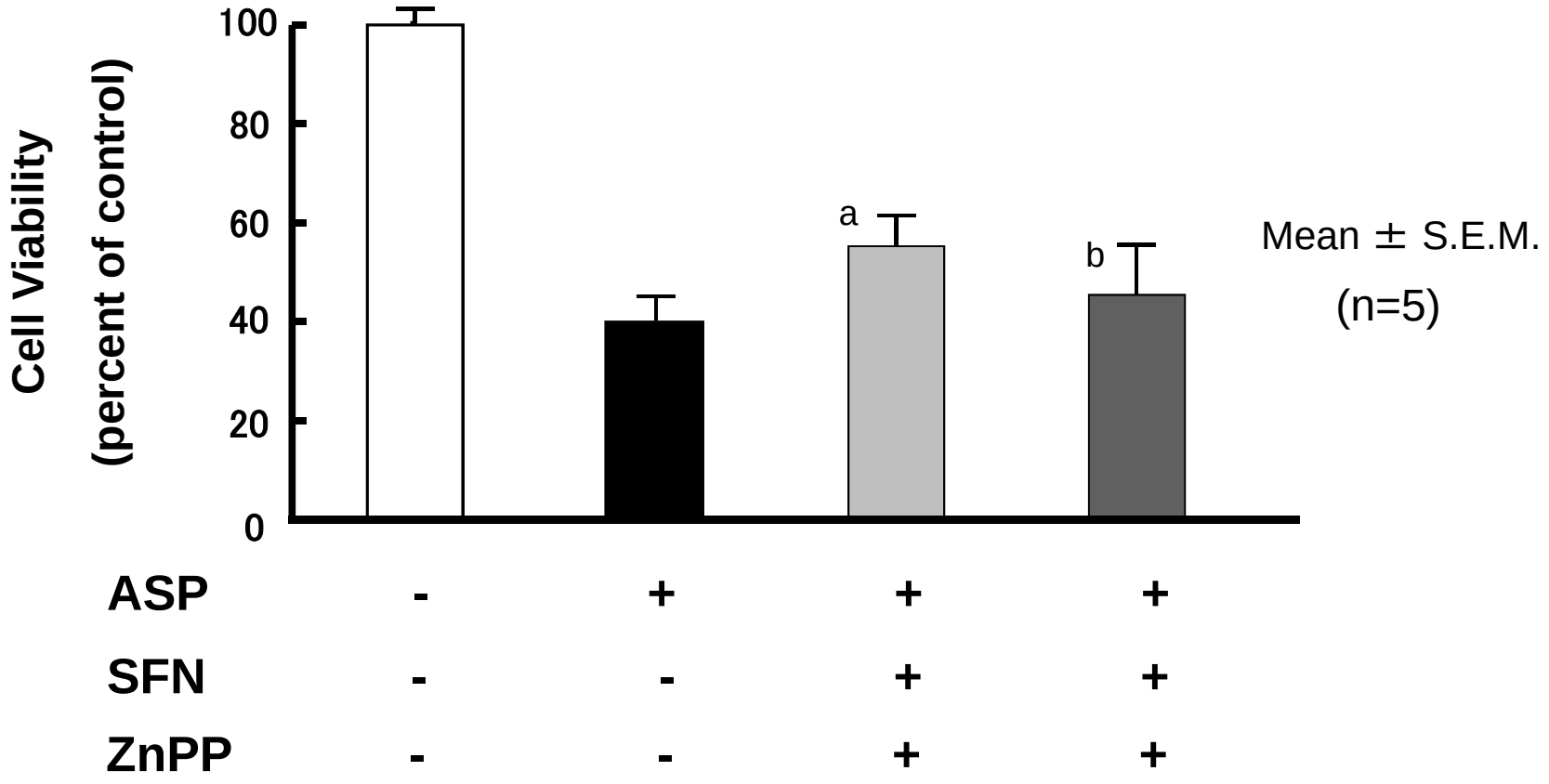


SFN (5μM) - + - +

Pretreatment with Sulforaphane Attenuated Aspirin-Induced Injury in IEC6 Cells



Protective Effects of SFN against Aspirin-Induced Injury was Attenuated by an HO-1 inhibitor



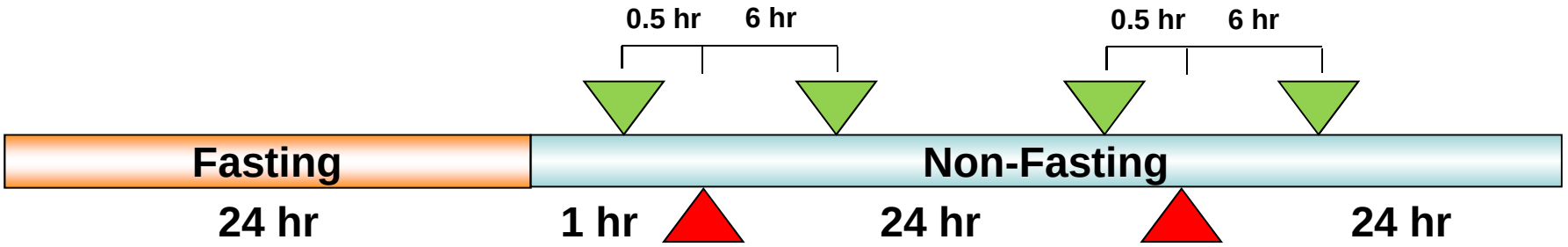
Summary (1)

SFN affords cytoprotection of small intestinal epithelial cells against aspirin *in vitro*, at least in part by enhancing HO-1.

In vivo Study

Effect of SFN on IND-induced Small Intestinal Injury

Mice: ddY♂ 7-weeks old

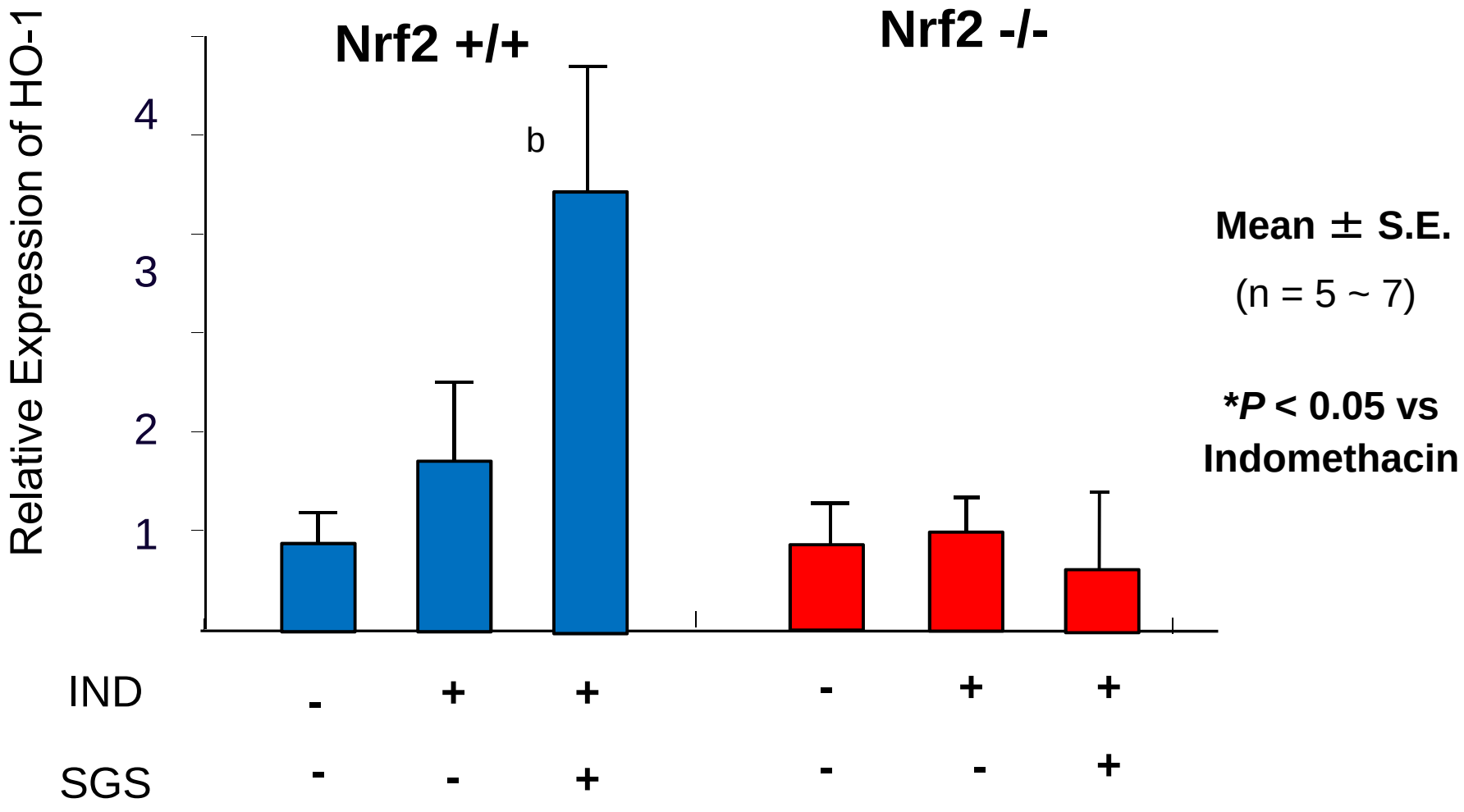


▲ : Indomethacin 20 mg/kg s.c.

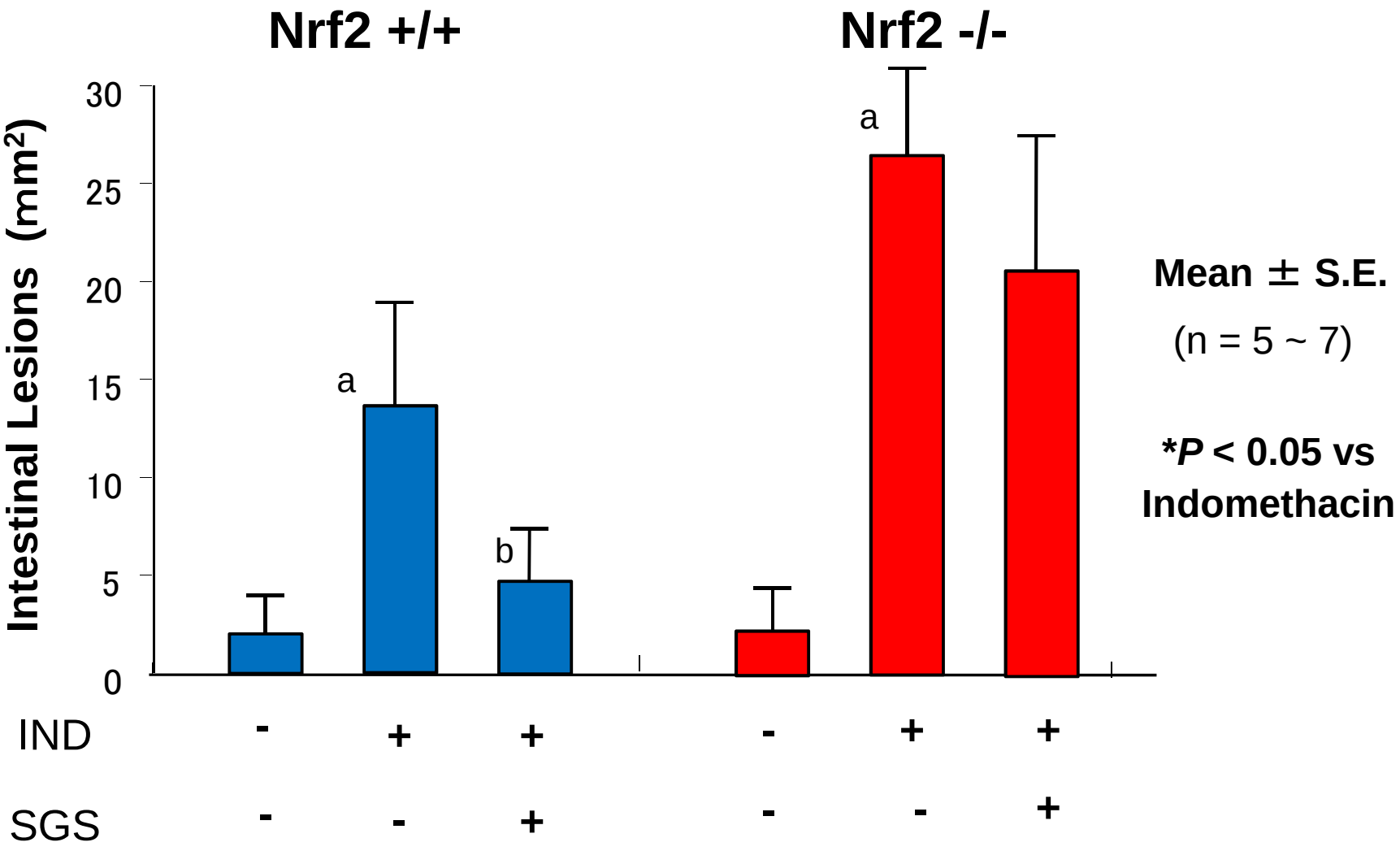
Miura N, et al. Biol Pharm Bull. 2007;30(3):495_501.

▼ : SGS (SFN GlucoSinolates) 8.5 mg/body, p.o.

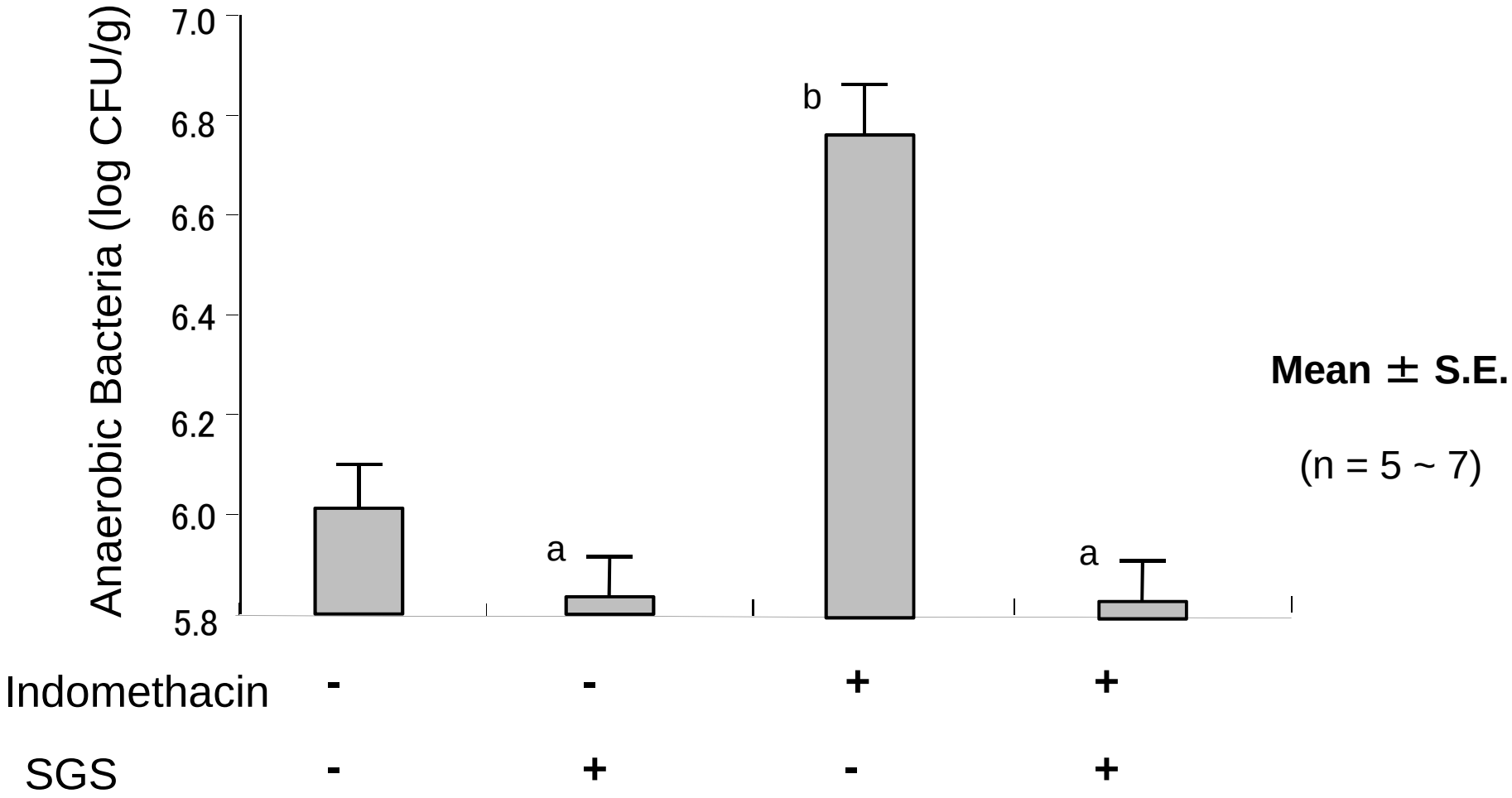
Sulforaphane Enhanced HO-1 Expression in nrf2+/+, but not in nrf2-/- Mice



Sulforaphane Mitigated IND-induced Injury in nrf2+/+, but not in nrf2-/- Mice



Sulforaphane Prevented IND-Induced Increase in Anaerobic Bacteria in Small Intestinal Mucosa



Conclusions(2)

1. Orally administered SGS protected small intestine from IND- induced injury in mice in vivo.

2. SGS affords protection of small intestine by

1) inducing nrf2-dependent antioxidant enzymes,
2) inhibiting mucosal invasion of anaerobic enterobacteria.

Effects of Various Compounds on NRF2-Mediated Protection of Gastrointestinal Tract against Oxidative Stresses

		Basic Study		Clinical Study	
		in vitro	in vivo	observational	intervention
Isothiocyanates	**Sulforaphane**	○	○		**Constipation H.pylori-Gastritis**
	Alyl-isothicyanate	○	○		
Polyphenols	Curcumin	○			IBS, UC
	Catechin	○	○	○	
	Quercetin	○	○	○	
	Resveratrol	○	○		UC
Carotenoids	Lycopene	○	○		
	Astaxanthin	○	○		GERD
Drugs	Lansoprazole	○	○		
	UDCA	○	○		Barret Esophagus
	Sofalcon	○	○		Gastric Ulcer
Hormones	Ghrelin		○		Diabetic Gastroparesis
	Melatonin	○	○		GERD, IBS, UC