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- and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996;**20**:1161-81.
31. Lucas LT, Gatehouse D, Shuker DE. Efficient nitroso group transfer from N-nitrosoindoles to nucleotides and 2'-deoxyguanosine at physiological pH. A new pathway for N-nitrosocompounds to exert genotoxicity. *J Biol Chem* 1999;**274**:18319-26.
32. Burney S, Caulfield JL, Niles JC, Wishnok JS, Tannenbaum SR. The chemistry of DNA damage from nitric oxide and peroxyxynitrite. *Mutat Res* 1999;**424**:37-49.
33. Lucas LT, Gatehouse D, Jones GD, Shuker DE. Characterization of DNA damage at purine residues in oligonucleotides and calf thymus DNA induced by the mutagen 1-nitrosoindole-3-acetonitrile. *Chem Res Toxicol* 2001;**14**:158-64
34. Vincent AL, Ash LR. Further observations on spontaneous neoplasms in the Mongolian gerbil, *Meriones unguiculatus*. *Lab Anim Sci* 1978;**28**:297-300.

35. Okamoto T, Isogai Y, Koizumi T, Fujishiro H, Sato Y. Studies on plant growth regulators, III. Isolation of indole-3-acetonitrile and methyl indole-3-acetate from the neutral fraction of the Moyashi extract. *Chem Pharm Bull* 1967;**15**:163-68.
36. Piacek-Llanes BG, Tannenbaum SR. Formation of an activated N-nitroso compound in nitrite-treated fava beans (*Vicia faba*). *Carcinogenesis* 1982;**3**:1379-84.
37. Yang D, Tannenbaum SR, Buchi G, Lee GC. 4-Chloro-6-methoxyindole is the precursor of a potent mutagen (4-chloro-6-methoxy-2-hydroxy-1-nitroso-indolin-3-one oxime) that forms during nitrosation of the fava bean (*Vicia faba*). *Carcinogenesis* 1984;**5**:1219-24.
38. Ochiai M, Wakabayashi K, Sugimura T, Nagao M. Mutagenicities of indole and 30 derivatives after nitrite treatment. *Mutat Res* 1986;**172**:189-97.
39. Wakabayashi K, Ochiai M, Saito H, Tsuda M, Suwa Y, Nagao M, Sugimura T. Presence of 1-methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxyl

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- ic acid, a precursor of a mutagenic nitroso compound, in soy sauce. *Proc Natl Acad Sci USA* 1983;**80**:2912-6.
40. Suzuki T, Mower HF, Friesen MD, Gilibert I, Sawa T, Ohshima H. Nitration and nitrosation of N-acetyl-L-tryptophan and tryptophan residues in proteins by various reactive nitrogen species. *Free Radic Biol Med* 2004;**37**:671-81.
41. Inoue M, Tsugane S. Epidemiology of gastric cancer in Japan. *Postgrad Med J* 2005;**81**:419-24.
42. Kobayashi T, Kikuchi S, Lin Y, Yagyu K, Obata Y, Ogiwara A, Hasegawa A, Miki K, Kaneko E, Mizukoshi H, Sakiyama T, Tenjin H. Trends in the incidence of gastric cancer in Japan and their associations with *Helicobacter pylori* infection and gastric mucosal atrophy. *Gastric Cancer* 2004;**7**:233-9.
43. Plummer M, Franceschi S, Munoz N. Epidemiology of gastric cancer. *IARC Sci Publ* 2004;**157**:311-26.

Table 1. *H. pylori* infection induced-gastritis in MGs.

Group	Treatment	Effective No.	Stomach wet weight (g)	Inflammation score
A	Broth	15	0.647 ± 0.097	0
B	NIAN + Broth	22	0.631 ± 0.094	0
C	<i>H. pylori</i>	18	1.432 ± 0.445*	2.22 ± 0.43*
D	NIAN + <i>H. pylori</i>	26	1.483 ± 0.445*	2.38 ± 0.64*

\*P&lt;0.01 vs. group A and B; Values for results are expressed as averages ± SD.

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Table 2. Incidence of glandular stomach adenocarcinoma in MGs.

Group	Treatment	Effective No.	No. of animals with glandular stomach adenocarcinoma (%)	
			Total	Moderately dif.
A	Broth	15	0 (0)	0 (0)
B	NIAN + Broth	22	0 (0)	0 (0)
C	<i>H. pylori</i>	18	0 (0)	0 (0)
D	NIAN + <i>H. pylori</i>	26	8 (31)*	1 (4)

Well dif., well differentiated adenocarcinoma; Moderately dif., moderately differentiated adenocarcinoma.

\*P&lt;0.05 vs. group A and C, and P&lt;0.01 vs. group B.

**Figure Legends**

**Figure 1.** Chemical structure of NAIN and experimental protocol for the carcinogenicity study. (A) Chemical structure of NIAN. (B) Male six-week-old MGs were orally administered NIAN (100 mg/kg) in 50% DMSO (groups B and D) or 50% DMSO alone (groups A and C) two times a week for three weeks. One week after the final administration, the animals were inoculated with *H. pylori* (ATCC 43504) (groups C and D) or sterilized broth (groups A and B).

**Figure 2.** Autoradiograms of NIAN-DNA adducts in glandular stomach of MGs or calf thymus DNA treated with NIAN. Adducts were analyzed by <sup>32</sup>P-postlabeling method, as described in the Materials and Methods. DNA samples were isolated from glandular stomach of MGs (A) or calf thymus DNA (B) after treatment with NIAN. DNA samples were also prepared from glandular stomach of MGs without NIAN treatment (C). Arrowheads indicate adducts.

**Figure 3.** Macro- and microscopic views of gastritis in MGs infected or uninfected with *H. pylori*. (A) Normal gastric mucosa

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in group A. (B) Severe infiltration of many inflammatory cells with development of heterophilic proliferative glands in group C; H&E staining x 40. Yellow boxes are shown at greater magnification below. x 200.

**Figure 4.** Histological findings of gastric adenocarcinoma in the animals treated with both NIAN and *H. pylori*. (A) Typical macrograph of a stomach. The yellow circle shows the suspected lesion of gastric cancer. (B) Well differentiated adenocarcinoma. (C) Moderately differentiated adenocarcinoma. (B,C) H&E staining x 400.

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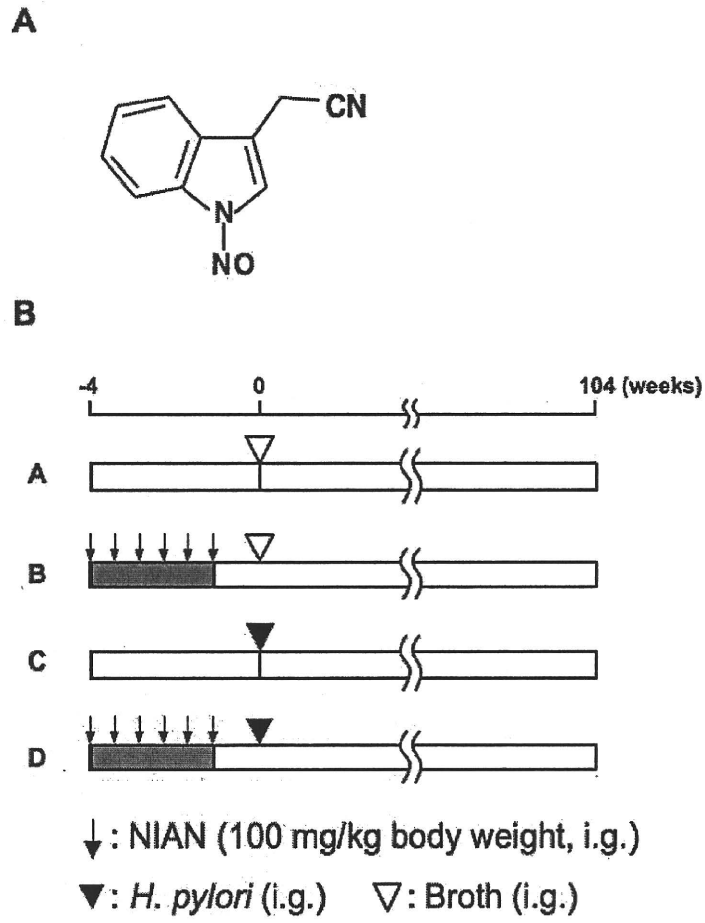
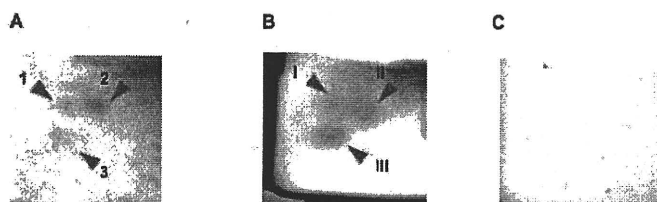


Figure 1



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Figure 2



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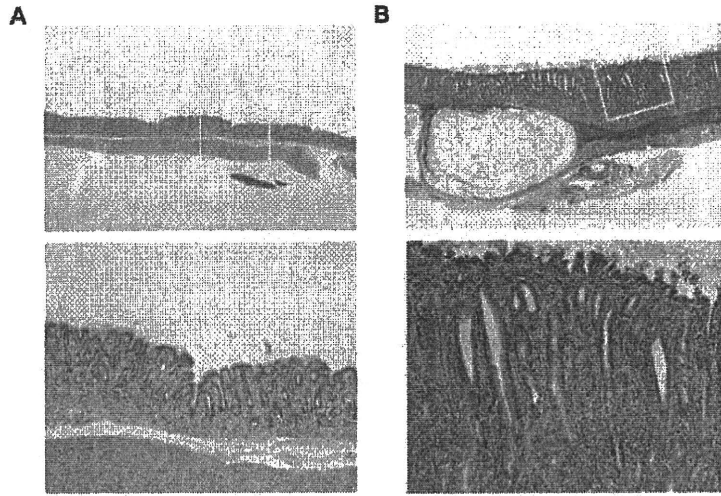


Figure 3

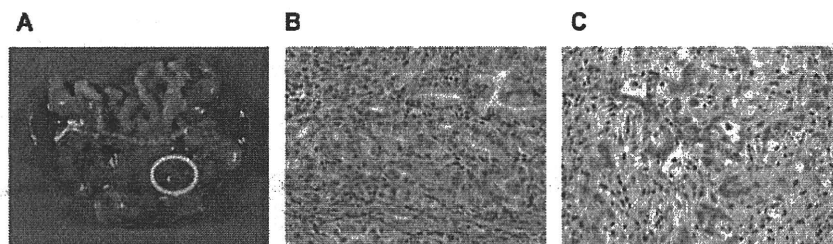


Figure 4

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