celecoxib plus indomethacin. Since many factors can affect the production of gastric lesions *in vivo* (mucosal blood flow and gastric motility for example), a number of interpretations for this phenomenon are possible. However, we consider that the direct cytotoxicity of NSAIDs, or direct cell damage at the gastric mucosa by NSAIDs in other words, can explain this phenomenon. In gastric lesions produced by a combination of the oral administration of celecoxib with the intraperitoneal administration of indomethacin or NCX 530, the direct cell damage at gastric mucosa should occur on account of the orally administered celecoxib. As shown *in vitro*, NCX 530 may suppress celecoxib-induced cell death at the gastric mucosa, meaning that NCX 530 does not actually produce gastric lesions when administered in conjunction with the celecoxib. This idea can also be used to explain the NCX 530-dependent suppression of the production of gastric lesions by ethanol or celecoxib plus indomethacin, given that, *in vitro*, NCX 530 protected the gastric mucosal cells not only from celecoxib but also from ethanol. Furthermore, observations that NCX 530 did not protect gastric mucosal cells from indomethacin *in vitro* may explain why the production of gastric lesions by the oral administration of high doses of indomethacin was not suppressed by NCX 530 *in vivo*. However, in Fig. 8, NCX 530 almost completely inhibited the production of gastric lesions by celecoxib *in vivo*, whereas the effect of this drug on celecoxib-induced cell death is partial *in vitro* (Fig. 2A and 3A). Previous papers reported that NCX 530 stimulated mucosal blood flow and mucus synthesis and did not so clearly increase gastric motility and adhesion of neutrophil as indomethacin (17, 39). We consider that these phenomenon are involved in the safety of NCX 530 on gastric mucosa *in vivo*.

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FIGURE LEGENDS

Fig. 1. Necrosis and apoptosis induced by NCX 530 or indomethacin.

Cultured guinea pig gastric mucosal cells were incubated with indicated concentrations of NCX 530 or indomethacin for 1 h (A) (necrotic conditions) or 16 h (B, C) (apoptotic conditions). Cell viability was determined by the MTT method (A, B). Values are mean ± S.E.M. (n=3). ***P<0.001; **P<0.01. Chromosomal DNA was extracted and analyzed by 2% agarose gel electrophoresis (C).

Fig. 2. Effect of NCX 530 on apoptosis induced by various gastric irritants.

Cultured guinea pig gastric mucosal cells were pre-incubated with indicated concentrations of NCX 530 for 1 h and further incubated with 0.1 mM celecoxib (A), 3% ethanol (B), 0.6 mM indomethacin (C) or 0.4 mM hydrogen peroxide (D) in the presence of indicated concentrations of NCX 530 for 16 h (apoptotic conditions). Cell viability was determined by the MTT method. Values are mean ± S.E.M. (n=3). **P<0.001; **P<0.01; *P<0.05.

Fig. 3. Effect of NCX 530 on necrosis induced by various gastric irritants.

Cultured guinea pig gastric mucosal cells were pre-incubated with indicated concentrations of NCX 530 for 1 h and further incubated with 0.18 mM celecoxib (A), 8% ethanol (B), 3 mM indomethacin (C) or 1 mM hydrogen peroxide (D) in the presence of

indicated concentrations of NCX 530 for 1 h (necrotic conditions). Cell viability was determined by the MTT method. Values are mean ± S.E.M. (n=3). ***P<0.001; **P<0.01; *P<0.05.

Fig. 4. Membrane permeabilization by NSAIDs.

Calcein-loaded liposomes were incubated for 10 min at 30°C with indicated concentrations of each NSAID (A) or 0.1 mM celecoxib plus indicated concentrations of NCX530 (B). The release of calcein from liposomes was determined by measuring fluorescence intensity. Melittin (10 μM) was used to determine the 100% level of membrane permeabilization (47).

Fig. 5. Effect of cycloheximide on cell viability in the presence of NCX 530.

Cultured guinea pig gastric mucosal cells were pre-incubated with indicated concentrations of cycloheximide for 1 h. Cells were further incubated with 2 mM NCX 530 and indicated concentrations of cycloheximide for 1 h (A) (necrotic conditions). Cells were pre-incubated with indicated concentrations of cycloheximide and 1 mM NCX 530 for 1 h. Cells were further incubated with 1 mM NCX 530, 0.18 mM celecoxib and indicated concentrations of cycloheximide for 1 h (B) (necrotic conditions). Cell viability was determined by the MTT method. Values are mean ± S.E.M. (n=3).

Fig. 6. Effect of ODQ on cell viability in the presence of NCX 530.

Cultured guinea pig gastric mucosal cells were pre-incubated with indicated concentrations of ODQ for 1 h. Cells were further incubated with indicated concentrations of NCX 530 and ODQ (A, C). Cells were pre-incubated with indicated concentrations of ODQ and 1 mM NCX 530 for 1 h. Cells were further incubated indicated concentrations of ODQ, NCX530 and celecoxib (B, D). Incubation was performed for 1 h (A, B) (necrotic conditions) or for 16 h (C, D) (apoptotic conditions). Cell viability was determined by the MTT method. Values are mean ± S.E.M. (n=3). ***P<0.001; *P<0.05.

Fig. 7. Production of gastric lesions by NCX 530 or indomethacin.

Rats were orally administered with NCX 530 or indomethacin as indicated. After 6 h, the stomach was removed and scored for hemorrhagic damage. Values are mean ± S.E.M. (n=5 - 6). **P<0.01. n. d.; not detected

Fig. 8. Production of gastric lesions by NCX 530 or indomethacin in combination with celecoxib.

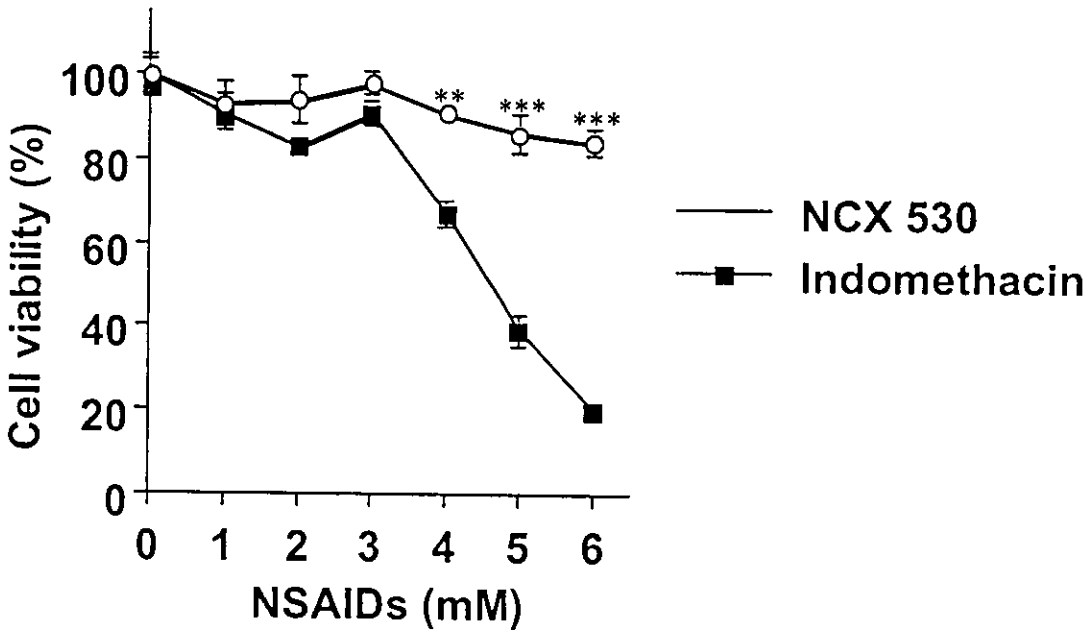
Rats were intraperitoneally administered with 5 mg/kg indomethacin or 7.1 mg/kg NCX 530 or vehicle. After 1 h, animals were administered orally with 15 mg/ml celecoxib or vehicle. After 6 h, the stomach was removed and scored for hemorrhagic damage. Values are mean ± S.E.M. (n=5 - 6). **P<0.01; *P<0.05. n. d.; not detected

Fig. 9. Effect of NCX 530 on production of gastric lesions by other gastric irritants.

Rats were intraperitoneally administered with 7.1 mg/kg NCX 530 or 5 mg/kg indomethacin or vehicle. After 1 h, animals were administered orally with ethanol (A) or 30 mg/kg indomethacin (B) or vehicle. After 6 h, the stomach was removed and scored for hemorrhagic damage. Values are mean ± S.E.M. (n=5 - 6). $*P<0.05$. n. d.; not detected

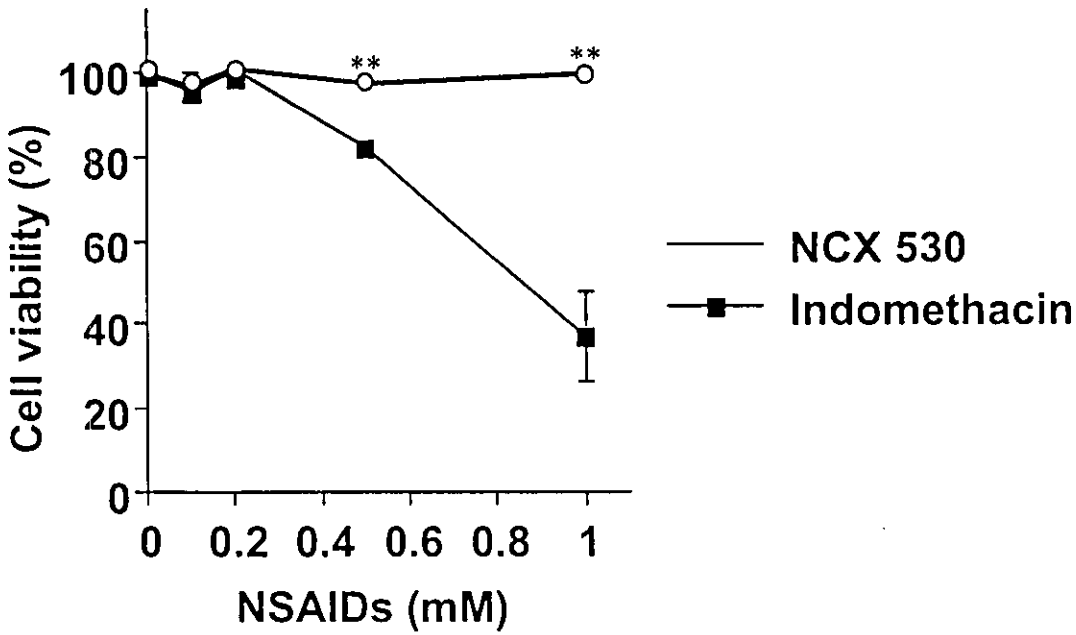
Tomisato et al. Fig. 1

A



Tomisato et al. Fig. 1

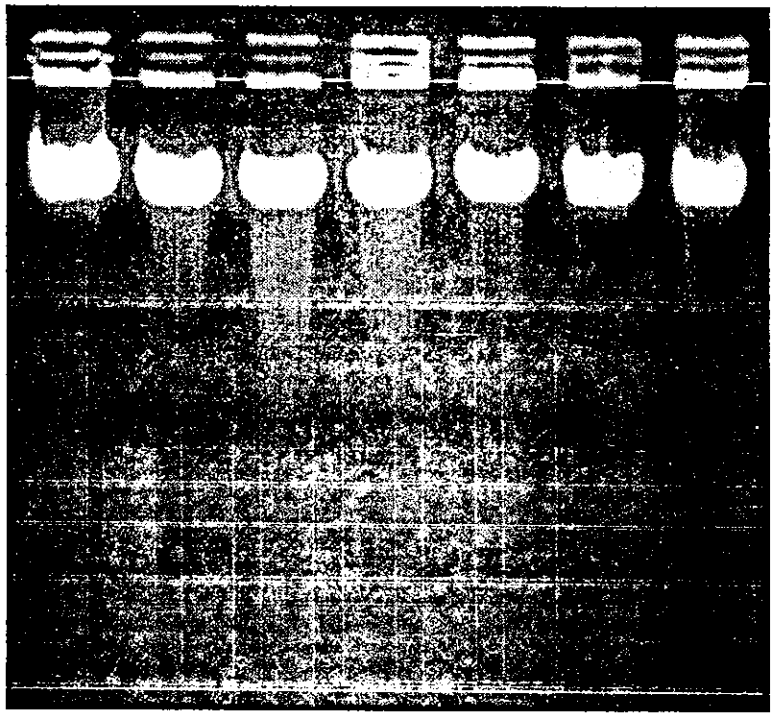
B



Tomisato et al. Fig. 1

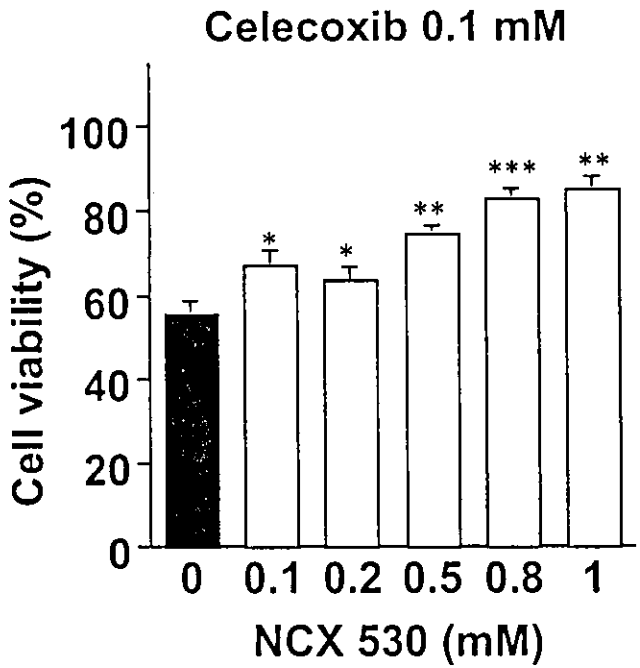
C

Indomethacin (mM)	-	0.5	0.8	1.0	-	-	-
NCX 530 (mM)	-	-	-	-	0.5	0.8	1.0



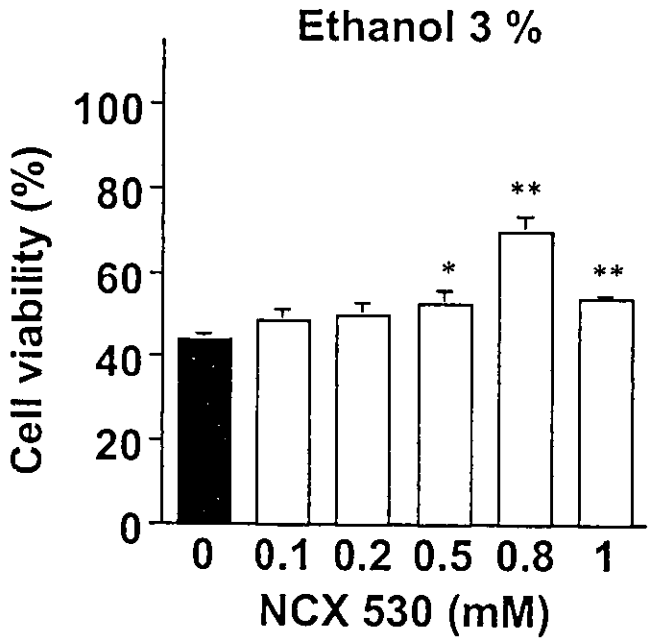
Tomisato et al. Fig. 2

A



Tomisato et al. Fig. 2

B



Tomisato et al. Fig. 2

C