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Characterization of the influence of nitric oxide donors on intestinal absorption of macromolecules

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Abstract

To characterize the influence of nitric oxide (NO) donors on the intestinal absorption of macromolecules, the relationship between the release rate of NO from NO donors and their absorption-enhancing effects and the effects of several scavengers and generators on the absorption-enhancing effects of NO donor were investigated. The $t_{1/2}$ values of the NO release rate from 3-(2-hydroxy-1-methylethyl-2-nitrosohydrazino)-1-propanamine (NOC5), 3-(2-hydroxy-1-methylethyl-2-nitrosohydrazino)-*N*-methyl-1-propanamine (NOC7) and *N*-ethyl-2-(1-ethyl-hydroxy-2-nitrosohydrazino)-ethanamine (NOC12) are 25, 5 and 100 min, respectively. The absorption-enhancing effects of NO donors on the absorption of fluorescein isothiocyanate dextrans with an average molecular weight of 4400 (FD-4) are NOC5 > NOC7 > NOC12 in the colon. The lowest enhancing effect of NOC12 may be due to the slow rate of NO release. The enhancing effect of NOC7 rapidly disappeared compared with the effect of NOC5. The results raise the possibility that the difference between NOC5 and NOC7 on enhancing effect is related to the $t_{1/2}$ of the NO release. The NOC7-induced enhancing effect was prevented by the co-administration of 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazole-1-oxyl-3-oxide sodium salt (C-PTIO), an NO scavenger; tiron, an O_2^- scavenger; mannitol, an $OH^\bullet$ scavenger, and deferoxamine, peroxynitrate scavenger. Pyrogallol, an O_2^- generator, potentiated the NOC7-induced enhancing effect. These results support a role for peroxynitrate, and possibly $OH^\bullet$, in the NO donor-induced intestinal enhancing effect.
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Keywords: NO donor; Absorption; Enhance; FD-4; Peroxynitrate

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1. Introduction

The oral bioavailability of highly polar drugs and macromolecules, including peptide and protein drugs, is very poor because they have low permeability across the intestinal mucosa and are extensively degraded proteolytically in the gastrointestinal tract (Lee and Yamamoto, 1990; Lee et al., 1991). To overcome these delivery problems, various approaches, such as the use of absorption enhancers (Muranishi, 1990), protease inhibitors (Saffran et al., 1988), and chemical modification (Yodoya et al., 1994; Asada et al., 1995; Tanaka et al., 1996) have been examined. A large number of absorption enhancers, including surfactants, bile salts, chelating agents and fatty acids, have been used to enhance the intestinal absorption of drugs (Lee and Yamamoto, 1990; Lee et al., 1991). Nishihata et al. (1981, 1983) demonstrated that sodium salicylate and 5-methoxysalicylate increased the rectal absorption of insulin. Murakami et al. (1984) reported enhanced rectal absorption of sodium ampicillin in the presence of various bile salts. Yamamoto et al. (1994) suggested the use of protease inhibitors to improve the intestinal absorption of drugs and macromolecules. It is known that these enhancers affect membrane fluidity, calcium ion mobility, non-protein and protein sulfydryl levels in the mucosal membrane, and the viscosity of the mucus layer (Lee and Yamamoto, 1990; Lee et al., 1991). However, some of these absorption enhancers cause damage and irritate the intestinal mucosal membrane (Swenson and Curatolo, 1992). Therefore, an important theme is the development of effective and safe absorption enhancers.

Salzman et al. (1995) reported that nitric oxide (NO) can regulate the permeability of Caco-2 monolayers. They suggested that NO donors induce an increase in the permeability of Caco-2 monolayers and that this effect is reversible. Utoguchi et al. (1998) demonstrated that the administration of a suppository containing an NO donor and insulin induced increases in the plasma insulin level. Our previous studies with an in situ method indicated that NO donors could enhance the absorption of fluorescein isothiocyanate dextrans not only from the colon but also from the jejunum (Numata et al., 2000). We also demonstrated the low cytotoxicity of NO donors and the reversibility of the enhancing effect. Our results were supported by recent studies using an Ussing chamber method (Yamamoto et al., 2001). However, few studies have been carried out on the relationship between the release of NO from NO donors and the enhancing effect in the intact intestine. Thus, the primary objective of this study was to elucidate the relationship between the release rate of NO from NO donors and the NO donor-induced enhancing effect. 3-(2-Hydroxy-1-methylethyl-2-nitrosohydrazino)-1-propanamine (NOC5), 3-(2-hydroxy-1-methylethyl-2-nitrosohydrazino)-*N*-methyl-1-propanamine (NOC7) and *N*-ethyl-2-(1-ethyl-hydroxy-2-nitrosohydrazino)-ethanamine (NOC12) were used as NO donors and FD-4 was chosen as a model of water-soluble compounds.

NO has the potential to directly influence the intestinal epithelial function (Borutaite and Brown, 1996). However, many effects attributed to NO are actually mediated by another reactive nitrogen metabolite, peroxynitrite. Peroxynitrite, a potent biological oxidant, is formed at near diffusion-limited rates when two radicals, NO and superoxide, react together (Pryor and Squadrito, 1995). Menconi et al. (1998) reported the role of peroxynitrite on the NO donor-induced hyperpermeability of water-soluble compounds across Caco-2 cell monolayers. As these studies were performed after long incubation (24 h) with NO donors, limited information is available on its immediate effects on the intestinal epithelium. This is surprising because the immediate effect of an absorption enhancer is likely to be more relevant than the long-term effect in vivo. In addition, few studies have examined the role of peroxynitrite in the enhancing effect in vivo. Thus, the second aim of this study was to assess the role of peroxynitrite in the NO donor-induced enhancing effect. This was accomplished by using an NO scavenger, a superoxide scavenger, a hydroxyl radical scavenger, a peroxynitrate scavenger and a superoxide generator. We also estimated the effect of NO donors on intestinal membrane toxicity by measuring ATP and the total purine contents in the intestine.

2. Materials and methods

2.1. Materials

NOC5, NOC7, NOC12, and 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazole-1-oxyl-3-oxide sodium salt (C-PTIO) were purchased from Dojindo Lab. (Kumamoto, Japan). FD-4, tiron, pyrogallol,

deferoxiamine and α,β-methylene adenosine 5′-diphosphate (α,β-methylene ADP) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Mannitol was obtained from Wako Pure Chemicals Ind. (Osaka, Japan). Tetrabuthylammonium bromide (TBAB) was from Nacalai Tesque Inc. (Kyoto, Japan). All other reagents used were of analytical grade.

2.2. Pharmacokinetic study

Male Wistar rats (SLC, Hamamatsu, Japan), weighing 220–270 g, were used in accordance with the Guidelines for Animal Experimentation of Mukogawa Women's University, which are based on the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science. The rats were fasted for 18–20 h before the experiments, with water freely available. After the animals had been anesthetized with sodium pentobarbital (30 mg/kg, i.p.), FD-4 solution (10 mg/mL, 0.2 mL) was injected in the left jugular vein. Blood (0.25 mL) was periodically taken from the right jugular vein. Absorption experiments were performed by an in situ closed loop method. After the animals were anesthetized with sodium pentobarbital, the intestine was exposed by a midline abdominal incision. The intestine was flushed with phosphate buffer saline (pH 7.4, 40 mL) through glass tubing attached to a plastic syringe, then the buffer remaining in the lumen was expelled with air. A single colonic loop (5 cm) was prepared by cannulating the animal with glass tubing. After the administration of compound solution (0.2 mL) into the loop, 0.25 mL of blood was periodically taken from the jugular vein. The plasma was separated by centrifugation, and the plasma concentration of FD-4 was determined using a fluorescence spectrophotometer (Jasco FP-315, Tokyo, Japan) at an excitation wavelength of 495 nm and emission wavelength of 515 nm after the addition of phosphate buffered saline (pH 7.4, 3 mL) to the plasma sample (100 μL). The detection limit for FD was 0.05 μg/mL. The calibration curves for FD-4 were linear up to 5 μg/mL. The correlation coefficient was in excess of 0.999.

2.3. Preparation of compound solution

FD-4 was dissolved in phosphate buffered saline (pH 7.4) to a final concentration of 10 mg/mL. In certain experiments, an NO donor (NOC5, NOC7 or NOC12; 100 mM) was added to the phosphate buffered saline containing FD-4. To investigate the effect of scavengers or generators on the absorption-enhancing effect of NOC7, 50 mM NOC7 was added to the above solution containing FD-4.

2.4. Degradation of NO donors

NOC solution (0.1 mL) dissolved in 0.1 M NaOH (20 mM) was added to 19.9 mL PBS (pH 7.4) at 37 °C. Aliquots (3 mL) of the PBS solution were withdrawn periodically for 6 h. The absorbance of the sample was immediately measured at 250 nm.

2.5. Assay of ATP and related adenine compounds in the colon

The amounts of ATP and related adenine compounds (ADP, AMP, adenine and adenosine) in the colon were measured. After 1 h treatment with NOC7 (100 mM) or tartric acid (500 mM), the colon was removed and washed with ice-cold saline and wiped with cotton. After weighing, the colon was homogenized with 6% perchloric acid solution (10% homogenate). The homogenate was centrifuged for 10 min at 13,000 rpm (4 °C), and the supernatant (1.6 mL) was neutralized with 3 M K_2CO_3 solution (0.32 mL). This solution was used as a sample to determine ATP and related compounds.

The derivatization of purines was performed by a method described previously with slight modification (Kawamoto et al., 1998). A 1.0 mL sample solution was added to a mixture of 40 μL of chloroacetaldehyde reagent, 75 μL α,β-methylene ADP (internal standard), and 385 μL of citrate phosphate buffer (pH 4.0). The mixed solutions were then incubated in a DTU-2C dry thermo unit (Taitec Co., Saitama, Japan) at 80 °C for 40 min. The reaction was terminated by placing the samples on ice. A 200 μL aliquot of the sample solution was injected into the HPLC system.

The HPLC system consisted of a pump (880-PU, Jasco, Tokyo, Japan), and a fluorescence detector (RF-535, Shimadzu, Kyoto, Japan), a 4.6 mM × 150 mM column packed with Cosmosil $5C_{18}$-MS (Nacalai Tesque Inc., Kyoto, Japan) equipped with a guard column (4 mM × 5 mM), which was packed

with TSKgel ODS-80TS (Tosoh, Tokyo, Japan). The wavelengths for excitation and emission were set at 270 and 410 nm, respectively. The flow rate was 1.0 mL/min and the separation was performed at ambient temperature. The eluents, 100 mM KH_2PO_4, 5 mM TBAB, and 2.0% CH_3CN adjusted to pH 3.3 with H_3PO_4 were prepared on the day of use and filtered through a 0.40 μm filter.

2.6. Data analysis

All results were expressed as the mean values ± S.D. The peak concentration (C_{max}) and time to reach C_{max} (T_{max}) were determined directly from the concentration–time profiles. The area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal method from time zero to the final sampling time.

F was calculated as follows:

$$F = \frac{\text{AUCcolon}_{0\text{–}6\,\text{h}}}{\text{AUCi.v.}_{0\text{–}6\,\text{h}}}$$

Statistical analyses were assessed using Student's unpaired t-test or analysis of variance (ANOVA:Bonferroni's method was used to compare individual data when significant F value was shown) depending on the design of experiments. P values of 0.05 or less were considered to indicate statistically significant differences.

3. Results

3.1. Degradation of NOCs and their effect on intestinal absorption of FD-4

Fig. 1 shows the time-course of the degradation of NOC5, NOC7 or NOC12. NOCs are additives of amine compounds and NO, and thus their degradation is very simple (Scheme 1). NOCs show absorbance at 250 nm, but the amine compounds after the liberation of NO from NOCs do not. Therefore, we measured the decrease of the absorbance at 250 nm in the sample. The degradation rates were different among the NOCs and the $t_{1/2}$ values of the degradation of NOC5, NOC7 and NOC12 were 25, 5 and 100 min, respectively.

Fig. 2 and Table 1 show the plasma concentration time profiles and the pharmacokinetic parameters, respectively, following the colonic administration of FD-4 with or without NOCs. After the co-administration of FD-4 and NOCs, remarkable absorption enhancement of FD-4 was observed. The values of the enhancing ratio ($F_{NOCs}/F_{control}$) were 34.9 in NOC5, 11.4 in NOC7 and 3.9 in NOC12.

3.2. Effect of NO, superoxide, hydroxyl radical and peroxynitrate scavengers on FD-4 absorption

In order to elucidate the role of NO and/or peroxynitrite as a possible mediator of increases in the intestinal absorption of FD-4, we investigated its absorption

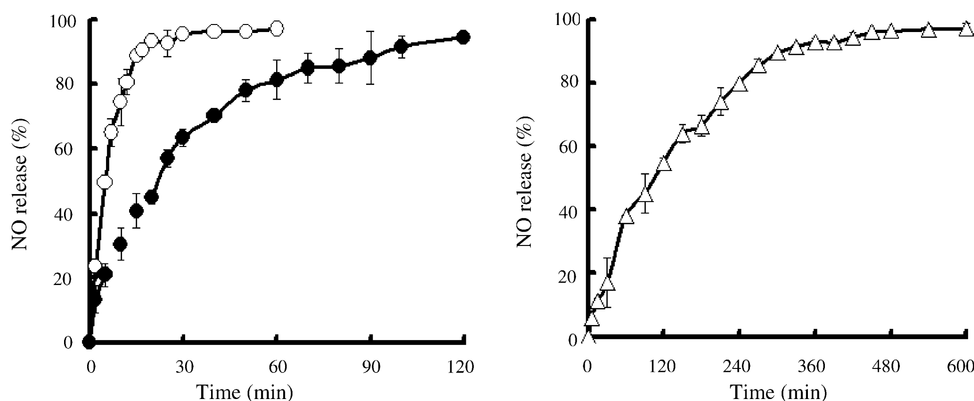
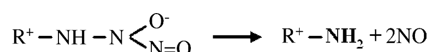


Fig. 1. Time-course of NO release from NOCs. NOC5 (●), NOC7 (○), and NOC12 (△). Each value represents the mean ± S.D. of four experiments.

Table 1
Effect of NO donor and radical scavengers or generator on pharmaockinetic parameters of FD-4

	$AUC_{0-6\,h}$ (μg/mL h)	C_{max} (μg/mL)	T_{max} (h)	F (%)[a]
i.v.	29.29 ± 4.20	–	–	100
Control	0.47 ± 0.06	0.10 ± 0.06	0.50 ± 0.25	1.6 ± 0.2
+NOC5 (100 mM)	16.37 ± 0.65*	5.08 ± 0.35*	1.5	55.9 ± 2.2*
+NOC7 (100 mM)	5.37 ± 0.65*	3.93 ± 0.15*	0.75	18.3 ± 2.2*
+NOC12 (100 mM)	1.81 ± 0.30*	0.77 ± 0.15*	0.5	6.2 ± 1.0*
NOC7 (50 mM)	2.81 ± 0.20	1.9 ± 0.12	0.75	9.6 ± 0.8
+C-PTIO (20 mM)	1.63 ± 0.21**	0.78 ± 0.10**	0.75	5.6 ± 0.7**
+Tiron (200 mM)	2.30 ± 0.22**	1.15 ± 0.06**	0.63 ± 0.25	7.9 ± 0.8**
+Man (500 mM)	1.74 ± 0.09**	1.00 ± 0.10**	0.67 ± 0.15	5.9 ± 0.3**
+DEF (10 mM)	2.56 ± 0.20	1.30 ± 0.20**	0.75	8.7 ± 0.7
+DEF (50 mM)	1.67 ± 0.30**	0.80 ± 0.08**	0.81 ± 0.13	5.7 ± 0.9**
+PG (5 mM)	5.09 ± 0.43**	3.54 ± 0.45**	0.67 ± 0.015	17.4 ± 1.5**
+PG (10 mM)	6.89 ± 0.61**	4.48 ± 0.21**	0.75	23.5 ± 2.1**

FD-4 (2 mg/rat) was administered in a jugular vein or colon loop.
[a] $F = [AUCcolon_{0-6\,h}/AUCi.v._{0-6\,h}] \times 100$.
* $P < 0.05$ compared with control.
** $P < 0.05$ compared with NOC7.



Scheme 1.

from the colon with the co-administration of NOC7 in the presence or absence of several scavengers. C-PTIO was firstly selected as a NO scavenger and evaluated. As shown in Fig. 3(A), the NOC7-induced enhancing effect was inhibited by co-administration with C-PTIO. The pharmacokinetic parameters are listed in Table 1. Next, tiron (4,5-hydroxy-1,3-benzene-disulfonic acid), a superoxide scavenger, was examined. Tiron attenuated the increases in the absorption which were observed with the co-administration of NOC7 in the absence of the superoxide scavenger (Fig. 3(B)). The effect of mannitol, a known scavenger of hydroxyl radical, was also investigated. As shown in Fig. 3(C), the NOC7-induced enhancing effect was inhibited by co-administration with mannitol. Deferoxamine, an iron chelator with properties similar to the peroxinitrate scavenger, decreased the enhancing effect of FD-4 absorption induced by NOC7 (Fig. 4(A)).

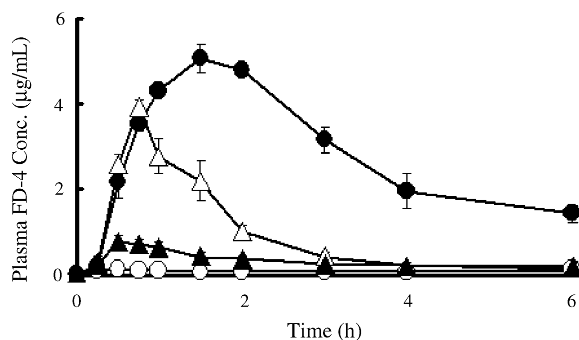


Fig. 2. Plasma concentration of FD-4 after administration to the colon with or without NOCs. Control (○), NOC5 (●), NOC7 (△), and NOC12 (▲). FD-4 (10 mg/mL) administered with or without NO donor (100 mM). Each value represents the mean ± S.D. of three to five rats.

To determine the effect of increasing extracellular levels of superoxide on the NO donor-induced enhancing effect, pyrogallol, a compound that undergoes spontaneous autoxidation under physiological conditions, was used. Pyrogallol (10 mM) did not affect the absorption of FD-4 from the colon (Fig. 4(B)). However, with the co-administration of NOC7, pyrogallol significantly potentiated the absorption promoting effect induced by NOC7 (Fig. 4(B)). Its effect increased with an increase of the pyrogallol dose.

3.3. Contents of ATP and its related purines in the colon

After 1 h pretreatment with NOC7 or tartaric acid, the concentrations of ATP and total purines in the colon were measured. Under control conditions, the ATP concentration was 8.1 nmol/mg protein. The intracellular levels of ATP after pretreatment with NOC7 did not

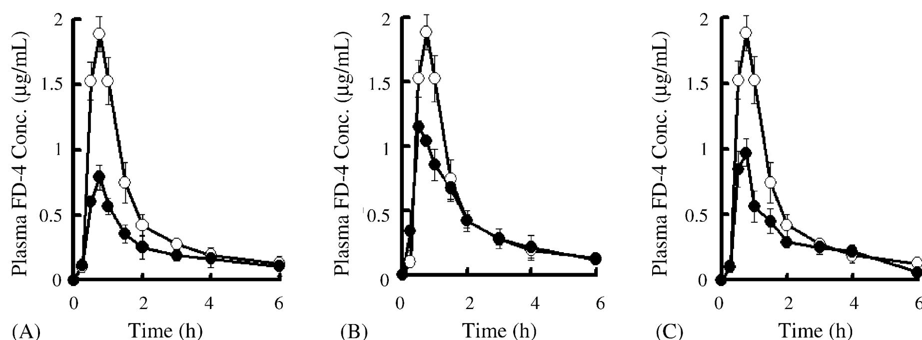


Fig. 3. Effects of the NO scavenger, C-PTIO (A), the superoxide scavenger, tiron (B), and the hydroxyl radical scavenger, mannitol (C), on plasma FD-4 concentration after colonic administration with NOC7. FD-4 (10 mg/mL) and NOC7 (50 mM) administered with (●) or without (◯) these scavengers (C-PTIO; 20 mM, tiron; 200 mM, mannitol; 500 mM). Each value represents the mean ± S.D. of three to five rats.

change compared with the control (Fig. 5). However, the values with tartaric acid pretreatment significantly decreased to 40% of the control values. For the total purine concentration (Fig. 5), the values after NOC7 pretreatment also were not different from the control, but the values significantly decreased after tartaric acid pretreatment.

4. Discussion

Recently, we demonstrated that NO donors (NOC7 and SNAP) enhanced the absorption of FITC-dextrans not only from the colon but also from the jejunum (Numata et al., 2000). We also demonstrated the low cytotoxicity of these NO donors. The same enhancing effects of NO donors were observed with NOC12 (Yamamoto et al., 2001) and sodium nitroprusside (Utoguchi et al., 1998). To further elucidate the effect of NO donors on the absorption of water-soluble macromolecules, we examined the relationship between the release rate of NO from NO donors (NOC5, 7 and 12) and the NO donor-induced enhancing effect.

NOC is one of the NONOates (1-substituted diazen-1-ium-1,2-diolates), which can undergo spontaneous degradation when placed in buffer solution, thus releasing NO (Hrabie et al., 1993; Keefer et al., 1996; Ramamurthi and Lewis, 1997). The kinetics of the

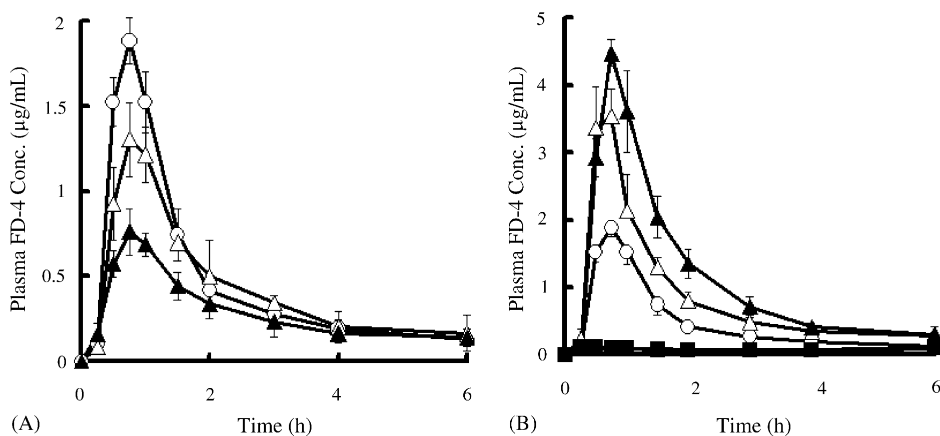


Fig. 4. Effects of the peroxynitrite scavenger, deferoxamine (A) and superoxide generator, pyrogallol (B), on plasma FD-4 concentration after colonic administration with NOC7. FD-4 (10 mg/mL) and NOC7 (50 mM) administered with or without deferoxamine or pyrogallol. NOC7 (◯), NOC7 with 10 mM deferoxamine or 5 mM pyrogallol (△), NOC7 with 50 mM deferoxamine or 10 mM pyrogallol (▲), control (◯) and control with 10 mM pyrogallol (■). Each value represents the mean ± S.D. of three to five rats.

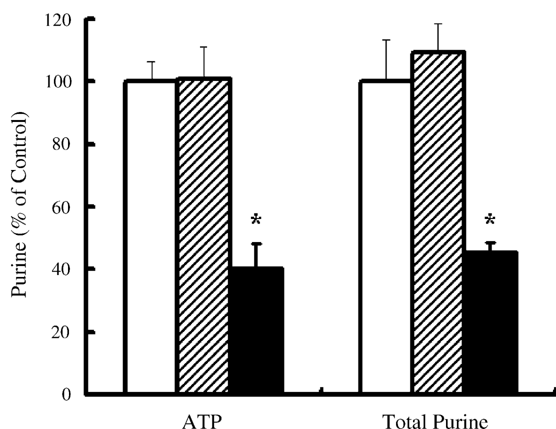


Fig. 5. Effect of NOC7 or tartaric acid on the ATP and total purine level in the colon 1 h after administration. After 1 h treatment with NOC7 (20 μmol) or tartaric acid (100 μmol), the colon was removed and the ATP and total purine levels were determined by HPLC. Control (open columns), NOC7 (hatched columns) and tartaric acid (filled columns). Each value represents the mean ± S.D. of three to five rats. $^{*}P < 0.05$, significantly different compared with the control.

release of NO from NOCs was estimated indirectly by measuring the amine compounds after the liberation of NO from NOCs. As indicated in Fig. 1, the degradation rates of NO differed among NOCs, in the order NOC7 > NOC5 > NOC12. NOCs induced a remarkable absorption enhancement of FD-4 after co-administration with FD-4 in the colon (Fig. 2 and Table 1). However, the effect of NOC12 was lower than that of other NOCs. This may have been due to the slow release rate of NO from NOC12. The enhancement effect of NOC7 rapidly disappeared compared with the effect of NOC5. These results suggested that there may be an optimum NO release rate from the NO donor for inducing the enhancement effect of the NO donor. Yamamoto et al. (2001) suggested the same enhancement effects of NOC5 and NOC12 in vitro. This difference may be due to the difference of elimination of NO or its relative nitrogen (perhaps peroxynitrate) from the action site in situ and in vitro. NO and peroxynitrate can permeate the intestinal membrane and be eliminated from the intestine though the bloodstream in situ, but not in vitro.

To characterize the molecular species mediating the absorption-enhancing effect of NOCs, several scavengers and generators were used. NOC7 was selected as the NO donor because of its short effect on the colon. C-PTIO, a scavenger of NO (Devlin et al., 1981), inhibited the absorption-enhancing effect of NOC7 after co-administration in the colon (Fig. 3(A)). The same inhibition effect of C-PTIO was observed in the jejunum (Numata et al., 2000). These results suggest that the absorption-enhancing effect of NOC7 may be caused by NO and/or its nitrogen metabolites in the intestine. NO has the potential to directly influence the intestinal epithelial function (Borutaite and Brown, 1996). However, it is increasingly apparent that many of the effects attributed to NO are actually mediated by another nitrogen metabolite, peroxynitrite. This extremely potent biological oxidant is formed at near diffusion-limited rates when two radicals, namely NO and superoxide, react with each other (Pryor and Squadrito, 1995). Tiron, the non-enzymatic superoxide scavenger (Devlin et al., 1981), attenuated the increases in permeability induced by the NO donor (Fig. 3(B)). To further investigate the contribution of superoxides, pyrogallol, a superoxide generator (Marklund and Marklund, 1974), was used. Pyrogallol potentiated the induction of hyperpermeability by NOC7 and its effect was dose-dependent (Fig. 4(B)). Pyrogallol did not affect the absorption of FD-4 from the colon. Menconi et al. (1998) also suggested the same result with Caco-2 monolayers. These results suggested that the endogenous production of superoxide participates in the development of hyperpermeability of the NO donor.

Deferoxamine, a peroxynitrate scavenger (Denicola et al., 1995), provided significant protection against hyperpermeability induced by NOC7 (Fig. 4(A)). Peroxynitrate (pK_a = 6.8) can be protonated to form peroxynitrous acid at physiological values of pH (7.4). Peroxynitrous acid can then yield a vibration-excited species with hydroxyl radical-like properties (Pryor and Squadrito, 1995). To investigate the participation of peroxynitrous acid, we used mannitol, a hydroxyl radical scavenger (Farias-Eisner et al., 1996). Mannitol reduced the hyperpermeability induced by NOC7 (Fig. 3(C)). Mannitol cannot cross the intestinal membrane (Cogburn et al., 1991). Thus, the absorption-enhancing effect of NOC7 involved the extracellularly generated hydroxyl radical-like species. These scavengers and generators seem to affect the NO release from NOC7. We measured the degradation of NOC7 in the presence of these compounds but could not determine the rate due to interference from these compounds at 250 nm. We tried to use other assay methods (Griess reaction method (Green et al., 1982) and

fluorescence method (Misko et al., 1993)). Unfortunately, NOC7 degradated immediately on addition of the reaction reagents. Menconi et al. (1998) suggested that some compounds used in this study slightly affected the release of NO from SNAP and SIN-1. Although the effect of these compounds on the release of NO from NOC7 was not clear, peroxynitrate may be partially involved in the hyperpermeable responses induced in situ by the NO donors.

The exact mechanism(s) of NO donors is unknown, but two possible mechanisms may be considered. One is a loss of cellular viability and the other is a dilution of the tight junction. We have previously demonstrated that NOC7 does not induce the releases of LDH and protein from the jejunum and colon (Numata et al., 2000). These results suggested that NO donor-induced hyperpermeability may not be simply due to a loss of cellular viability. Lindmark et al. (1998) and Hayashi et al. (1999) reported that absorption enhancers (medium chain fatty acids, acylcarnitines and organic acids), which induced dilution of the tight junction, decreased the intracellular ATP level. ATP-induced alterations in cytoskeletal integrity can result in dysregulation of the normal functioning of tight junctions (Hindshaw et al., 1993), thereby leading to enterocytic hyperpermeability via the paracellular pathway (Canfield et al., 1991; Unno et al., 1996). Salzman et al. (1995) suggested that the intracellular levels of ATP in Caco-2 monolayers decreased after 24 h incubation with NO donors (SNAP and SIN-1). In our study, tartaric acid reduced the ATP and total purine levels in the colon (Fig. 5). However, NOC7 did not reduce the ATP and total purine levels. From these results, we conclude that the mechanism(s) of the hyperpermeability induced by NOC7 are partially different and more complex mechanisms than previously recognized enhancers.

In conclusion, our results indicate that the optimum NO release rate from an NO donor may induce the absorption-enhancing effect of the NO donor in the intestine, which, in turn, is mediated, at least in part, by the formation of the NO metabolite, peroxynitrate.

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