

Mitochondrial disorders in NSAIDs-induced small bowel injury

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Recent studies using small bowel endoscopy revealed that non-steroidal anti-inflammatory drugs including low-dose aspirin, can often induce small bowel injury. Non-steroidal anti-inflammatory drugs-induced small bowel mucosal injury involves various factors such as enterobacteria, cytokines, and bile. Experimental studies demonstrate that both mitochondrial disorders and inhibition of cyclooxygenases are required for development of non-steroidal anti-inflammatory drugs-induced small bowel injury. Mitochondrion is an organelle playing a central role in energy production in organisms. Many non-steroidal anti-inflammatory drugs directly cause mitochondrial disorders, which are attributable to uncoupling of oxidative phosphorylation induced by opening of the mega channel called mitochondrial permeability transition pore on the mitochondrial membrane by non-steroidal anti-inflammatory drugs. Bile acids and tumor necrosis factor- α also can open the permeability transition pore. The permeability transition pore opening induces the release of cytochrome c from mitochondrial matrix into the cytosol, which triggers a cascade of events that will lead to cell death. Therefore these mitochondrial disorders may cause disturbance of the mucosal barrier function and elevation of the small bowel permeability, and play particularly important roles in early processes of non-steroidal anti-inflammatory drugs-induced small bowel injury. Although no valid means of preventing or treating non-steroidal anti-inflammatory drugs-induced small bowel injury has been established, advances in mitochondrial studies may bring about innovation in the prevention and treatment of this kind of injury.

Key Words: oxidative phosphorylation, uncoupling, mitochondrial permeability transition, aspirin

It has been known since many years ago that non-steroidal anti-inflammatory drugs (NSAIDs) can injure the small bowel as well as upper gastrointestinal tract. Allison *et al.*⁽¹⁾ reported that among 249 autopsied cases that used NSAIDs, small intestinal injuries were found in 21 cases (8.4%), while among 464 cases that did not use NSAIDs, these injuries were found only in 3 cases (0.6%). However, details of this injurious effect of NSAIDs had remained unclarified in the absence of high accuracy small bowel testing methods. In the 21st century, introduction of new modalities such as capsule endoscopy⁽²⁾ and balloon endoscopy⁽³⁾ began to shed light on this “dark continent”, yielding findings such as the potential of NSAIDs to induce small bowel injury frequently regardless of the length of oral NSAID treatment period.

Furthermore, it has been demonstrated that 40–90% of NSAID users had mucosal defects (erosion, ulcer, etc.) which could lead to bleeding, perforation or stenosis.^(4–7) Now, close attention is paid to this condition. Although onset of NSAIDs-induced small bowel mucosal injury is considered to involve many factors,^(8–10) its details remain unclarified. This paper will outline the signifi-

cance of mitochondrial disorders during onset of NSAIDs-induced small bowel mucosal injury.

Mechanism for NSAIDs-Induced Small Bowel Mucosal Injury

NSAIDs, including low-dose aspirin, can induce diverse kinds of mucosal injury such as redness, erosion and ulcer (Fig. 1), affecting the entire small bowel. A mechanism of primary importance for onset of such injury is inhibition of cyclooxygenase (COX) by NSAIDs and collapse of the mucosal defensive system due to reduction of prostaglandin (PG) level following COX inhibition.⁽¹¹⁾ Tanaka *et al.*⁽¹⁰⁾ demonstrated that NSAIDs can induce small intestinal damage when both COX-1 and COX-2 are inhibited. Other than this mechanism, COX-independent topical effect of NSAIDs on small bowel epithelium is considered to play an important role in early processes of injury.⁽¹²⁾ This direct activity involves effects on mitochondria. That is, NSAIDs can

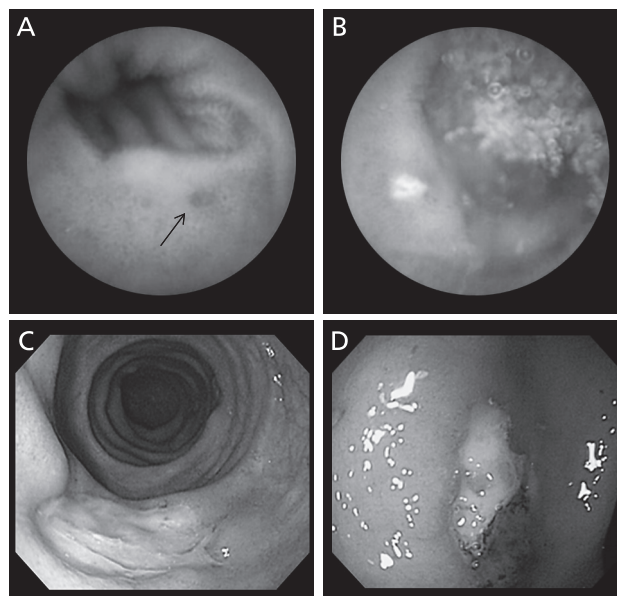


Fig. 1. NSAIDs-induced small bowel injury. A, B: Capsule endoscope pictures (low-dose aspirin-induced small bowel injury). C, D: Double-balloon endoscope pictures (sulindac-induced small bowel ulcer). A: redness (arrow), B, C, D: ulcer.

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injure epithelial cell mitochondrial function, inducing compromised tight junction function, apoptosis, and necrosis.

As a result of such initial disorders, permeability across the small bowel mucosa increases, facilitating invasion of small bowel by luminal injury factors such as enterobacteria⁽¹³⁾ and bile.⁽¹⁴⁾ Gram-negative bacteria having invaded the small bowel mucosa activate toll-like receptor 4, a receptor for lipopolysaccharide, and induce excessive expression of cytokines such as tumor necrosis factor- α through activation of the transcription factors nuclear factor kappa B, leading to neutrophil infiltration.^(15,16) Eventually, the small bowel which has weakened due to PG deficiency cannot suppress stimulation by various injury factors, possibly leading to formation of macroscopic ulcers. As described above, injury of mitochondria by NSAIDs is a key event which can induce a series of inflammatory reactions.

Mitochondrial Structure and Function

Mitochondrion is an organelle playing a central role in energy production in organisms. There are about 100–2,000 mitochondria in each cell. Structurally, the mitochondrion is surrounded by double membranes (outer and inner membranes), and the inner membrane partitions the central matrix from the intermembranous space (the space between inner and outer membranes).⁽¹⁷⁾ The inner membrane of the mitochondrion has numerous folds called “cristae”.

The mitochondrion takes up pyruvic acid, fatty acid, oxygen, adenosine diphosphate (ADP) and phosphoric acid from the surrounding cytoplasm. Within the mitochondrial matrix, pyruvic acid and fatty acid are converted into acetyl CoA. Acetyl CoA then enters the citric acid cycle and is degraded into nicotinamide adenine dinucleotide (NADH) and carbon dioxide.

NADH is subsequently transferred into the inner membrane, where it is converted into NAD, while donating electron to the electron transport chain composed of three respiration enzyme complexes (NADH dehydrogenase complex, cytochrome complex and cytochrome oxidase complex). The electron transport chain releases proton (H⁺) from the matrix to the intermembranous space. As a result, there arises a pH and potential gradient between the intermembranous space and the matrix. The energy derived from this gradient is utilized by adenosine triphosphate (ATP) synthetase to phosphorylate ADP and to synthesize ATP (oxidative phosphorylation).⁽¹⁸⁾ The electron transport chain is coupled to oxidative phosphorylation. Uncoupling agents, which inhibit this series of reactions, suppress the formation of ATP and induce cell death such as apoptosis and necrosis.

NSAIDs-Induced Small Bowel Injury and Mitochondrial Disorders

Somasundaram *et al.*⁽¹⁹⁾ observed the small bowel epithelium following oral indomethacin treatment under an electron microscope and reported morphological changes of the mitochondrion in early processes of small bowel ulceration. They found

mitochondrial vacuolation 1 h after indomethacin treatment and mitochondrial swelling and loss of cristae at 2 h. Similar morphological changes were noted also after treatment with dinitrophenol (an uncoupling agent).

In a study *in vitro*, NSAIDs such as indomethacin, aspirin, naproxen, and piroxicam uncoupled oxidative phosphorylation of isolated rat liver mitochondria in micromolar and NSAIDs at higher concentrations inhibited respiration in coupled mitochondria,⁽¹⁹⁾ suggesting that the changes in mitochondria observed soon after indomethacin treatment are attributable to its activity to uncouple oxidative phosphorylation and/or inhibit electron transport. The above-mentioned morphological changes of mitochondria were reproduced also following non-oral treatment with indomethacin, but they were absent in animals with ligated bile duct. Aspirin exerted uncoupling activity *in vitro* but did not induce morphological changes of mitochondria in epithelial cells of the small intestine or small bowel ulceration when administered orally.

When aspirin was administered directly into the small bowel, severe mucosal injury was induced in the area distal to the site of administration.⁽¹⁹⁾ Considering that aspirin is immediately absorbed in the stomach and duodenum without entering the enterohepatic circulation and that indomethacin does not induce ulcers in the bile duct-ligated rats,⁽¹⁴⁾ these results strongly suggest that mitochondrial disorders through the uncoupling of oxidative phosphorylation and/or inhibition of respiration by NSAIDs are indispensable for onset of small bowel ulcers, and it seems likely that such topical effect of NSAIDs on small bowel epithelium is dependent on local drug concentration (concentration within the small bowel) and frequency of exposure to the drug. Other studies also demonstrated importance of mitochondrial disorders by NSAIDs on their intestinal toxicity.^(20,21)

As described above, aspirin has been believed to be less harmful to the small intestine for a long time. However, in 2007, Leung *et al.*⁽²²⁾ reported a case of small intestinal ulcers in a patient taking low-dose aspirin. So we evaluated ulcerogenicity of aspirin to the small intestine. Capsule endoscopy identified red spots and mucosal breaks (erosions/ulcers) in 100% (11/11) and 90.9% (10/11) of patients who taking low-dose enteric-coated aspirin, respectively.⁽⁵⁾ One reason for this very high incidence of injuries by low-dose enteric coated aspirin seems to be intensification of mitochondrial disorders due to exposure of the small bowel mucosa to high concentrations of aspirin dissolved within the small bowel. Further studies including evaluation of the intestinal toxicity of buffered aspirin are needed to support the hypothesis that the enteric-coated formulation of aspirin might be a principal cause of the damage.

Somasundaram *et al.*⁽²³⁾ conducted a detailed study on the role of mitochondrial disorders in NSAIDs-induced small bowel mucosal injury. They demonstrated: (1) uncombined treatment with dinitrophenol elevated the permeability across small bowel mucosa and induced relatively mild neutrophil infiltration, although it did not induce ulceration; (2) non-oral treatment with aspirin reduced the PG level in small bowel mucosa, but did

Table 1. Effects of drug administration routes on onset of small bowel ulcer

| Drugs and administration route | COX inhibition | Mitochondrial disorders | Ulceration |
|--|----------------|-------------------------|------------|
| Oral treatment with indomethacin | + | + | + |
| Non-oral treatment with indomethacin | + | + | + |
| Non-oral indomethacin + bile duct ligation | + | – | – |
| Oral treatment with aspirin | + | – | – |
| Aspirin administration into small bowel | + | + | + |
| Dinitrophenol administration into small bowel | – | + | – |
| Non-oral aspirin + dinitrophenol administration into small bowel | + | + | + |

COX, cyclooxygenase.

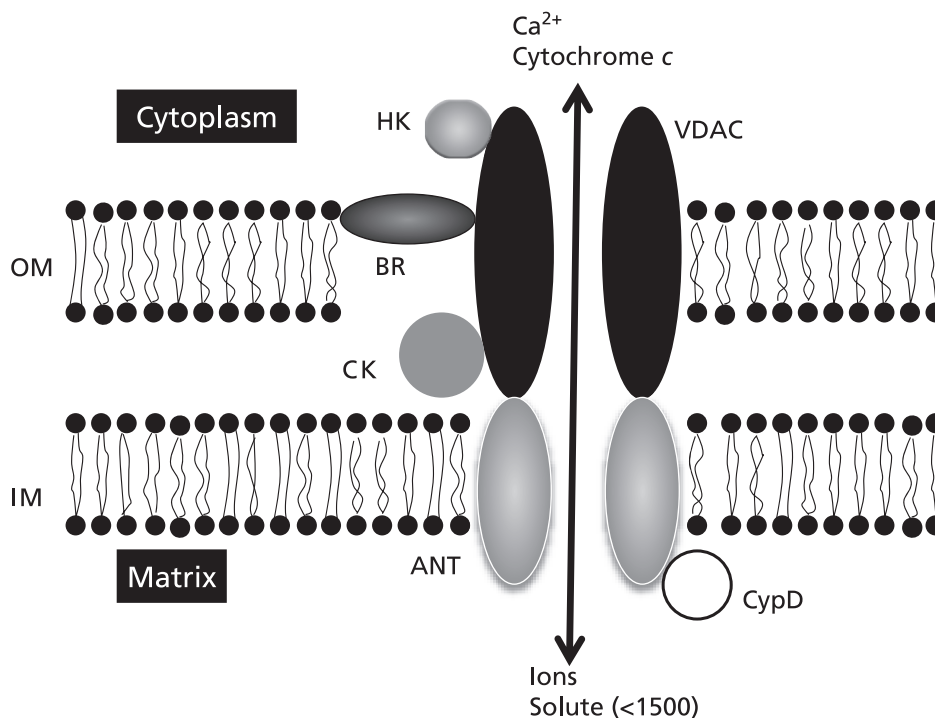


Fig. 2. Structure and function of mitochondrial permeability transition pore. HK, hexokinase; BR, benzodiazepine receptor; CK, creatine kinase; ANT, adenine nucleotide translocase; VDAC, voltage-dependent anion channel; CypD, cyclophilin D, OM, outer membrane; IM, inner membrane.

not affect small bowel permeability or induce ulceration; and (3) treatment with dinitrophenol in combination with non-oral aspirin resulted ulceration. These findings suggest that mitochondrial disorders due to uncoupling of oxidative phosphorylation disturb the mucosal barrier function and elevate the small bowel permeability, but that PG deficiency through COX inhibition is additionally needed for ulceration to occur (Table 1).

Mitochondrial Permeability Transition and NSAIDs

Mitochondrial permeability transition (elevation of permeability across the mitochondrial membrane) is a phenomenon of sharp elevation in the permeability of substances (below 1,500 Da in molecular weight) across the mitochondrial membrane.⁽²⁴⁾ This phenomenon is initiated by opening of the mega-channel called mitochondrial permeability transition pore (PTP; composed of voltage-dependent anion channel (porin), adenine nucleotide translocase, cyclophilin D, etc.) on the mitochondrial membrane (Fig. 2). When the PTP is opened, low-molecularweight substrates can freely penetrate the mitochondrial matrix, carrying along with them water and resulting in mitochondrial swelling and the release of cytochrome *c* into the cytosol. Cytochrome *c* release triggers a cascade of events that will lead to either apoptosis (in ATP-replete cells) or necrosis (in ATP-depleted cells).⁽²⁵⁻²⁷⁾

As described above, NSAIDs have uncoupling activity. This activity is considered to be attributable, at least partially, to induction of PTP. Masubuchi *et al.*⁽²⁸⁾ reported that diclofenac induced mitochondrial swelling and depolarization of membranes, resulting in release of Ca^{2+} (accumulated within mitochondria) and suppression of ATP formation by cells. They added that these effects of diclofenac were suppressed by cyclosporine A (a PTP inhibitor). Salicylic acid, an active metabolite of aspirin, was shown to open PTP in kidney cells by Al-Nasser *et al.*⁽²⁹⁾ and in liver cells by Trost *et al.*⁽³⁰⁾ On the basis of the findings from these studies, it is now considered that the high cytotoxic activity of NSAIDs is partially explained by their effect on PTP. So far as NSAIDs-

induced small bowel mucosal injury is concerned, opening of PTP seems to be involved in elevation of the intestinal permeability, epithelial cell apoptosis, and necrosis. The mechanism by NSAIDs cause mitochondrial dysfunction is still unclear. Lal *et al.*⁽³¹⁾ reported that several NSAIDs including ketorolac and diclofenac open PTP only under conditions of oxidative stress and in a Ca^{2+} dependent fashion.

Mitochondrial Disorders Due to Other Factors

Bile plays an important role in the mechanism for NSAIDs-induced small bowel mucosal injury.^(14,19) Bile acids such as chenodeoxycholate are known to open PTP.⁽³²⁾ Furthermore, many apoptotic signaling molecules act as PTP inducers, and these include lipid mediators like ceramides and arachidonic acid.^(33,34) The latter is particularly interesting in the context of Ca^{2+} -dependent cell death. Indeed, it has been shown that arachidonic acid-selective, Ca^{2+} -dependent phospholipase A2 is essential for the cytotoxic action of tumor necrosis factor- α ,⁽³⁵⁾ which triggers PTP opening^(36,37) through activation of phospholipid hydrolysis⁽³⁴⁾ and that arachidonic acid signals apoptosis^(38,39) through a mitochondrial effect that can be amplified by inhibition of COX.⁽³³⁾ It seems therefore likely that various factors other than NSAIDs are also involved in mitochondrial disorders which arise during the course of small bowel ulceration.

Rebamipide, an anti-ulcer agent,⁽⁴⁰⁻⁴²⁾ suppressed indomethacin-induced mitochondrial permeability transition in gastric epithelial cells.⁽⁴³⁾ Recently, it was reported that this drug was useful in preventing diclofenac-induced small bowel injury.⁽⁴⁴⁾ This finding seems to be significant when arguing about the features of NSAIDs-induced small bowel mucosal injury and drugs useful in preventing or treating such injury.

Conclusions

Close attention has recently been paid to small bowel diseases

in the field of gastroenterology. NSAIDs-induced small bowel injury is a representative of such small bowel diseases. The mechanism for NSAIDs-induced small bowel injury involves enterobacteria, bile, intestinal hypermotility, cytokines, neutrophils, etc. As described in this paper, mitochondrial disorders in small bowel epithelial cells also play an important role in early processes of this kind of injury. Although no valid means of preventing or treating NSAIDs-induced small bowel injury has been established, advances in mitochondrial studies may bring about innovation in the prevention and treatment of this kind of injury.

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Abbreviations

| | |
|--------|---------------------------------------|
| ADP | adenosine diphosphate |
| ATP | adenosine triphosphate |
| COX | cyclooxygenase |
| NADH | nicotinamide adenine dinucleotide |
| NSAIDs | non-steroidal anti-inflammatory drugs |
| PG | prostaglandin |
| PTP | permeability transition pore |

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