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Mechanisms of Damage Involved in Small Intestinal Tract Caused by Nonsteroidal Anti-Inflammatory Drugs

Mecanismos de daño en el intestino delgado inducidos por anti-inflamatorios no esteroideos

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Abstract:

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used type of drugs, as they are very effective as anti-inflammatory, anti-pyretic and analgesic. Their use has increased dramatically over the years even without a medical prescription¸ however the use of NSAIDs is limited due to the adverse effect in the gastrointestinal tract, heart and kidney. This research work is focused on the adverse effect in the gastrointestinal tract. The mechanisms involved in gastric damage caused by NSAIDs are well known compared to those in the intestinal area. Research on NSAID-induced intestinal damage is very limited, despite its higher mortality rate, partially due to the difficulty of reaching this area. Therefore, this review mentions some of the mechanisms involved in small intestinal damage to help as a reference to continue doing research about new strategies to come up with an effective clinical therapy.

***Key words**:*
NSAIDs; intestinal damage; prostaglandin; oxidative stress

Resumen:

Los medicamentos anti-inflamatorios no esteroideos (AINE) se encuentran entre los fármacos más utilizados, ya que son muy eficaces como anti-inflamatorios, antipiréticos y analgésicos. Su uso ha aumentado dramáticamente a través de los años, incluso sin una prescripción médica, sin embargo, el uso de AINEs es limitado debido a los efectos adversos en el tracto gastrointestinal, en el corazón y en el riñón. En esta revisión nos centramos en el efecto adverso en el tracto gastrointestinal. Los mecanismos implicados en el daño gástrico causado por los AINEs son bien conocidos, en contraste con aquellos en el área intestinal. La investigación sobre el daño intestinal inducido por AINEs es muy limitada, a pesar de su mayor tasa de mortalidad, en parte debido a la dificultad de llegar a esta área. Por lo tanto, en esta revisión, mencionamos algunos de los mecanismos implicados en el daño del intestino delgado como referencia para continuar la investigación de estrategias novedosas para obtener una terapia clínica efectiva.

***Palabras Clave**:*
AINEs; daño intestinal; prostaglandinas; estrés oxidativo

INTRODUCTION

The human body is constantly exposed to infectious agents and damaging substances that put our health at risk, resulting in some sort of disease.[1] The gastrointestinal (GI) tract is in close contact with antigens from the external environment due to the role it plays.[2] To ensure internal homeostasis, the GI tract performs absorption and digestion of nutrients, transport of electrolytes and water, as well as secretion of proteins and water to the intestinal lumen. The GI tract is also a highly important defense system, avoiding the entrance of substances from the intestinal lumen that can be harmful (pathogens, antigens and/or pro-inflammatory factors), but at the same time allowing the passage of other beneficial substances that favor the development of the intestinal immune system. This permeability is adaptable and is regulated as a response to extracellular stimuli, such as nutrients, cytokines and bacteria.[3] Both, defensive and digestive functions, are carried out as part

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of the "gastrointestinal barrier" or "mucosal barrier", local defense and neurohormonal mechanisms converge to perform, in a coordinated way, to ensure the optimal function of the barrier.[4,5]

The factors that affect the integrity of the GI barrier are several types of stress, such as psychological stress, prolonged intense exercise, heat stress, and stress caused by the ingestion of irritants (alcohol and caffeine, to mention some). Mucosal injury is presented when noxious factors "overwhelm" mucosal defense mechanism. Moreover, some types of drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs), also contribute to the damage of the GI mucosa. [6,7]

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

NSAIDs are known for their antipyretic, analgesic and anti-inflammatory actions. Their use has increased dramatically in the recent years due to the growth of the elderly population and the easy access to these drugs, even without a medical prescription. Twenty five percent of all the reports of secondary effects of all drugs are due to NSAIDs.[8] In the United States alone, more than 100,000 people are hospitalized due to some sort of complication caused by these drugs, from which more than 15,000 unfortunately die every year.[9] Meanwhile, reports of 2009 showed that about 490,000 patients were hospitalized from ulcers in the gastrointestinal tract alone, from which almost 3,700 of them lost their lives.[10] In Mexico, gastric and duodenal ulcers were the 20th cause of general mortality in 2011, a figure that has not changed over the years. The lack of epidemiology studies in Mexico and other countries is partially due to small intestinal damage which is largely asymptomatic and there is not an easy method of diagnosis because is a very difficult area to reach. It is hard to estimate the amount of the population that are suffering from these complications.[11,12]

The intake of NSAIDs constitutes one of the most frequent causes of ulcers, and it represents an elevated risk to develop gastrointestinal complications. Conventional NSAIDs inhibit prostaglandin-endoperoxide synthase or cyclooxygenase-1 and -2 (COX1 and COX2). NSAIDs inhibition of COX enzymes, along with luminal aggressors mediate the gastrointestinal damage resulting in erosions and ulcers, with potential complications of bleeding, protein loss, stricture formation and perforation. NSAIDs decrease prostaglandins in mucosal levels (driven by inhibition of COX1), cause a defect on the quality of the gastric mucus and increase the production of acid, reduce submucosal blood flow causing local ischemia, alter epithelial proliferation, decrease bicarbonate production, which all correlate with gastric damage. The suppression of COX renders the intestinal mucosa more susceptible to injury and less efficient in undergoing repair. However, decreased levels of prostaglandins are found to be less important in the pathogenesis of small intestinal damage.[7,13,14]

MECHANISMS BY WHICH NSAIDs CAUSE INJURIES IN THE GASTROINTESTINAL TRACT

The mechanisms underlying the ability of NSAIDs to cause ulceration in the stomach and proximal duodenum are well understood, and this injury can largely be prevented through suppression of gastric acid secretion (mainly with proton pump inhibitors). In contrast, the pathogenesis of small intestinal injury induced by NSAIDs is less well understood, involving more complex mechanisms than those in the stomach and proximal duodenum. A critical feature of some NSAIDs that appears to be essential for induction of significant intestinal ulceration is their reabsorption in the ileum and subsequent secretion back into the duodenum via the enterohepatic recirculation. NSAIDs that do not undergo enterohepatic recirculation do not cause significant intestinal damage in animal models.[14,15]

Several research groups have analyzed the mechanism by which NSAIDs induce intestinal damage based on recent insights from experimental models in rodents typically exposed to diclofenac, indomethacin, or naproxen, and all agree that the inhibition of COX does not seem to be the only mechanism involved.[9,13,15]

Enterohepatic circulation

Enterohepatic circulation occurs when substances are metabolized in the liver, excreted into the bile, and passed into the intestinal lumen; then, those compounds are reabsorbed across the intestinal mucosa and returned to the liver via portal circulation.

Enterohepatic circulation is related with drug biotransformation. Drug biotransformation is a multi-step process where drug compounds go through phase I (hydrolysis and oxy-reduction reactions), others phase II (conjugated reactions in which the original compound is linked to a polar group such as glucuronic acid, sulfate, taurine, glycine, or glutathione to increase the solubility of the metabolite in the bile) and another portion of drugs go through both phases.

Indomethacin is a type of NSAID that is metabolized in the liver through phase I and II reaction; during phase II, indomethacin is conjugated with a glucuronic group for its excretion in bile and enterohepatic cycling. This cycle implies the participation of new metabolic reactions mediated by enzymes or bacteria of the intestinal flora, that alter the chemical structure of the conjugated indomethacin excreted in bile to the parent drug again, so that it can increase indomethacin biological activity and absorption. Indomethacin when is excreted in bile, reaches the intestine being exposed to β-glucuronidase enzymes of the intestinal flora, that hydrolyze the conjugate (indomethacin linked to glucuronic acid) leaving indomethacin in its free form. Once in its free form, it is absorbed in the intestine entering the bloodstream reaching the liver, where, once again, is conjugated

with glucuronic acid creating a cycle. In this case, it has been reported that intestinal damage induced by indomethacin depends of enterohepatic circulation; furthermore, ligation of bile duct prevents the damage induced by NSAIDs. NSAIDs interact with the intestinal mucus layer and the cell surface phospholipid bilayer compromising the hydrophobic lining, which leads to mucosal exposure to luminal aggressors (bacterial and bile).[13,16-20]

Uncoupling of mitochondrial oxidative phosphorylation

NSAIDs that have been associated with enteropathy are uncouplers of oxidative phosphorylation in mitochondria, dissociating respiration from energy production. This feature which is related to their molecular structure and typical for lipophilic weak acids.[21] The uncoupling of mitochondrial oxidative phosphorylation by NSAIDs was demonstrated by electron microscopy in the small intestine of mice given conventional acidic NSAIDs and similar changes were found in gastric biopsies from patients.[21]

The mechanisms are not fully understood, but it is known that indomethacin direct contact with intestinal mucosa induces uncoupling of mitochondrial oxidative phosphorylation which impairs tight junction and generates water flows into the matrix, causing characteristics and pathognomonic swelling of mitochondria in enterocytes. There is also a release of intra-mitochondrial Ca^{2+} into cytoplasm with depletion of reduced glutathione, depletion of $NSD(P)H_2$, generation of superoxide anion (O^{2-}) and release of pro-apoptogenic proteins. Free radicals accumulate within the mitochondria setting up a vicious cycle as this activates uncoupling proteins in the inner mitochondrial membrane. The uncoupling finally leads to depletion of cellular ATP levels, with loss of integrity of the intercellular junctions in the gastrointestinal tract (leading to increased mucosal permeability), and apoptosis and cell death in the end.[13,21]

Oxidative stress

Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses.[22] Reactive oxygen species (ROS) are produced in response to injury tissue and can take oxidative damage to proteins, lipids, and DNA. Oxidative stress could result from: (1) the presence of xenobiotics, (2) the activation of the immune system in response to invading microorganisms, and (3) radiation.[23]

NSAIDs-induced mucosal injury have been also related to the imbalance of oxidative stress and anti-oxidative system, and this can be improved by anti-oxidant drugs.[24] It seems that NSAID-induced neutrophil infiltration in intestinal mucosa, then neutrophils lead to the liberation of oxygen-derived free radicals[20] affecting mitochondrial dysfunction, then, the combination of these factors induce lipid peroxidation and oxidative stress.

Severe injury to the small intestine has been estimated to account for one-third of all NSAID-associated complications. These can lead to ulcer formation, mucosal bleeding, inflammation and perforation. Typically, ulcers present necrotic or apoptotic injury of enterocytes that may involve deeper layers of the mucosa, with loss of intestinal microvilli and an acute inflammatory cell infiltrate. The increasing use of novel diagnostic tools including video capsule endoscopy and double-balloon endoscopy has revealed that, unexpectedly, approximately two thirds of both long-term and short-term NSAID users exhibit mild or more severe forms of drug-induced lesions in the small intestine. Despite its high incidence, there are currently no approved effective therapies to prevent or treat NSAID enteropathy. This lack of enteric-protective therapies is in part due to an incomplete understanding of the underlying mechanisms of NSAID-induced GI damage.[21,25,26]

Some of the effects caused by NSAID-induced damage in stomach and small intestine are summarized in table 1.

PROTON PUMP INHIBITORS AND SMALL INTESTINAL DAMAGE TREATMENT

Unfortunately, the successfully used current therapies to treat gastric injury do not protect from small intestinal damage. Acid suppressants such as proton pump inhibitors (PPIs) prevent NSAID-induced damage to upper GI tract, but these agents are not effective against small intestinal damage, as hydrochloric acid is not produced in the small intestine and gastric acid is neutralized by bicarbonate in the duodenum.[27] In fact, in animal models and in patients, these therapies have been shown to aggravate the extent of enteropathy.[14,21] Recent video capsule endoscopy studies suggest a very high incidence of small intestinal damage (about 60%) in young, healthy, human subjects taking both an NSAID and a PPI for 2 weeks.[25,28,29] This suggests that the PPI conferred little, if any, protection to the mid- and distal small intestine, which are major sites of NSAID-induced bleeding.[30] Wallace et al. (2011) demonstrate how NSAIDs (naproxen) co-administrated with a PPI (omeprazole), given in a dose of 10 mg/kg twice a day (p.o.) for 5 days, exacerbates the intestinal damage. The mechanism underlying this effect of PPIs was a dramatic shift in types of bacteria colonizing the intestine, specifically, a marked loss of *Bifidobacteria* species.[11,13,31-33]

INTESTINAL MICROBIOTA

The composition of the intestinal microbiota is a key factor in the pathophysiology of NSAID-induced small intestinal damage. Animal and human studies have documented dramatic shifts in the types and numbers of bacteria in the intestine during

chronic NSAID use leads to increase susceptibility to mucosal injury. In general, there is an increase in the numbers of Gram-negative bacteria and a decrease in Gram-positive bacteria, resulting in dysbiosis.[12] Dysbiosis is an abnormality present in the composition of the microbial community in which the population of bacteria beneficial for the host's health decreases and the population of pathogenic bacteria typically present in small numbers increases[24]. In some studies, there appeared to be an enrichment of specific bacteria, such as *Enterococcus faecalis*, *Clostridium*, *Bacteroides* and *Escherichia coli*; interestedly, β-glucuronidase has been shown to be expressed in these bacterial.[31] Moreover, an inhibitor of bacterial β-glucuronidase could significantly reduce the severity of diclofenac-induced small intestinal injury in mice[32,33] and *Lactobacillus casei* prevented aspirin-induced small intestinal damage in 13 patients. However, more studies need to be done.[34]

IN VIVO MODELS

Some animal research models have been used to find insight on enteropathy caused by this type of drugs are NSAID-induced models (both chronic and acute administration of NSAIDs). In these models, the use of acute administration of NSAIDs to mice and rats (consisting on administering a single high dose p.o) is very important to be able to visualize, in a relatively quick way, the injuries that a certain drug may cause. In contrast, the use of chronic administration of NSAID (usually indomethacin, diclofenac, naproxen, among others) at low doses for a few days or even weeks, simulate what occurs in the clinic.[13,33,35]

CONCLUSION

In summary, the use of NSAIDs increases yearly, even without any medical prescription. Even though these type of drugs are very effective (anti-inflammatory, anti-pyretic and analgesic), they account for many side effects, highlighting those in the gastrointestinal tract. Some of these complications are erosions and ulcers, with potential complications of bleeding, protein loss, stricture formation and perforation.

The mechanisms underlying the ability of NSAIDs to cause ulceration in the stomach and proximal duodenum are well understood, and this injury can largely be prevented through suppression of gastric acid secretion (mainly with proton pump inhibitors). Contrary to what occurs in the small intestine, where the mechanisms involved in the pathogenesis of small intestinal injury induced by NSAIDs is less well understood.[13,15] Despite its clinical relevance and high mortality rates, there is no proven effective therapies or treatment for NSAID-induced small intestinal damage, and the current therapies available exacerbate the damage instead of solving it. With that being said, this health problem has to be urgently addressed and novel strategies to treat NSAID-induced intestinal damage have to be searched, keeping in mind that the mechanisms involved in NSAID-induced gastrointestinal damage differ in stomach and intestine. Therefore, NSAID-induced enteropathy cannot longer be treated with the same agents that NSAID-induced gastropathy is managed.

Table 1: Effects caused by NSAIDs and their contribution to gastric or intestinal damage.

Type of damage	Effects caused by NSAIDs
Gastric	↑ROS ↑iNOS ↑cell death Uncoupling of mitochondria oxidative phosphorylation ↓PGs ↓Mucus synthesis ↑Proinflammatory cytokines Disrupt phospholipid monolayer and bilayer ↓Microvascular flow ischemia ↑Lipid peroxidation ↑Neutrophil infiltration
Intestinal	↑ROS ↑iNOS ↑cell death ↓ATP Uncoupling of mitochondria oxidative phosphorylation ↑Endoplasmic reticulum stress ↑CHOP ↑P-JNK ↑Dysbiosis ↑Electrophile stress Disrupt phospholipid monolayer and bilayer ↓Microvascular flow ischemia ↑Lipid peroxidation

NSAIDs (Nonsteroidal anti-inflammatory drugs); ROS (Reactive oxygen species); iNOS (inducible Nitric oxide synthases**);** PGs (Prostaglandins); CHOP (C/EBP-Homologous Protein); P-JNK (Phosphorylated c-Jun NH2-terminal kinase).

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