論 文 の 内 容 の 要 旨

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論文題目

The inhibition effect of lyophilized aspirin/trehalose on the aspirin-induced gastric mucosal injury

（アスピリン誘発性胃粘膜障害に対する凍結乾燥アスピリン/トレハロースの抑制効果）

Aspirin, one of non-steroidal anti-inflammatory drugs (NSAIDs), has been widely used for its analgesic, anti-inflammatory and anti-thrombotic effects. However, the serious gastrointestinal damage remains a significant problem. The inhibition of cyclooxygenase (COX) enzymes and subsequent reduction of gastric mucosal protective prostaglandin (PG) were first proposed to be the reason of aspirin-induced gastropathy. Recently, there is increasing evidence that COX-independent effects, such as direct irritation, influence of inflammatory mediators and the involvement of microcirculation, might also be important for aspirin-induced gastropathy.

Numerous strategies have been undertaken to decrease the incidence of gastropahty, including co-administration of protective substances such as sucralfate, proton pump inhibitors or prostaglandin analogues. However, the protection effects were not totally satisfied. Except co-administration, investigators also tried to develop safer NSAIDs, for instance, selective COX-2 inhibitors, however

some of this type of drugs were withdrawn from the market because of the adverse effect on cardiovascular system. Among these various strategies, the combination of NSAIDs with small protective molecules such as nitric oxide, hydrogen sulfide, and phosphatidylcholine, which help to maintain mucosal integrity, has been highly emphasized recently.

Trehalose, a natural disaccharide, is widely used as a food ingredient and in the cosmetics industry. Recent researches discovered several novel anti-inflammatory and cell-protective functions of trehalose. Trehalose could protect the protein integrity, reduce oxidative damage to cells, inhibit inflammatory cytokine production, and suppress apoptosis. Therefore, trehalose is expected to be a potential powerful therapeutic material for various diseases. Our research group had previously undergone several clinical trials of trehalose on adhesion prevention after abdominal surgery, inhibition of xenosis during dental treatment, and suppressing inflammation and vasospasm induced in an experimental subarachnoid hemorrhage model by its protective effects of cell membranes. Our group further hypothesized that lyophiliaztion of aspirin with trehalose, by which the specific interaction mediated by hydrogen bonding changes, may decrease the severity of aspirin-induced gastropathy.

The aim of this study was to determine whether lyophilization of aspirin with trehalose would decrease the incidence of aspirin-induced gastropthy. In this present study, the author investigated the effect of lyophilized aspirin/trehalose compared with other NSAIDs compounds on gastric mucosa *in vitro* and *in vivo*.

In the first chapter, the effects of NSAIDs on gastric cells were evaluated *in vitro*. A human gastric cell line (AGS) cultured in normal F–12 medium was exposed to aspirin, lyophilized aspirin, mixture of (mix) aspirin/trehalose, and lyophilized aspirin/trehalose at the aspirin concentration of 1–10 mM for 24 hr. After exposure, cytotoxicity was measured by MTT assay, and apoptosis-inducing potency was evaluated by DNA fragmentation analysis and TUNEL assay.

In the MTT assay, lyophilized aspirin/trehalose induced less cell death compared to other three test compounds at the same aspirin concentration. DNA fragmentation in AGS cells treated with aspirin, lyophilized aspirin or mix aspirin/trehalose was shown a ladder pattern on agarose gel at 10 mM aspirin concentration. On the contrary, there was no fragmented DNA pattern shown in any concentrations of lyophilized aspirin/trehalose treatment. Quantification of apoptosis by TUNEL assay also revealed that lyophilized aspirin/trehalose caused less apoptosis than other three test compounds at

the same concentration. These results indicated that lyophilized aspirin/trehalose was much less cytotoxic and low apoptosis-inductive *in vitro* compared to aspirin, lyophilized aspirin and mix aspirin/trehalose, suggesting that the cytoprotective effect did not appear either by simple lyophilization of aspirin nor by mixture of trehalose with aspirin.

In the second chapter, the effects of aspirin, mix aspirin/trehalose, and lyophilized aspirin/trehalose on gastric mucosa were evaluated in a rodent acute gastric ulceration model. Acute gastric ulceration was induced by a single oral administration of 200mg/kg aspirin, 960mg/kg mix aspirin/trehalose (200 mg/kg aspirin), 960mg/kg lyophilized aspirin/trehalose (200mg/kg aspirin), or vehicle (0.5% carboxymethylcellulose aqueous solution) in rats. Rats (n=6–8) were euthanized 5 hr after drug administration, and the stomach was exicised and grossly evaluated for the ulcerative lesions. The tissues were prepared for histology for gastric injuries and immunohistochemistry. *In situ* TUNEL assay and cleaved caspase-3 immunohistochemistry were performed to evaluate the apoptosis-inducing potency of each treatment. The acute ulceration was also induced in other groups of rats (n=6–8) by the same regimens, and the stomach was excised for the mRNA expression of inflammatory mediators by real-time polymerase chain reaction (PCR) and detection of mucosal PGE_2 concentration by EIA kit.

In this rodent acute ulceration model, lyophilized aspirin/trehalose was proved to induce less extent of gastropathy than aspirin and mix aspirin/trehalose macroscopically and microscopically. The results of *in situ* TUNEL assay and cleaved caspase-3 immunohistochemistry revealed that lyophilized aspirin/trehalose was less potent to induce apoptosis despite profound inhibition of mucosal prostaglandin synthesis. The inhibition of PGE_2 synthesis elicited by the lyophilized aspirin/trehalose was comparable with that of aspirin, which indicated the lyophilization procedure did not interfere the ability to inhibit COX activity. Moreover, lack of ulceration with presence of profound suppression of gastric PG synthesis suggested that lyophilized aspirin/trehlaose exerted protective effects that counteracted the potential damaging effects of COX inhibition. However, there were no significant differences between each group in the relative mRNA expression of inflammatory mediators, which might be due to the sampling time of gastric mucosa.

In the third chapter, the effects of aspirin and lyophilized aspirin/trehalose on gastric mucosa were evaluated in a canine ulceration model. Thirteen beagle dogs were assigned into aspirin (n=5), lyophilized aspirin/trehalose (n=5), and control groups (n=3). The dogs received oral administration of 25 mg/kg aspirin, lyophilized aspirin/trehalose (25 mg/kg aspirin), or vehicle every 12 hr for 28

consecutive days. The NSAID-induced gastropathy was evaluated on gastroscopy and scored using the modified Lanza scale 7 days before drug administration and 5, 14, 28 days after initiation of drug administration. Another 6 dogs were used to measure the plasma aspirin concentration by high-performance liquid chromatography (HPLC) at 1, 2, 4, 6, 8, 12,16, 24 hr after aspirin or lyophilized aspirin/trehalose administration.

The gastric lesion scores of the aspirin group rapidly increased after administration on day 5 and maintained high on day 14, while that of the lyophilized aspirin/trehalose group was significantly lower. The scores of the aspirin group slightly decreased from day 14 to day 28, and this decrease may be due to the adaptation effect of long-term aspirin administration. Although there was no significant difference in scores between the two groups on day 28, the aspirin group attained a higher lesion score than the lyophilized aspirin/trehalose group. In addition, the plasma aspirin level increased rapidly after administration and peaked 2 hr after administration in both groups. The mean peak plasma aspirin concentration and the area under curve showed no significant difference between both groups. These results indicated that lyophilized aspirin/trehalose induced less gastric ulceration than aspirin alone while maintaining therapeutic concentrations of plasma aspirin -.

In conclusion, lyophilized aspirin/trehalose could reduce gastric mucosal injuries mainly through the inhibition of apoptosis while maintaining the anti-inflammatory effect. These effects were proved both *in vitro* and *in vivo*, and also appear in a canine model. These results indicated that lyophilized aspirin/trehalose might be a solution to decrease aspirin-induced gastropathy, and encouraged the clinical application, both for medical and veterinary medicine.