Amino Acids Suppress Apoptosis Induced by Sodium Laurate, An Absorption Enhancer

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ABSTRACT: The formulation containing sodium laurate (C12), an absorption enhancer, and several amino acids such as taurine (Tau) and L-glutamine (L-Gln) is a promising preparation that can safely improve the intestinal absorption of poorly absorbable drugs. The safety for intestinal mucosa is achieved because the amino acids prevent C12 from causing mucosal damages via several mechanisms. In the present study, the possible involvement of apoptosis, programmed cell death, in mucosal damages caused by C12 and cytoprotection by amino acids was examined. C12 induced DNA fragmentation, a typical phenomenon of apoptosis, in rat large-intestinal epithelial cells while the addition of amino acids significantly attenuated it. C12 alone significantly increased the release of cytochrome *C*, an apoptosis-inducing factor, from mitochondria, which could be via the decrease in the level of Bcl-2, an inhibiting factor of cytochrome *C* release. The enhancement of cytochrome *C* release by C12 led to the activation of caspase 9, an initiator enzyme, and the subsequent activation of caspase 3, an effector enzyme. On the other hand, Tau or L-Gln significantly suppressed the release of cytochrome *C* from mitochondria and attenuated the activities of both caspases, which could be attributed to the maintenance of Bcl-2 expression. J Pharm Sci 98:4629–4638, 2009

Keywords: sodium laurate; absorption enhancer; amino acids; apoptosis; cytochrome C; caspase; intestinal absorption; toxicity; intestinal epithelia; excipients

INTRODUCTION

The improvement of bioavailability of poorly absorbable drugs by using absorption enhancers has been very attractive. Many studies have been devoted to discover and/or develop the compounds and/or formulations that would enhance the mucosal absorption of drugs,[1–11] but their practical use has been very limited because of the potential local toxicity.[1,2] Therefore, it has been really desired to develop the preparation that would be safe enough to be practically applied. We have already reported that the formulation containing sodium laurate (C12), an absorption enhancer, and a given amino acid such as taurine (Tau) or L-glutamine (L-Gln), would be a promising safe preparation that is able to improve the absorption of phenol red, a poorly absorbable dye, and rebamipide, classified into biopharmaceutical classification system class IV, from the large intestine and/or rectum.[3,12–15] The amino acids protected the intestinal epithelial cells

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from the local toxicity caused by C12, while the preparation maintained the enhancement of drug absorption by C12. Although the mechanisms behind the local toxicity by C12 and cytoprotective effect by these amino acids are not fully understood yet, it has been clarified that C12 increased intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and induced the release of histamine, and that several amino acids suppressed $[Ca^{2+}]_i$ and release of histamine.[12] The involvement of induced heat shock protein 70 (HSP70) in the cytoprotective action was also confirmed in the case of L-Gln.[12] Furthermore, we examined the mechanisms by which C12 increased and these amino acids decreased $[Ca^{2+}]_i$, because high level of $[Ca^{2+}]_i$ is well known to trigger off the cytotoxicity.[16–18] C12 was found to increase $[Ca^{2+}]_i$ due to the influx of extracellular Ca^{2+} through Ca^{2+} channel, and the release of Ca^{2+} from the endoplasmic reticulum.[19] Amino acids such as Tau and L-Gln decreased $[Ca^{2+}]_i$ by the activation of plasma membrane Ca^{2+}-ATPase and the enhancement of mitochondrial uptake of Ca^{2+}.[19] Mitochondria is well known to be one of the internal Ca^{2+} store,[20,21] and the two amino acids, especially Tau, would decrease $[Ca^{2+}]_i$ by enhancing the mitochondrial uptake of Ca^{2+}.[19] On the other hand, the entry of Ca^{2+} into mitochondria beyond its capacity would result in mitochondrial disruption and apoptosis, even though mitochondria can function as buffering and/or decreasing mechanism.[22,23] C12 alone also significantly increased the mitochondrial uptake of Ca^{2+}, but the increase did not lead to the reduction of $[Ca^{2+}]_i$ at all, and rather resulted in the cell damage that was evidenced by the enhanced elution of proteins.[19] It was reported that Tau enhanced the mitochondrial uptake of Ca^{2+} via uniport system (mCU)[24] and mitochondrial Ca^{2+}-ATPase,[25] and this enhancement is thought to lead to cytoprotection by enhancing the total buffering capacity of cells under Ca^{2+}-overloaded condition.[25] The excessive increase in $[Ca^{2+}]_i$ induces apoptosis[22,23,26,27] and mitochondria is a key organelle for one of the two major pathways inducing apoptosis.[26–28]

Apoptosis, programmed cell death, is a physiological process that occurs in normal turnover of cells, especially in actively proliferating cells such as epithelial cells of gastrointestinal tract.[29,30] Apoptosis is also induced in certain pathological states such as infarction, neurodegenerative diseases and viral or chemical toxicity. The process of apoptosis involves a cascade of biochemical events, leading finally to characteristic changes in nuclear morphology and DNA fragmentation.[26] It has been reported that a short-chain fatty acid, butyrate, induces apoptosis accompanied with DNA fragmentation in Caco-2 cells[31] and that oleic acid induces apoptosis in HeLa cells,[32] suggesting that C12, a sodium salt of medium chain fatty acid, might also induce apoptosis.

In this study, therefore, the possible involvement of apoptosis in the cell damage caused by C12 alone and the cytoprotective action by amino acids such as Tau and L-Gln were examined by utilizing the epithelial cells of rat large intestine.

MATERIALS AND METHODS

Materials

C12, L-Gln, L-arginine (L-Arg) and Tau were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). Ribonuclease A, proteinase K, dithiothreitol (DTT), phenylmethanesulfonyl fluoride (PMSF), 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), rotenone, oligomycin, leupeptin, aprotinin, phenylarsine oxide (PAO) and 7-amino-4-methylcoumarin (AMC) were purchased from Sigma Chemical Co. (St. Louis, MO). Acetyl-Asp-Glu-Val-Asp-(4-methylcoumarinyl-7-amine) (MCA) and acetyl-Leu-Glu-His-Asp-MCA were purchased from Peptide Institute, Inc. (Minoo, Japan). Agarose was obtained from Cambrex (North Brunswick, NJ). All other reagents were analytical grade commercial products.

Animals

Male Wistar rats weighing about 200 g (Japan SLC, Hamamatsu, Japan) were used throughout the present study. Our investigations were performed after approval by our local Ethical Committee at Okayama University and in accordance with *Principles of Laboratory Animal Care (NIH publication #85-23)*.

Treatment of Rat Large Intestine with C12 and Amino Acids

In situ closed loop study was performed to treat the epithelial cells of rat large intestine, a colon segment, with 10 mM C12 in the presence or absence of 10 mM each amino acid for 90 min as in the case of in situ loop absorption experiments.[3,12]