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Protective effect of α-mangostin against iodixanol-induced apoptotic damage in LLC-PK1 cells

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ABSTRACT

Radiographic contrast media facilitate the visibility of internal body structures, but its use to patients with lowered renal function needs to be careful because of severe side effect in kidney. The present study aims to evaluate potential protective effect and mechanism of Alpha mangostin (α-mangostin) against contrast-induced apoptotic damage in LLC-PK1 cells. As a result, α-mangostin in non-toxic concentrations improved the viability of the iodixanol-treated cells up to 90.42% against contrast-induced damage in LLC-PK1 cells. Iodixanol treatment increased the phosphorylation of p38, ERK and cleavage of caspase-3 in LLC-PK1 cells, which were significantly decreased by co-treatment with α-mangostin (2.5 and 5 μM). The protective effect of α-mangostin on contrast-induced apoptotic damage was mediated by the inhibition of MAPKs and caspase activation.

Radiographic contrast media have been prescribed to facilitate the visibility of internal body structures in X-ray-based imaging techniques such as computed tomography and radiography.[1] It is commonly classified as iodine or barium compounds, and iodixanol used in the present study is a nonionic hydrophilic compound.[1] Injection of iodinated radiographic contrast media is generally considered safe, but intravascular administration of radiographic contrast media is the third most common cause of hospital-acquired acute kidney injury.[2] When radiographic contrast media are injected intravenously or intra-arterially, they reported to induce acute renal failure within 72 h in patients with chronic kidney disease and diabetes.[1,3] Therefore, radiographic contrast-induced acute kidney injury has become one of the significant source of hospital morbidity and mortality.[2]

The mechanisms of radiographic contrast-induced acute kidney injury have not yet fully elucidated, but the several factors have been known to cause renal hemodynamics, tubular epithelial cell toxicity by disruption of cell integrity, oxygen radical generation, apoptosis, intratubular obstruction, and hemoglobin oxygen saturation curve shifts.[2,4] The reasons of these adverse events were likely to be multifactorial.

Based on these potential mechanisms, several natural substances were previously studied for the treatment to radiographic contrast-induced acute kidney injury. Earlier studies shown that propolis,[5] lycopene,[6] curcumin,[7] dongchongxiacao,[8] phyllanthus emblica[9] and *N*-acetylcysteine[10] can prevent radiographic contrast-induced damage. Interestingly, they all have strong antioxidant capacity.

Xanthones are phytochemicals which exhibit a variety of biological activities. Over 68 xanthone-type compounds have identified from mangosteen (*Garcinia mangostana* Linn).[11] Of these, alpha mangostin (α-mangostin, Fig. 1A)), beta-mangostin, gamma-mangostin, garcinone E, and gartanin are the most abundant and most frequently studied.[11] α-Mangostin not only inhibits fatty acid synthase[12] and adipocytes differentiation in 3T3-L1 cells,[12,13] but also regulates hepatic steatosis and obesity through SirT1-AMPK and PPARγ pathways in high-fat diet-induced obese mice.[14] α-Mangostin induced apoptosis in breast cancer cells[12] and inhibits cell invasion and migration in mammary,[15] human pancreatic,[16] prostate[17] and skin cancer cells[18] as well.

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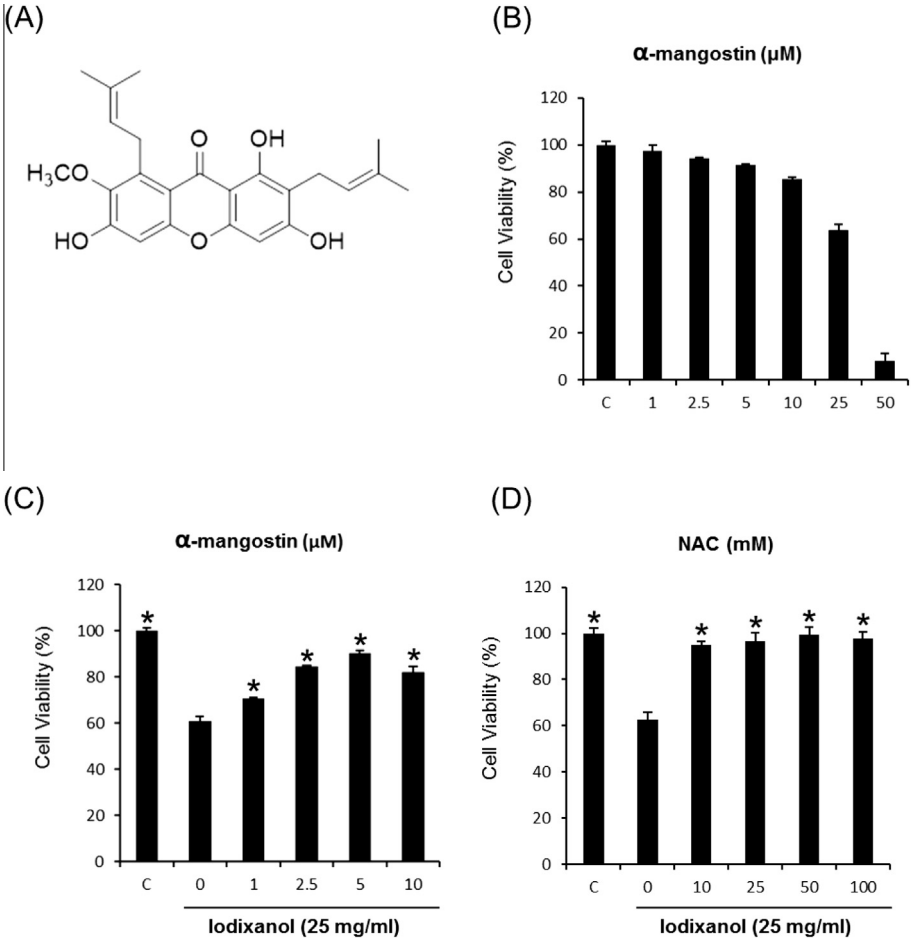

Figure 1. Effect of α-mangostin on the iodixanol-induced damage in LLC-PK1 cells. (A) Structure of α-mangostin. (B) Cytotoxic effect of α-mangostin on cell viability in LLC-PK1 cells. (C) Protective effect of α-mangostin against iodixanol-induced damage in LLC-PK1 cells. (D) Protective effect of *N*-acetyl cysteine against iodixanol-induced damage in LLC-PK1 cells. NAC, *N*-acetyl cysteine. $^{*}p$ <0.05 compared to the iodixanol-treated value.

Among the several efficacies of α-mangostin, it suppresses TNF-α, IL-6 and IL-8 expression in LPS-stimulated various cell lines,[19] and it induces cell-cycle arrest[20] and apoptosis via the mitochondrial pathway[20,20,21] in various types of human cancer cells. In addition, it has antioxidative effect by reducing ROS formation.[22] In light of these anti-inflammatory and antioxidative effects, we speculated that α-mangostin might be effective for the radiographic contrast-induced acute kidney injury.

To evaluate the cytotoxic dose of α-mangostin, we treated with various concentration of α-mangostin in LLC-PK1 cells. α-Mangostin was purified (>98.0% of purity) at the College of Pharmacy, Dongguk University (Seoul, South Korea) according to the previously reported protocol.[23] As a result, α-mangostin has no cytotoxic effect whose concentration was below 10 μM (Fig. 1B), and we used these concentrations of α-mangostin in the following experiments. Reduced cell viability by 25 mg/mL iodixanol was recovered by the co-treatment with α-mangostin in a dose-dependent manner (Fig. 1C). Particularly, the reduced LLC-PK1 cell viability by iodixanol was recovered up to 90.42% after co-treatment with 5 μM α-mangostin, and it was stronger effect than that of *N*-acetyl cysteine (Fig. 1D).

Depending on the above protective effects of α-mangostin, we further verified the effect of α-mangostin on MAPKs protein expressions in the iodixanol-induced damage in LLC-PK1 cells. The phosphorylation of p38, ERK and cleavage of caspase-3 were increased markedly in LLC-PK1 cells treated with 25 mg/mL iodixanol, which were significantly decreased after co-treatment with α-mangostin (2.5, 5 μM) (Fig. 2A and B). The effect of α-mangostin on the activation caspase-3 enzyme activity was also confirmed with iodixanol-induced damage in LLC-PK1 cells. The activation of caspase-3 which supports Western blot result was also increased significantly by 25 mg/mL iodixanol treatment, whereas co-treatment of α-mangostin (2.5, 5 μM) completely inhibited iodixanol-induced caspase-3 activity to near basal levels (Fig. 2C).

To explore whether α-mangostin could decrease iodixanol-induced apoptosis in LLC-PK1 cells, we treated α-mangostin (2.5, 5 μM) to LLC-PK1 and stained it with annexin V Alexa Fluor 488 and propidium iodide. As shown in Figure 3A, the number of dead and apoptotic cells, which were stained with red or green colors, was increased by iodixanol treatment, whereas it was decreased after co-treatment with α-mangostin in a doe dependent manner (2.5, 5 μM) (Fig. 3B).

Although the fundamental mechanisms of toxic effects of radiographic contrast media in the kidney are not completely understood, several factors such as renal vasoconstriction, renal tissue damage, intrarenal hypoxia, and apoptotic tubular cells and increased in levels of inflammation and oxidative stress have been identified.[2,4,24] Our present cell viability test indicate that iodixanol is toxic to LLC-PK1 cells by causing 39% decrease in cell viability at 25 mg/mL. In addition, other in vitro studies have also suggested that iodixanol may have a toxicity to LLC-PK1 and human kidney 2 cells (HK-29) by causing 34% and 20% decrease respectively in cell viability at 100 mg/mL,[25] though the cells and used experimental conditions were different to ours. Also, ROS play an important