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In vivo anti-inflammatory and antioxidant properties of ellagitannin metabolite urolithin A

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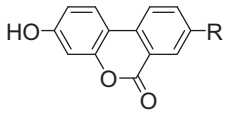
ABSTRACT

Urolithin A is a major metabolite produced by rats and humans after consumption of pomegranate juice or pure ellagitannin geraniin. In this study, we investigated the anti-inflammatory effect of urolithin A on carrageenan-induced paw edema in mice. The volume of paw edema was reduced at 1 h after oral administration of urolithin A. In addition, plasma in treated mice exhibited significant oxygen radical antioxidant capacity (ORAC) scores with high plasma levels of the unconjugated form at 1 h after oral administration of urolithin A. These results indicate strong associations among plasma urolithin A levels, the plasma ORAC scores, and anti-inflammatory effects and may help explain a mechanism by which ellagitannins confer protection against inflammatory diseases.

Ellagitannins are natural antioxidants, which are found in many medicinal plants and foods such as pomegranates, raspberries, blackberries, and walnuts.[1] Various biological studies of ellagitannins have demonstrated antioxidant,[2] antiviral,[3] antimutagenic,[4] antimicrobial,[5–7] anti-inflammatory,[8] and antitumor activities[9,10] and the absorption and metabolism of ellagitannins have recently been reported in animal and human studies. Consumption of ellagitannin-rich beverages, such as pomegranate juice, results in the production of ellagitannin metabolites, ellagic acid and 3,8-dihydroxy-6*H*-dibenzo[*b,d*]pyran-6-one (urolithin A) (Fig. 1).[11–14] Furthermore, we have isolated and characterized seven urinary and gut microbial metabolites in rats including urolithin A after the ingestion of geraniin, which is a typical ellagitannin found in *Geranium thunbergii*.[15] Urolithin A has been found to be the main metabolite in plasma after the administration of geraniin in rats[16] and pomegranate juice in humans[13] and it is the most potent antioxidant among major ellagitannin metabolites.[16]

Free radical-mediated peroxidation of membrane lipids and oxidative damage of DNA are involved in a variety of pathological complications such as cancer, atherosclerosis, and neurodegenerative diseases. Because of their antioxidant activity, ellagitannins may play a vital role in protecting against these oxidative stress-mediated pathological conditions. We previously reported that urolithin A exhibited more potent antioxidant activity than intact ellagitannins, as indicated by oxygen radical absorbance capacity (ORAC) measurements, suggesting that urolithin A may be a key mediator of ellagitannin protection. In addition, because oxidative stress plays an important role in the pathogenesis of inflammation, the ability of antioxidants to scavenge reactive oxygen species (ROS) may also provide anti-inflammatory activity. Specifically, ellagic acid, an ellagitannin metabolite, has been shown to inhibit activated biomarkers of inflammation, such as tumor necrosis factor-α and interleukin (IL)-1β.[17] Recently, urolithin A has been shown to inhibit prostaglandin E2 production induced by IL-1β[18] and attenuate the effect of colonic inflammation in a colitis rat model.[19] In the present



Urolithin A: R = OH
Urolithin B: R = H

Figure 1. Chemical structures of urolithins A and B.

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study, we investigated in vivo anti-inflammatory and antioxidant properties of the ellagitannin metabolite urolithin A in a carrageenan-induced paw edema model in mice[20] and with an ORAC assay in order to clarify the possible role of ellagitannin metabolites as biological antioxidants after consumption of ellagitannins.

Carrageenan-induced inflammation is a useful model to evaluate the effect of potential anti-inflammatory agents after oral administration.[21] Paw edema was induced in the right hind paw of ICR mice by the subcutaneous injection of 1% λ-carrageenan in physiological saline (50 μL). The inflammation level was quantified by the volume of paw edema. Urolithin A prepared by chemical synthesis[15] in 0.5% carboxymethylcellulose suspension was orally administered to the mice at 1 or 6 h before carrageenan injection. The anti-inflammatory effects of urolithin A on carrageenan-induced edema in mice are summarized in Figure 2. The volume of paw edema of mice treated with urolithin A at 1 h before carrageenan injection decreased to 35%, 26%, and 34% relative to the control group after 3, 6, and 24 h of inflammatory induction, respectively (Fig. 2A). The differences in mean values of the control group were statistically significant at $p < 0.05$; however, treatment with urolithin A at 6 h before inflammatory induction by carrageenan showed no effect (Fig. 2B). The edema induced by carrageenan injection is believed to be biphasic in nature. The initial phase, beginning 1 h after carrageenan administration, is due to the release of histamine and serotonin. The second phase, occurring 2–5 h after carrageenan ingestion, is induced by the release of bradykinin, proteases, prostaglandin, and lysozyme.[22] Our data suggest that treatment with urolithin A at 1 h before inflammatory induction is effective on both phases of inflammation induced by carrageenan.

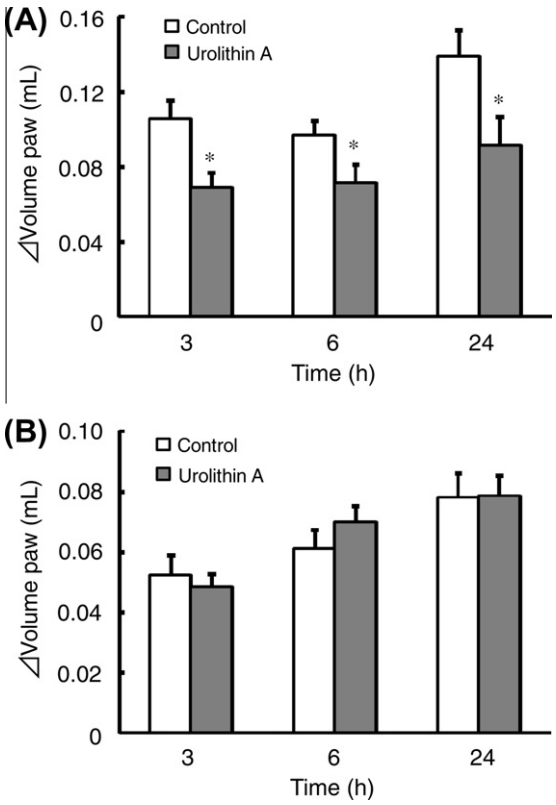

Figure 2. Anti-inflammatory effects of urolithin A on paw edema induced by carrageenan in mice at 1 (A) and 6 (B) h after oral administration. Data are expressed as means of the difference between the final and initial volumes ± SEM ($n = 10$). Mean value was significantly different from control: $^{*}p < 0.05$.

Peripheral inflammatory responses have been mechanistically linked to enhanced production of ROS, such as superoxide anion, peroxynitrite anion, hydroxyl radical, and hydrogen peroxide radical, at the inflamed site.[23] Systematic comparison studies on the antioxidant and anti-inflammatory effects of phytochemicals have recently been performed.[24–26] Natural antioxidants such as polyphenols may protect against oxidant-mediated inflammation and tissue damage by their ability to scavenge free radicals. The antioxidant capacity of urolithin A proved more potent than that of the intact ellagitannins, such as geraniin and corilagin, as measured by the ORAC assay,[16] so that urolithin A is predicted to directly contribute to suppression of carrageenan-induced inflammation after oral administration. The ORAC method is based on the inhibition of peroxyl radical-induced oxidation and has the advantage of utilizing a biologically relevant radical source.[27,28]

We investigated the association between the plasma ORAC scores and plasma levels after oral administration of urolithin A in mice. Mouse plasma samples collected at 1 and 6 h after administration were employed for the ORAC assay[29] and estimation of plasma urolithin A levels.[30] The ORAC scores were increased to 142% in plasma of mice at 1 h after administration compared to those of control plasma samples obtained before administration (Fig. 3). The scores were reduced to 118% of the control scores at 6 h.

Plasma levels of urolithin A analyzed by the HPLC-ESI-MS/MS method are shown in Table 1. Total urolithin A levels reached 3.9 μM at 1 h after ingestion and decreased to 1.3 μM at 6 h. On the other hand, the related metabolite, 3-hydroxy-6*H*-dibenzo[*b*,*d*]pyran-6-one (urolithin B) (Fig. 1), which may be a gut microbial metabolite derived from urolithin A in mammals,[31] could not be detected in any plasma samples. We recently demonstrated that urolithin A plasma levels in rats reached a maximum of 0.45 μM at 6 h after ingestion of 5 mg/head of ellagitannin geraniin.[16] Furthermore, Seeram et al. reported that plasma levels of urolithin A in humans reached 0.04 and 0.11 μM at 0.5 and 6 h, respectively, after consumption of pomegranate juice (180 mL

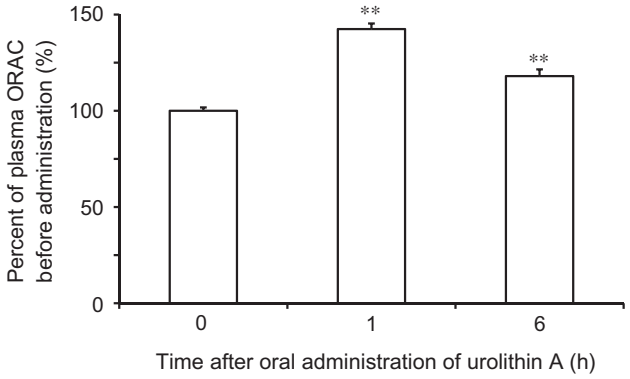

Figure 3. Plasma Oxygen Radical Absorbance Capacity (ORAC) scores after urolithin A intake by mice. Data are expressed as means ± SEM ($n = 7–10$). Mean value was significantly different from the value at 0 h: $^{**}p < 0.01$.

Table 1
Plasma levels of total or free urolithin A treated with or without β-glucuronidase[a]

Time (h)	Total urolithin A (μM)	Free urolithin A (μM)	Percentage of free urolithin A[b] (%)
1	3.88 ± 0.25	2.85 ± 0.32	77.2 ± 10.9
6	1.27 ± 0.06	0.83 ± 0.05	65.7 ± 4.4

[a] Data are expressed as means ± SEM ($n = 5–10$).
[b] Free urolithin A/total urolithin A × 100.