Potential of the ellagic acid-derived gut microbiota metabolite – Urolithin A in gastrointestinal protection

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Abstract

Urolithin A (UA) is a metabolic compound generated during the biotransformation of ellagitannins by the intestinal bacteria. The physiologically relevant micromolar concentrations of UA, achieved in the plasma and gastrointestinal tract (GI) after consumption of its dietary precursors, have been revealed to offer GI protection. The health benefit has been demonstrated to be principally related to anticancer and anti-inflammatory effects. UA has been shown to possess the capability to regulate multiple tumor and inflammatory signaling pathways and to modulate enzyme activity, including those involved in carcinogen biotransformation and antioxidant defense. The purpose of this review is to gather evidence from both in vitro and in vivo studies showing the potential of UA in GI protection alongside suggested mechanisms by which UA can protect against cancer and inflammatory diseases of the digestive tract. The data presented herein, covering both studies on the pure compound and in vivo generated UA form its natural precursor, support the potential of this metabolite in treatment interventions against GI ailments.

Key words: Urolithin A; Colonic metabolite; Gut microbiota; Colorectal cancer; Inflammatory bowel diseases; Hepatocellular carcinoma; Pancreatic ductal adenocarcinoma; Barrett’s esophagus; Ellagitannins

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Core tip: Urolithin A (UA) - an ellagitannin metabolite generated by the intestinal bacteria at physiologically relevant micromolar concentrations, offers gastrointestinal (GI) protection. The health benefit is principally related to anticancer and anti-inflammatory effects. UA can regulate multiple tumor and inflammatory signaling pathways and enzyme activities, including those involved in carcinogen biotransformation and antioxidant defense. The presented herein data support the potential of both pure compound and in vivo generated UA in treatment interventions against GI ailments.
INTRODUCTION

The human gastrointestinal tract (GI) is colonized by a large number of microbes, which is ten times higher than the total number of human cells\(^1\). The intestinal microbiota, being an integral part of the host system, has emerged as a key modulator of health and disease risk. Indeed, microbes that reside in the human gut form a co-metabolism structure with the host to participate in various metabolic processes, including biotransformation of food substances\(^2\). There is increasing evidence of the involvement of gut microbiota on the health-beneficial effects of food\(^3\). Given this, microbial metabolites have recently been profoundly studied in phytopharmacology as potential sources of novel therapeutics. It has been demonstrated that phytochemicals, which are poorly absorbed in the intestine, undergo microbial mediated biotransformation there, such as ring cleavage, demethylation, dihydroxylation, deglycosylation, etc. The generated metabolites can be absorbed and distributed into various tissues, which often correlates with lowered disease risk\(^4\). Ellagitannins (ETs), a class of hydrolyzable tannins found mainly in pomegranate, berries and nuts, are hydrolyzed spontaneously into ellagic acid (EA) during digestion in the upper GI tract. EA can be transformed by the gut microbiota to urolithins D, C, A and B in the intestine and then transported into blood circulation through intestinal epithelial cells as their lipophilicity increases\(^5\). Absorbed urolithins, which reach micromolar concentrations in the bloodstream, undergo phase I and II metabolism including methylation, glucuronidation, and sulfation\(^6\). The presence of urolithins, including urolithins A, in peripheral plasma and their glucuronides and methyl glucuronides in bile after the administration of ETs to pigs, confirms a very active enterohepatic circulation\(^7\). A regular intake of ET-rich food has been reported to enable the achievement of physiologically relevant concentrations of urolithins and their phase II derivatives in plasma. However, it has been found that their concentration in plasma after consumption of dietary precursors varies considerably between individuals\(^8,9\). In addition, significant differences among subjects in the ability to produce the final urolithins and the health benefits associated with consumption of ET-containing food products have been reported due to the variable compositions of their gut microbiota. In respect to the microbial metabolism, individuals are stratified into three urolithin metabotypes: metabotype A – individuals producing only urolithin A (UA, 25%-80% of the volunteers), metabotype B, yielding isourolithin A and/or urolithin B in addition to UA (10%-50%), and metabotype 0, which is not able to produce any urolithin (5%-25%). Remarkably, metabotype B has been reported to be associated with gut microbial dysbiosis and colorectal cancer (CRC) in patients (reviewed in\(^10\)). Three urolithin-producing bacteria from the human gut belonging to the genus Gordonibacter (G. pamelaeae and G. urolithinifaciens) and Ellagibacter isourolithinifaciens have been identified to correlate with metabotype A positively\(^11\). Recently, Gaya et al\(^11\) have reported that also Bifidobacterium pseudocatenulatum strain can produce UA.

UA (3,8-dihydroxy-dibenzo[\(c,d\)]pyranone), including its glucuronide derivative, has been found to be the predominant isomeric form of urolithins found in the plasma and urine following the consumption of ETs (reviewed in\(^10\)). It has been demonstrated that this metabolite is also accumulated in organs. The presence of UA in rodent colon, intestinal, prostate and even brain tissues has been reported, whereas UA glucuronide was primarily detected in liver and kidney tissues\(^5,12-14\). UA has been demonstrated both in in vitro and in vivo experiments to possess a broad spectrum of bioactivities, including antioxidative, anti-inflammatory, antiproliferative and apoptosis-inducing that might contribute to potential cancer chemoprotection (reviewed in\(^10\)). Since bioavailability of urolithins and their anti-inflammatory, antioxidant and antiproliferative properties are higher relative to parent compounds, the biological activity of EA and ETs including their chemopreventive and chemotherapeutic effects, have been suggested to be mediated by these metabolites\(^10\).

The purpose of this review is to gather evidence from in vitro and in vivo and clinical studies showing the potential of UA in GI protection alongside suggested
mechanisms by which UA can protect against cancer and inflammatory diseases of the digestive tract.

**INTESTINE**

Given the location of UA generation, it can be expected that UA exerts its effects within the intestinal tract and intestinal walls. This may include inhibitory effects on CRC and suppressive effects on inflammatory bowel diseases (IBDs)\(^\text{[17]}\). Results of human and animal studies and an experiment performed in the combined SHIME/Caco-2 cell system have indicated that UA can occur along the GI tract, from the duodenum to the rectum, upon long-term ETs consumption due to enterohepatic circulation\(^\text{[10,19]}\). Moreover, free availability of UA in inflammatory microenvironmental sites due to tissue deconjugation, especially within the intestinal tract, demonstrated in a systemic inflammation rat model, supports its beneficial effects on IBDs or in colon cancers\(^\text{[18]}\). Based on these findings, it is likely that UA provides significant protection against common intestinal pathologies.

**CRC**

Numerous studies have given evidence of the beneficial effects of this metabolite against CRC, which is the third most commonly diagnosed malignancy and the second leading cause of cancer death worldwide\(^\text{[20]}\). Most of these reports documenting chemopreventive effects are based on in vitro studies. UA has been demonstrated to cause a dose-dependent\(^\text{[21-24]}\), a time-dependent\(^\text{[25,26]}\) and both a concentration- and time-dependent proliferation decrease of CRC cell lines\(^\text{[27,28]}\). Moreover, it has been revealed that a mixture containing mostly UA (85%) and its precursors - urolithin C and EA, at concentrations detected in colon tissues of individuals with metabotype A following the intake of ET-rich food, exerted inhibitory activity against colon cancer stem cells (CSCs), which are considered to be involved in the control of cancer metastasis and the acquisition of chemoresistance\(^\text{[29]}\). The inhibition of cell proliferation was accompanied by cell cycle arrest in the G2/M and S stages\(^\text{[26-28]}\), and in the G2/M phase, followed by induction of apoptosis\(^\text{[21-23]}\). Since the delay in the G2/M transition has been reported to be a hallmark of topoisomerase II inhibition\(^\text{[25]}\), potent catalytic inhibition of this human enzyme by UA could be suggested. However, competition tests performed for submicromolar concentrations did not confirm this property of UA, which was inactive up to 5 μmol/L\(^\text{[30]}\). Several reports indicate that UA-induced apoptosis is associated with increased caspases activity\(^\text{[22,23]}\). More recent evidence\(^\text{[26]}\) showing a dose-dependent anti-clonogenic effect of long-term exposure to UA in CRC cell lines, indicates that a decrease in the colony formation is exerted through the senescence induction via the p53/p21 pathway, rather than by cell cycle arrest or apoptosis, which required much higher concentrations\(^\text{[24]}\). These data are consistent with previous results\(^\text{[32]}\), also suggesting that the antiproliferative effect of long-term exposure to UA in a colon cancer cell line was mediated through the p53/p21-dependent senescence-like growth arrest. This action was synergistic with the standard chemotherapeutic drug oxaliplatin\(^\text{[31]}\). González-Sarrías et al\(^\text{[33]}\) have also demonstrated that UA at concentrations achievable in the human colorectum, can potentiate the anticancer effects of 5-fluorouracil (5-FU) on human colon cancer cells. Interestingly, unchanged tannins such as proanthocyanidins extracted from grape seeds, have also been demonstrated to exert anti-tumorigenic effects on colon cancer cells through inhibition of cellular proliferation\(^\text{[34,35]}\), induction of apoptosis and cell cycle arrest, inhibition of the formation of spheroid derived stem-like colon cancer cells\(^\text{[35]}\) as well as by enhancing the impact of 5-FU chemotherapy. However, the activity of PCs has been limited to the proximal jejunum, due to its degradation in the distal region of the small intestine\(^\text{[36]}\). It has now been suggested that the anticancer effects of UA may also result from autophagy induction since, at concentrations found in the intestine after dietary polyphenols’ consumption, it triggered autophagy, and as a result, inhibited CRC cell growth and metastasis\(^\text{[36]}\). Kojadinovic et al\(^\text{[37]}\) have demonstrated that both long- and short-term exposure of colorectal adenocarcinoma Caco-2 cells to a mixture of UA and UB, at concentrations reached in the lumen of the gut, contributed to the reduction of oxidative stress, thus preventing the damage caused by reactive oxygen species. The colon cancer chemopreventive property of UA has been supported through the inhibition of CYP1 enzymes involved in the metabolic activation of dietary carcinogens\(^\text{[23,27]}\). On the other hand, González-Sarrías et al\(^\text{[33]}\) have revealed the induction of CYP1A1 gene expression and activity in the Caco-2 cells after exposure to UA, suggesting that this could reflect the mobilization of detoxification mechanisms. The CYP1A1 induction was accompanied by increased...
mRNA expression of an enzyme catalyzing the glucuronidation reaction, which is generally considered to facilitate detoxification of potential colon carcinogens[43].

Although there are numerous in vitro studies supporting UA as a CRC chemopreventive agent, there is a lack of clinical evidence in this area. The only study by Nuñez-Sánchez et al[38] has revealed that the consumption of an ET-containing pomegranate extract counterbalanced the expression of several CRC-related genes in cancerous colon tissues; however, this effect was not associated with the UA level in these tissues.

Data on the chemopreventive activities of UA against CRC are presented in Table 1.

**IBDs**

Chronic inflammation is regarded to be involved in approximately 20% of all human cancers. The tight link between long-standing inflammation of the GI tract and carcinogenesis is most apparent in CRC. IBDs, and particularly ulcerative colitis, are thought to be at increased risk for CRC. It is supported by the results of epidemiological studies indicating that IBDs and CRC have a similar prevalence worldwide[25]. The anti-inflammatory effects of UA, which has been demonstrated in lipopolysaccharide (LPS)-induced inflammation in RAW 264.7 murine macrophages[49], may be especially relevant in the intestine, where it can reach considerable micromolar concentrations through a diet rich in ETs. Giménez-Bastida et al[41] have demonstrated that UA can contribute to the prevention of intestinal inflammations also in colon fibroblasts - cells playing a pivotal role in the inflammatory response since the increased permeability of the tight junctions then allows for direct contact of luminal content with the cells resident in the lamina propria. UA at a concentration that is achieved in the colon through the diet has been found to inhibit two critical processes of the intestinal inflammatory response: fibroblast migration and monocyte adhesion that was accompanied by the downregulated expression of their modulatory factors. The effects were due to the attenuation of inflammatory signaling and the upregulation of the tumor suppressor genes[42]. Of note, procyanidins have been reported to decrease the severity of selected markers of DSS-induced colitis; however, only in the distal ileum and proximal colon. The declined bioactivity of procyanidins in the large bowel has been suggested to be due to their degradation by endogenous microflora[49]. In addition, UA has been shown to modulate the gut microbiota composition favorably in DSS-treated rats, and the authors of the experiment have suggested the prevention of dysbiosis as an additional protective mechanism of UA against intestinal inflammatory diseases[42]. The importance of functional dynamics of gut microbiota and their metabolites has recently become the object of studies, including those concerning intestine health[42,44,45]. The black raspberries diet is also a source of UA that has been recently become the object of studies, including those concerning intestine health[41,44,45].

In a dextran sodium sulfate (DSS)-induced colitis rat model, pre- and co-treatment with UA mitigated systemic inflammation and colitis effects against intestinal pathogens[40]. Giménez-Bastida et al[41] have demonstrated that UA can contribute to the prevention of intestinal inflammations also in colon fibroblasts - cells playing a pivotal role in the inflammatory response since the increased permeability of the tight junctions then allows for direct contact of luminal content with the cells resident in the lamina propria. UA at a concentration that is achieved in the colon through the diet has been found to inhibit two critical processes of the intestinal inflammatory response: fibroblast migration and monocyte adhesion that was accompanied by the downregulated expression of their modulatory factors. The reduction of the colon fibroblast migration, mediated by UA, might protect against excessive fibrosis as mechanisms controlling wound healing become disordered in IBDs. Overall, UA contributed to the prevention against the detrimental effect of inflammation on the cells’ viability, which was slightly improved following exposure to the metabolite[41]. In a dextran sodium sulfate (DSS)-induced colitis rat model, pre- and co-treatment with UA attenuated the severity of colon injury as preserved mucosal architecture and a decreased epithelial cell loss have been observed, which was accompanied with improved hematological parameters. The effects were due to the attenuation of inflammatory signaling and the upregulation of the tumor suppressor genes[42]. Of note, procyanidins have been reported to decrease the severity of selected markers of DSS-induced colitis; however, only in the distal ileum and proximal colon. The declined bioactivity of procyanidins in the large bowel has been suggested to be due to their degradation by endogenous microflora[49]. In addition, UA has been shown to modulate the gut microbiota composition favorably in DSS-treated rats, and the authors of the experiment have suggested the prevention of dysbiosis as an additional protective mechanism of UA against intestinal inflammatory diseases[42]. The importance of functional dynamics of gut microbiota and their metabolites has recently become the object of studies, including those concerning intestine health[42,44,45]. The black raspberries diet is also a source of UA that has been recently become the object of studies, including those concerning intestine health[41,44,45].
| Cell line | Mechanism | Ref. |
|-----------|-----------|-----|
| SW620     | ↓Proliferation, MMP-9 activity, Autophagy, LC3, G2/M arrest, Apoptosis, necrosis | Zhao et al [21] |
| HT-29     | ↓Proliferation, MMP-9 activity, Autophagy, LC3, G2/M arrest, Apoptosis, necrosis | Cho et al [22] |
| HT-29     | ↓Proliferation, Apoptosis, necrosis, G2/M arrest, p21 expression, MMP, caspase 8 and 9 activity | Kasimsetty et al [23] |
| HT-29     | ↓Proliferation, G0/G1, G2/M, G0, CYPI activity, Apoptosis, caspase-3 like activity | Tortora et al [24] |
| Caco-2, HT-29, SW480 | ↓Proliferation, G2/M arrest (SW480, Caco-2), G2/M arrest, Gene expression in Caco-2: ABCG2, TP53, c-MYC, CDKN1A, BIRC5, Gene expression in SW480: c-MYC, CDKN1A, BIRC5, CASP3, EGFR, Gene expression in HT-29: CDKN1A, S arrest (SW480, Caco-2), G2/M arrest | González-Sarrías et al [25] |
| HCT-116, Caco-2, HT-29 | ↓Colonies, G2/M arrest (Caco-2, HCT-116), Senescence-associated β–galactosidase activity (HCT-116), p53 and p21 expression (HCT-116) | Giménez-Bastida et al [26] |
| CSCs (Caco-2 and primary human colon tumor cells) | ↓Colonospheres number, ALDH<sup>hi</sup> cells, Primary human colon tumor cells: Colonospheres number, Colonospheres size, ALDH<sup>hi</sup> cells, Cell growth, p53, p21, TIGAR expression, 5-FU- and 5'-DFCR-induced cytotoxicity G2/M arrest (Caco-2, SW480), Caspase 9 activity (Caco-2) | Núñez-Sánchez et al [27] |
| HCT116 | ↓ROS, CAT activity | Norden et al [28] |
| Caco-2, HT-29, SW480 | ↓Proliferation, 5-FU- and 5'-DFCR-induced cytotoxicity, G2/M arrest (Caco-2, SW480), Caspase 9 activity (Caco-2) | González-Sarrías et al [29] |
| Caco-2 | ↓NS cell vitality, ROS, CAT activity | Kojadinovic et al [30] |
| Caco-2 | ↓Proliferation | González-Sarrías et al [31] |
Kinase signaling pathway gene expression:
- ↓FGFR2, ↓DUSP6, c-MYC, Fox, CD44
- ↑CD44, CTNNB1 CDKN1A, EGFR, TYMS

Human tissue samples / pomegranate extract

1mixture containing urolithin A, 5-DFCR; 5-deoxy-5-fluorocytidine; 5DFUR; 5-deoxy-5-fluorouridine; ALDH: Aldehyde dehydrogenase; CAT: Catalase; CYP450: Cytochrome P450; GPx: Glutathione peroxidase; LC3: GFP-microtubule-associated protein 1 light chain 3; MMP-9: Matrix metalloproteinase-9; NS: Non significant change; ROS: Reactive oxygen species; SOD: Superoxide dismutase; TIGAR: TP53 induced glycolysis regulatory phosphatase.

signaling such as Toll-like receptor activation or cytokine release in the tumor microenvironment. Thus, antagonizing AhR by UA has been found to be beneficial to maintain balanced AhR activity for overall homeostasis and to prevent pathological conditions correlating with elevated AhR activity such as cancer[47]. Data on the anti-inflammatory activity of UA relevant to the intestine are presented in Table 2.

LIVER

According to the “World Cancer Report 2014”[48] published by the International Agency for Research on Cancer, liver cancer is the second largest cause of cancer death. The most common primary liver malignancy is hepatocellular carcinoma (HCC) which develops under the influence of chronic inflammation and oxidative stress[49]. Given the antioxidant, anti-inflammatory and antiproliferative activity of UA and its active enterohepatic circulation the chemopreventive potential of UA has also been studied with regard to hepatoprotection. In hepatic carcinoma cell lines, physiologically achievable micromolar concentrations of UA were sufficient to inhibit cell proliferation and induce cytotoxicity in a dose-dependent manner[50,51]. UA suppressed cell proliferation via caspase-3 dependent apoptosis[50,51] accompanied by down-regulation of the Bcl-2/Bax ratio. Moreover, Wang and co-workers[50] have shown that the antiproliferative effect of UA on HepG2 cells was due to the synergistic action of p38-MAPK activation and suppression of Wnt/β-catenin signaling. UA decreased protein expression of β-catenin and its downstream transcriptional factor c-Myc and Cyclin D1 thereby promoting p53 protein expression, which also resulted from a direct induction of p38-MAPK phosphorylation by UA. The capability of UA to affect Wnt signaling was demonstrated previously by Sharma et al[52], who observed this pathway inhibition with IC50 at 9 μg/mL (39 μmol/L) in a human 293T cell line. Given the fact that over 90% of colon cancers are believed to originate from activating mutations in the Wnt pathway[53], this finding by Wang et al[50] has proven the anticancer activity of UA against CRC. Additionally, since inflammatory response plays a crucial role at all stages of tumor development and HCC is an inflammation-driven disease with up to 90% of cases occurring on the background of chronic liver inflammation[54], the anti-inflammatory role of UA in HepG2 cells by blocking the NF-κB signaling pathway and therefore suppressing the release of inflammatory mediators supports its chemopreventive potential against HCC. Furthermore, the anti-inflammatory effect was accompanied by decreased intracellular ROS production and increased level of intracellular SOD and GPx activity[50]. The antioxidant property of UA has been supported by preventing in a dose-dependent manner the oxidative injury and cytotoxicity in cultured rat primary hepatocytes[55].

The leading risk factor for the development of HCC is the hepatitis B virus (HBV) infection. Accumulated evidence attributes the HBV-encoded x protein (HBx) as a multifunctional oncoprotein in the development of HCC. Previous studies have demonstrated that HBx can upregulate the proteins of the Lin28 family, known inhibitors of let-7a, classified as tumor-suppressor miRNAs[56,57]. Qiu et al[51] have revealed the capacity of UA to act against HBx relevant cell proliferation and invasion via regulation of the Lin28a/let-7a axis. In this study, UA also inhibited the expression of let-7a downstream targets, such as the high mobility group AT-hook 2 and K-ras, through regulation of the epithelial-to-mesenchymal transition occurring in the initiation of invasion and metastasis of tumor progression[51].

OTHERS

The capability of UA to target kinases downstream of KRAS, in particular, the PI3K/AKT/mTOR signaling pathways, has been demonstrated to be a promising therapeutic approach in pancreatic ductal adenocarcinoma (PDAC) treatment. UA treatment has inhibited human PDAC cell proliferation, migration, and enhanced
Table 2  Anti-inflammatory activity of urolithin A relevant to the intestine

| Cell line/model | Mechanism | Ref. |
|-----------------|-----------|-----|
| CCD18-Co/cytokine-induced inflammation IL-1β or TNF-α | ↓IL-1β or TNF-α-stimulated migration | Giménez-Bastida et al[41] |
|               | ↓IL-1β-stimulated adhesion | |
| Fischer rats/DSS-induced colitis | ↑HCT, RBCs | Larrosa et al[42] |
|               | ↑Bifidobacteria | |
|               | ↑E. coli, enterobacteria, and total aerobic bacteria | |
|               | ↑CAT activity | |
|               | ↓NO, iNOS | |
|               | ↓PGE2, COX-2, PTGES | |
|               | ↑Rb1, p53 | |
|               | ↓CD40, IL-1β and IL-4 | |
| C57BL/6 x FVB F1/BRB intervention | ↑Shannon diversity index | Gu et al[44] |
|               | ↑Bacteroidetes, Barnesiella | |
|               | ↑Firmicutes, Clostridiurn, Turicibacter, Lactobacillus | |
| C57BL/6 mice/LPS | ↑IL-6, TNF-α level | Singh et al[45] |
|               | ↑Colonic and hepatic Cyp1A1 activity in WT not in AhR−/− mice | |
|               | ↑Colonic Cldn4, Nrf2, and NQO1 in WT not in AhR−/− and Nrf2−/−/− mice | |
|               | ↑Colonic Cldn4, Nrf2, and NQO1 in WT not in AhR−/− and Nrf2−/−/− mice | |
| HT-29, Caco-2 | ↑Cldn4, Occln, and ZO1 | Singh et al[46] |
|               | ↑Cldn4, Occln, and ZO1 | |
|               | ↑LPS-induced leakage of FITC-dextran | |
|               | ↑Cyp1A1, Cyp1A1 protein expression, and activity | |
|               | ↑AhR-reporter, nuclear translocation of AhR | |
| C57BL/6 mice/TNBS | ↑Cldn4 expression not affected in Ahr SiRNA and CYP1A1 SiRNA cells | |
|               | ↑Nrf2 levels, nuclear translocation of Nrf2 | |
|               | ↑Colonic MPO level | |
|               | ↑Serum IL-6, TNF-α, CXCL1, and IL-1β levels | |
|               | ↑Colon length, Cldn4 | |
|               | ↑Tissue damage and inflammation scores | |
| AhR-knockdown caco-2 cells/TCDD/IL-1β/TMF | ↑IL-6, CYP1A1, PTGS2 in siControl | Muku et al[47] |
|               | ↑IL-6, CYP1A1, PTGS2 in siAhR | |

AhR: Aryl hydrocarbon receptor; BMDMs: Mouse bone marrow derived macrophages; BRB: Black raspberries; CAT: Catalase; Cldn4: Claudin 4; COX-2: Cyclooxygenase 2; CXCL1: Chemokine (C-X-C motif) ligand 1; CYP: Cytochrome; EGF: Epidermal growth factor; HCT: Haematocrit; IGF: Insulin-like growth factor; IL: Interleukin; iNOS: Inducible nitric oxide synthase; LPS: Lipopolysaccharide; M-CSF: Macrophage colony-stimulating factor; MPO: Myeloperoxidase; ns: non significant change; NO: Nitric oxide; Nrf2: Nuclear factor erythroid 2-related factor 2 ; NQO1: NAD(P)H dehydrogenase [quinone] 1; Occln: Occludin; siAhR: AhR knockdown by siRNA Caco-2 cells; siControl: Caco-2 cells transfected with siControl; PAI-1: Plasminogen activator inhibitor-1; PDGF: Platelet-derived growth factor; PGE2: Prostaglandin E2; PTGES: Prostaglandin E synthase; PGF: Placental growth factor; PTGS2: Prostaglandin-endoperoxide synthase; RBCs: Red blood cells; TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin (agonist of AhR); TMF: 6,2',4'-trimethoxyflavone (AhR antagonist); TNBS: 2,4,6-Trinitrobenzenesulfonic acid; TNF-α: Tumor necrosis factor α; WT: Wild type; VEGF: Vascular endothelial growth factor; ZO-1: Zonula occludens-1.

Apoptosis by blocking the phosphorylation of AKT and p70S6K in vitro. Interestingly, UA only slightly affects pAKT and p70S6K expression in normal epithelial cell lines,
thereby suggesting its selective potential against cancer cells. The mechanism of the anticancer action of UA through the inhibition of AKT and p70S6K phosphorylation, reduction of proliferation, and enhancement of cellular apoptosis has been confirmed in both xenograft and transgenic mouse models of pancreatic cancer. Additionally, UA treatment has reprogrammed the tumor microenvironment, as evidenced by reduced levels of infiltrating immunosuppressive cell populations such as myeloid-derived suppressor cells, tumor-associated macrophages, and regulatory T-cells thus inhibiting tumor growth resulting in enhanced survival [58].

In Barrett’s esophagus (BE) patients, a 26-wk intervention with lyophilized black raspberries significantly increased GST-pi, a marker of detoxification, in BE epithelium, with over 50% of subjects responding favorably. The presence of UA-glucuronide and sulfate in the urine of the BE patients may support the involvement of UA in the observed beneficial effect on BE epithelium [59]. Data on the chemopreventive activities of UA against the above mentioned other cancers of the alimentary tract are presented in Table 3.

CONCLUSION

The above reviewed studies have revealed the multidirectional role of the gut bacterial metabolite UA in the regulation of multiple tumor and inflammatory signaling pathways and the modulation of enzyme activity, including those involved in carcinogen biotransformation and antioxidant defense. These mechanisms have been demonstrated to contribute to the alleviation of inflammation and counteraction of the growth of cancer cells in various structures and organs of GI. Based on the above, UA can be considered as a potential candidate to use as an anti-cancer or anti-inflammatory agent in treatment interventions related to GI ailments. Since UA concentrations in human serum and tissues depend on their gut microbial composition, the direct supplementation of UA would overcome the individual variation in microbiota among populations and offer health benefits.
| Cell line/model | Mechanism | Ref. |
|-----------------|-----------|-----|
| Liver | ↓Cell survival | Wang et al [50] |
| | ↓Wnt/β-catenin signalling; β-catenin | |
| | ↓c-MYC, cyclin D1, phospho-c-Jun | |
| | ↓IL-6, IL-1β, NF-κB, C/EBP-2, iNOS | |
| | ↓TP53, BAX, PI3K, p53, phosphor-p53 | |
| | ↑Caspase-3, phospho-p38 | |
| | ↑H2O2-induced ROS, MDA level | |
| | ↑SOD level, GPx activity in H2O2-induced oxidative stress | |
| HepG2.2.15 | ↓Cell proliferation | Qiu et al [51] |
| | ↑Cleaved caspase-3, Bax | |
| | ↓Lin28a, Zcchc11, Sp-1 | |
| | ↓Let-7a | |
| | ↓HMGA2, K-ras | |
| | ↑Let-7a | |
| Cultured rat hepatocytes/t-BHT | ↓Cytotoxicity | Olennikov et al [55] |
| | ↑LDH, MDA level | |
| Pancreas | | |
| The human PDAC cell lines (MIAPaCa2, PANC1, AsPC1, CFPAC1, Capan1, Capan2, SW1990, HPAC, BxPC3) | ↓Cell proliferation, migration, apoptosis | Totiger et al [58] |
| | ↑pAKT (T308), p70S6K (T421/S424), PDK1, pGSK3β, p4E-BP1 | |
| PDAC xenografts in mice | ↓Tumor volume | Totiger et al [58] |
| | ↑pAKT (T308), p70S6K (T421/S424) | |
| | ↓Ki67 | |
| | ↑Cleaved caspase-3 | |
| PKT mice | ↑Tumor weight | Totiger et al [58] |
| | ↑Survival | |
| | ↑pAKT (T308), p70S6K (T421/S424) | |
| | ↓Ki67 | |
| | ↑Cleaved caspase-3 | |
| | ↑F4/80-, Fox-P3, CD3+ positive cells | |
| Esophagus | | |
| BE patients/LBR intervention | ↑GST-pi level in BE biopsies | Kresty et al [59] |

BE: Barrett's esophagus; Bcl-2: B-cell lymphoma 2; GST: Glutathione S-transferase; HMG2: High-mobility group AT-hook 2; MDA: Malondialdehyde; t-BHT: tert-butyl hydroperoxide; LB: Lyophilized black raspberries; LDH: Lactate dehydrogenase; MMP: Matrix metalloproteinase; PDAC: Pancreatic ductal adenocarcinoma; ROS: Reactive oxygen species; SOD: Superoxide dismutase; HMGA2: High mobility group AT-hook 2.

REFERENCES

1. Cani PD. Human gut microbiome: hopes, threats and promises. Gut 2018; 67: 1716-1725 [PMID: 29934437 DOI: 10.1136/gutjnl-2018-316723]
2. Wang XQ, Zhang AH, Miao JH, Sun H, Yan GL, Wu FF, Wang XJY. Gut microbiota as important modulator of metabolism in health and disease. RSC Advances 2018; 8: 42380-42389 [DOI: 10.1039/C8RA08094A]
3. Peiróten Á, Bravo D, Landete JM. Bacterial metabolism as responsible of beneficial effects of phytoestrogens on human health. Crit Rev Food Sci Nutr 2019; 1-16 [PMID: 31161778 DOI: 10.1080/10408398.2019.1622505]
4. Dey P. Gut microbiota in phytopharmacology: A comprehensive overview of concepts, reciprocal interactions, biotransformations and mode of actions. Pharmacol Res 2019; 147: 104367 [PMID: 31344423 DOI: 10.1016/j.phrs.2019.104367]
5. Espín JC, Larrosa M, García-Conesa MT, Tomás-Barberán F. Biological significance of urolithins, the gut microbial ellagic Acid-derived metabolites: the evidence so far. Evid Based Complement Alternat Med 2013; 2013: 270418 [DOI: 10.1155/2013/270418]
6. Nuñez-Sánchez MA, García-Villalba R, Monedero-Saiz T, García-Talavera NV, Gómez-Sánchez MB, Sánchez-Alvarez C, García-Albert AM, Rodríguez-Gil FJ, Ruiz-Marín M, Pastor-Quirante FA, Martinez-
Diaz F, Yáñez-Gascón MJ, González-Sarrias A, Tomás-Barberán FA, Espin JC. Targeted metabolic profiling of pomegranate polyphenols and urolithins in plasma, urine and colon tissues from colorectal cancer patients. Mol Nutr Food Res 2014; 58: 1199-1211 [PMID: 2435260 DOI: 10.1002/mnfr.201300931]

García-Niño WR, Zaraleta C. Ellagic acid: Pharmacological activities and molecular mechanisms involved in liver protection. Pharmacol Res 2015; 97: 84-103 [PMID: 2594101 DOI: 10.1016/j.phrs.2015.04.008]

Tomás-Barberán FA, González-Sarrias A, García-Villalba R, Núñez-Sánchez MA, Selma MV, García-Conesa MT, Espin JC. Urolithins, the rescue of "old" metabolites to understand a "new" concept: Metabolites as a nexus among phenolic metabolism, microbiota dysbiosis, and host health status. Mol Nutr Food Res 2017; 61 [PMID: 27158799 DOI: 10.1002/mnfr.201500991]

Ismail T, Calcagnini C, Díaz AR, Fimognari C, Turrini E, Catanzaro E, Akhtar S, Sesíli P. Ellagitannins in Cancer Chemoprevention and Therapy. Toxins (Basel) 2016; 8 [PMID: 27187472 DOI: 10.3390/toxins8050151]

Espin JC, García-Barrón R, Cerdá B, López-Bote C, Rey AI, Tomás-Barberán FA. Iberian pig as a model to clarify obscure points in the bioavailability and metabolism of ellagitannins in humans. J Agric Food Chem 2007; 55: 10476-10485 [PMID: 17990850 DOI: 10.1021/jf0723864]

Selma MV, Beltrán D, Luna MC, Romo-Vaquero M, García-Villalba R, Mira A, Espin JC, Tomás-Barberán FA. Isolation of Human Intestinal Bacteria Capable of Producing the Bioactive Metabolite Isourolithin A from Ellagic Acid. Front Microbiol 2017; 8: 1521 [PMID: 28824607 DOI: 10.3389/fmicb.2017.01521]

Gerhauser C. Impact of dietary gut microbial metabolites on the epigenome. Philos Trans R Soc Lond B Biol Sci 2018; 373 [PMID: 2965596 DOI: 10.1098/rstb.2017.0159]

García-Mantrana I, Calatayud M, Romo-Vaquero M, Espin JC, Selma MV, Collado MC. Urolithin Metabolites Can Determine the Modulation of Gut Microbiota in Healthy Individuals by Tracking Walnuts Consumption over Three Days. Nutrients 2019; 11 [PMID: 31623169 DOI: 10.3390/nu11102484]

Gaya P, Peiró M, Medina M, Álvarez I, Landete JM. Bifidobacterium pseudocatenulatum INIA P815: the first bacterium able to produce urolithins A and B from ellagic acid. J Funct Foods 2018; 45: 95-99 [DOI: 10.1016/j.jff.2018.03.040]

Seeram NP, Zhang Y, Sartippour M, Henning SM, Lee R-P, Harris DM, Moro A, Heber D. Pharmacokinetics and tissue disposition of urolithin A, an ellagitannin-derived metabolite, in mice. FASEB J 2007; 21: A1081-A1081

Kajawowska M, Jourdes M, Kurpic M, Szulec M, Szafer H, Chmielarz P, Keiniger G, Krajka-Kuźniak V, Mikołajczak PL, Teissedre PL, Jodyns-Liebert J. Neuroprotective Effects of Pomegranate Juice against Parkinson's Disease and Presence of Ellagitannins-Derived Metabolite-Urolithin A-In the Brain. Int J Mol Sci 2019; 21 [PMID: 31821676 DOI: 10.3390/ijms21102020]

Kawahata K, Yoshio A, Terao J. Role of Intestinal Microbiota in the Bioavailability and Physiological Functions of Dietary Polyphenols. Molecules 2019; 24 [PMID: 30696935 DOI: 10.3390/molecules24020370]

García-Villalba R, Vissenackens H, Pittard J, Romo-Vaquero M, Espin JC, Grootaert C, Selma MV, Raes K, Smagghe G, Possemiers S, Van Camp J, Tomás-Barberán FA. Gastrointestinal Simulation Model TWIN-SHIME Shows Differences between Human Urolithin-Metabotypes in Gut Microbiota Composition, Pomegranate Polyphenol Metabolism, and Transport along the Intestinal Tract. J Agric Food Chem 2017; 65: 5480-5493 [PMID: 28616977 DOI: 10.1021/acs.jafc.7b02049]

Ávila-Gálvez MA, Giménez-Bastida JA, González-Sarrias A, Espin JC. Tissue conjugation of urolithin A a glucuronide to free urolithin A in systemic inflammation. Food Funct 2019; 10: 3135-3141 [PMID: 31041969 DOI: 10.1039/c9fo00298g]

World Health Organization. Colorectal cancer. Available from: http://go.in.cancer.org/today/data/factsheet/cancers/10_8_9-Colorectum-fact-sheet.pdf

Zhao W, Shi F, Guo Z, Zhao J, Song X, Yang H. Metabolite of ellagitannins, urolithin A induces autophagy and inhibits metastasis in human SW620 colorectal cancer cells. Mol Carcinog 2018; 57: 193-200 [PMID: 28976622 DOI: 10.1002/mc.22736]

Cho H, Jung H, Lee Y, Hi YC, Kwik HK, Kwang HT. Chemopreventive activity of ellagitannins and their derivatives from black raspberry seeds on HT-29 colon cancer cells. Food Funct 2015; 6: 1675-1683 [PMID: 25906041 DOI: 10.1039/c5fo0274e]

Kasimsetty SG, Bialonska D, Reddy MK, Ma G, Khan SI, Ferreira D. Colon cancer chemopreventive activities of pomegranate ellagitannins and urolithins. J Agric Food Chem 2010; 58: 2180-2187 [PMID: 20112993 DOI: 10.1021/jf903762a]

Tortora K, Femia AP, Romagnoli A, Sinoe I, Khatib M, Mulinacci N, Giovannelli L, Caderni G. Pomegranate By-Products in Colorectal Cancer Chemoprevention: Effects in Apc-Mutated Fire Rats and Mechanistic Studies In Vitro and Ex Vivo. Mol Nutr Food Res 2018; 62 [PMID: 28946904 DOI: 10.1002/mnfr.201700401]

González-Sarrias A, Azorín-Ortuño M, Yáñez-Gascón MJ, Tomás-Barberán FA, García-Conesa MT, Espin JC. Dissimilar in vitro and in vivo effects of ellagic acid and its microbiota-derived metabolites, urolithins, on the cytokchrome P450 1A1. J Agric Food Chem 2009; 57: 5623-5632 [PMID: 19469472 DOI: 10.1021/jf900726c]

González-Sarrias A, Núñez-Sánchez MA, Tomé-Carneiro J, Tomás-Barberán FA, García-Conesa MT, Espin JC. Comprehensive characterization of the effects of ellagic acid and urolithins on colorectal cancer and key-associated molecular hallmarks: MicroRNA cell specific induction of CDKN1A (p21) as a common mechanism involved. Mol Nutr Food Res 2016; 60: 701-716 [PMID: 26634414 DOI: 10.1002/mnfr.201500730]

González-Sarrias A, Giménez-Bastida JA, Núñez-Sánchez MA, Larrus M, García-Conesa MT, Tomás-Barberán FA, Espin JC. Phase-II metabolism limits the antiproliferative activity of urolithins in human colon cancer cells. Eur J Nutr 2014; 53: 853-864 [PMID: 24077694 DOI: 10.1007/s00394-013-0599-4]

Giménez-Bastida JA, Ávila-Gálvez MA, Espin JC, González-Sarrias A. The gut microbiota metabolite urolithin A, but not other relevant urolithins, induces p53-dependent cellular senescence in human colon cancer cells. Food Chem Toxicol 2020; 139: 111260 [PMID: 32179165 DOI: 10.1016/j.fct.2020.111260]

Núñez-Sánchez MA, Karmokar A, González-Sarrias A, García-Villalba R, Tomás-Barberán FA, García-Conesa MT, Brown K, Espin JC. In vivo relevant mixed urolithins and ellagic acid inhibit phenotypic and molecular colon cancer stem cell features: A new potentiality for ellagitannin metabolites against cancer. Food Chem Toxicol 2016; 92: 8-16 [PMID: 26999223 DOI: 10.1016/j.fct.2016.03.011]
Metastatic Potential of HCC via the NF-κB/miR-497/SALL4 Axis. Li Q, Liu J, Huang A, Liu X. Inflammatory Micro-environment Contributes to Stemness Properties and, Wang Y, Tan X, Ke K, Zheng X, Wang F, Lan S, Liao N, Cai Z, Shi Y, Zheng Y, Lai Y, Wang L, Zhao B.

Available from: https://www.who.int/cancer/publications/WRC_2014/en/, Wild CP. International Agency for Research on Cancer, WHO. World Cancer Report 2014, Stewart BW, Giles RH.

J Agric Food Chem 2010; 58: 3965-3969 Effects of urolithin A, the colonic metabolite of ellagic acid, on hepatocellular carcinomas HepG2 cells. Wang Y 2014; Nat Hydrocarbon Receptor Antagonist. Muku GE, Murray IA, Espín JC, Perdew GH. Urolithin A Is a Dietary Microbiota-Derived Human Aryl Hydrocarbon Receptor Antagonist. Metabolites 2018; 8 [PMID: 30501068 DOI: 10.3390/metabolites8060168] Stewart BW, Wild CP. International Agency for Research on Cancer, WHO. World Cancer Report 2014. Available from: https://www.who.int/cancer/publications/WRC_2014/en/, Singh S, Roberts CR, Sanchez W. Chemosensitization strategies in hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 2014; 11: 45-54 [PMID: 23938452] Wang Y, Qua Z, Zhou B, Liu C, Ruan J, Yan Q, Liao J, Zha F. In vivo antiproliferative and antioxidant effects of urolithin A, the colonic metabolite of ellagic acid, on hepatocellular carcinomas HepG2 cells. Toxicol In Vitro 2015; 29: 1107-1115 [PMID: 25910917 DOI: 10.1016/j.tiv.2015.04.008] Qiu Z, Zhou J, Zhang C, Cheng Y, Hu J, Zheng G. Antiproliferative effect of urolithin A, the ellagic acid-derived colonic metabolite, on hepatocellular carcinoma HepG2 cells: a dual-target ( Caveolin-1 and 7a-axis. Braz J Med Biol Res 2018; 51: e7220 [DOI: 10.1590/1414-431x20187220] Sharma M, Li L, Celver J, Killian C, Kvoovor A, Seeram NP. Effects of fruit ellagitannin extracts, ellagic acid, and their colonic metabolite, urolithin A, on Wnt signaling. J Agric Food Chem 2010; 58: 3965-3969 [PMID: 20614760 DOI: 10.1021/jf902857v] Giles RH, van Es JH, Clevers H. Caught up in a Wnt storm: Wnt signaling in cancer. Biochim Biophys Acta 2003; 1653: 1-24 [PMID: 12781368 DOI: 10.1016/S0304-419x(03)00005-2] Zhao B, Wang Y, Tan X, Ke K, Zheng X, Wang F, Lan S, Liao N, Cai Z, Shi Y, Zheng Y, Lai Y, Wang L, Li Q, Liu J, Huang A, Liu X. Inflammatory Micro-environment Contributes to Stemness Properties and Metastatic Potential of HCC via the NF-κB/MR-497/SALL4 Axis. Mol Ther Oncolytics 2019; 15: 79-90 [PMID: 31650026 DOI: 10.1016/j.omto.2019.08.009]
55 Olennikov DN, Kashchenko NI, Chirikova NK. In Vitro Bioaccessibility, Human Gut Microbiota Metabolites and Hepatoprotective Potential of Chebulic Ellagitannins: A Case of Padma Hepaten® Formulation. *Nutrients* 2015; 7: 8456-8477 [PMID: 26473917 DOI: 10.3390/nu7105406]

56 Zhang B, Han S, Feng B, Chu X, Chen L, Wang R. Hepatitis B virus X protein-mediated non-coding RNA aberrations in the development of human hepatocellular carcinoma. *Exp Mol Med* 2017; 49: e293 [PMID: 28186085 DOI: 10.1038/emm.2016.177]

57 Lamontagne J, Steel LF, Bouchard MJ. Hepatitis B virus and microRNAs: Complex interactions affecting hepatitis B virus replication and hepatitis B virus-associated diseases. *World J Gastroenterol* 2015; 21: 7375-7399 [PMID: 26139985 DOI: 10.3748/wjg.v21.i24.7375]

58 Totiger TM, Srinivasan S, Jala VR, Lamicichane P, Dosch AR, Gaidarski AA, Joshi C, Rangappa S, Castellanos J, Vemula PK, Chen X, Kwon D, Kashikar N, VanSaun M, Merchant NB, Nagathihalli NS. Urolithin A, a Novel Natural Compound to Target PI3K/AKT/mTOR Pathway in Pancreatic Cancer. *Mol Cancer Ther* 2019; 18: 301-311 [PMID: 30404927 DOI: 10.1158/1535-7163.MCT-18-0464]

59 Kresty LA, Fromkes JJ, Frankel WL, Hammond CD, Seeram NP, Baird M, Stoner GD. A phase I pilot study evaluating the beneficial effects of black raspberries in patients with Barrett’s esophagus. *Oncotarget* 2018; 9: 35356-35372 [PMID: 30450163 DOI: 10.18632/oncotarget.10457]
