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Original article

Fate and distribution of kynurenic acid administered as beverage

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ABSTRACT

Background: Kynurenic acid (KYNA) is a biologically active metabolite of tryptophan exerting action on several receptors located in the brain and periphery. KYNA can be synthesized endogenously or supplied in the diet. It was documented that KYNA is present in various types of food. However, its presence in beverages was not yet investigated. Here, we measured content of KYNA in tea and coffee as well as analyzed distribution and fate of intragastrically administered labelled KYNA in mice.
Methods: 16 and 13 studied samples of tea and coffee, respectively were of commercial origin. Tea and coffee infusions were prepared according to the producers' guidelines. KYNA content in beverages was measured by means of HPLC detection. Adult male mice were used for analysis of fate of intragastrically administered labelled KYNA and collected samples were analyzed using liquid scintillation counter.
Results: KYNA was identified in all studied beverages. Amounts of KYNA found in various types of beverages differed significantly. The highest content of KYNA in tea and coffee was 8.7 μg/100 ml and 0.63 μg/100 ml, respectively. It was found that KYNA administered intragastrically as a liquid is absorbed from the digestive system and readily excreted in urine. The atypical kinetics of KYNA distribution were found in intestinal content of cecum, where it appeared later and persisted longer than in other tissues.
Conclusions: Our data show that tea and coffee intake may contribute to KYNA content in the human organism. The distribution pattern of KYNA delivered as a liquid suggests that it either directly affects digestive system's functioning and intestinal microbiome composition, or participates in the whole body pool of KYNA.

Introduction

Kynurenic acid (KYNA) is a metabolite of tryptophan which is formed enzymatically along kynurenine pathway. In the previous years, KYNA attracted researchers' interest due to its intriguing pharmacological properties, expressed mostly in the brain. It was found that it is an antagonist of glutamate ionotropic receptors and nicotinic α-7 receptor, which are present mainly in the central nervous system [1–3]. KYNA's anticonvulsant and neuroprotective properties were also discovered, however, it was also stated that it poorly penetrates the blood-brain barrier [4,5]. Later research indicated that KYNA also acts as an agonist on G protein-coupled receptor (GPR35) [6]. However, its action on aryl hydrocarbon receptor (AHR) is controversial [7,8]. Interestingly, outstandingly high expression of GPR35 receptors was determined in the human digestive system [6]. Moreover, the immunoreactivity of glutamate receptor subunits AMPA and NMDA was detected on neurons in both submucosal and myenteric plexuses and additionally glutamate-evoked depolarizing responses were demonstrated [9]. These findings led to further examination of KYNA properties and concentration outside of the central nervous system.

It was found that KYNA possesses anti-ulcerative properties against ulcers induced by restraint-cold stress and ethanol [10]. It also protects against gastric and duodenal ulcers, duodenal hyperemia and peritoneal ascites evoked by administration of an extract prepared from poisonous Atlantic mussels in mice [11]. In an animal model of colon obstruction, KYNA was found to inhibit elevated tone of colon and the motility index of giant colonic contractions. Moreover, it reduced the pace of growth in xanthine oxidoreductase and myeloperoxidase activity in the colon [12]. Similarly, KYNA decreased motility and reduced inflammatory activity in experimental colitis in rats [13,14]. Very recently, the potent effect of KYNA on adipose tissue and energy homeostasis was evidenced [15]. Thus, it has been suggested that the dietary KYNA intake may impact pathophysiological processes in the body [16]. The presence of KYNA in various types of food and animal feed

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was ascertained – interestingly, its amounts differed significantly between the analysed products [17–20]. Its content is remarkably higher in plants than in meat [16]. However, the highest amounts of KYNA were found in bee products, such as bee pollen, propolis and honey [17]. Recently, an exceptionally high content of KYNA was also reported in chestnut honey [21].

Since it was proven that KYNA can be absorbed from the gastrointestinal tract when administered intragastrically [17], it might be concluded that KYNA is both an endogenous and exogenous compound which is constantly present in tissues and body fluids of animals and humans [16].

On the other hand, the presence of KYNA has never been systematically studied in beverages, except for water extracts of medicinal herbs [22]. Since water intake is necessary for well-being of humans, this study concentrates on the content of KYNA in tea and coffee, both being highly popular beverages all over the world. Moreover, its distribution after intragastric application as a liquid was studied in mice, because this way it may immediately affect specific receptors present on the superficial tissue layers of the digestive system.

Materials and methods

Animals

Adult male Swiss mice weighing 25–28 g were used. Animals were kept under standard laboratory conditions with access to food and water *ad libitum*. Mice were fasted for 12 h before KYNA administration. All procedures were approved by the I Local Ethics Committee for Animal Experiments in Lublin (30/2010) and were in agreement with Directive 2010/63/EU on the protection of animals used for scientific purposes. In total, 36 animals were used.

Chemicals

5,7-^{3}H-Kynurenic acid (specific activity 50–60 Ci/mmol) purchased from American Radiolabeled Chemicals, Inc. St. Louis, MO, USA, was used. All samples of beverages were of commercial origin. The names of producers or suppliers were specified in table legends. All other chemicals were obtained from commercial suppliers and were of the highest purity.

Administration and determination of labeled KYNA

5,7-^{3}H-Kynurenic acid was dissolved in saline (1 mCi/ml) and administered intragastrically by oral gavage in a volume of 1 ml per 100 g of body weight. A ball tip needle was used. A conscious mouse was manually immobilized and substance was administered as a bolus, slowly to avoid improper administration into the lungs. Groups consisted of 6 animals. Mice were decapitated 1, 3, 6, 12 and 24 h after KYNA administration. Tissue was removed and rapidly dissected. Each sample was weighed, sonicated in 2 vol (w/v) water, and after adding trichloroacetic acid (TCA) (50%) – centrifuged (7800 x *g*, 10 min). Blood samples were centrifuged (1400 x g, 10 min), supernatant was deproteinated by TCA (50%) and centrifuged (7800×g, 10 min). Urine was collected immediately before decapitation. Mouse was held over a Petri plate and encouraged to micturate by holding its tail back and gentle handling. The content of radioactivity in urine was determined without prior preparation.

Samples in a volume of 50 μl were applied to glass microfiber filters (GF/C, Whatman), dried at room temperature and subjected to infrared radiation for 15 min. Thereafter, filters were placed in scintillation vials containing 2 ml of scintillation liquid and analysed in liquid scintillation counter (LS6000SE, Beckman Coulter, Fullerton, CA, USA).

Preparation of beverages

Tea and coffee infusions were prepared according to the producers' guidelines. Since 1–2 teaspoons of tea are a usually recommended dose, 3-6 g of tea leaves were weighed. Distilled water in recommended volume (100–200 ml) was used for steeping. Its temperature was adjusted according to each producer's recommendations. Steeping time depended on tea category (specified in Table 1). Tea beverage was stirred and 2 samples from each beaker (duplicates) were taken for KYNA determination. Whole coffee beans were milled. Samples of milled coffee beans and instant coffee were weighed (4–5 g). Boiled distilled water in recommended volume (100–200 ml) was used for brewing. Brewing time was 5 min. Coffee beverage was stirred, filtered and 2 samples from each beaker (duplicates) were taken for KYNA determination.

Table 1
Kynurenic acid (KYNA) content in tea and rooibos tea.

Symbol	Brand/type	Type/country of origin	Steep time [min]	KYNA [μg/100 ml] mean ± SD	$p < 0.05$ *vs.*
A	Ginkaku Sencha	green/Japan	2	8.693 ± 2.115	C,D,E,F,G,H,I, J,K,L,M,N,O,P
B	Japan Tamaryokucha	green/Japan	3	7.539 ± 0.993	C,D,E,F,G,H,I, J,K,L,M,N,O,P
C	Ceylon Kenilworth	black/Sri Lanka	3	5.415 ± 0.168	A,B,H,I,J,K, L,M,N,O,P
D	Kenya Original GFOP Milima	black/Kenya	3	5.282 ± 0.292	A,B,H,I,J,K, L,M,N,O,P
E	China Green Yunnan	green/China	3	3.766 ± 0.193	A,B,O,P
F	Asairi Houjicha gojobashi	green/Japan	1.5	3.714 ± 0.610	A,B,O,P
G	Assam TGFOP 1 Tezpore & Gogra	black/India	3	3.420 ± 0.470	A,B,O,P
H	Assam TGFOP 1 Tezpore	black/India	5	3.145 ± 0.770	A,B,C,D,P
I	Xue Long	white/China	4	2.902 ± 0.110	A,B,C,D,O,P
J	China Huang Da Cha	yellow/China	3	2.896 ± 0.771	A,B,C,D,P
K	Gunpowder Temple of Heaven	green/China	3	2.861 ± 0.253	A,B,C,D,P
L	China Pai Mu Tan	white/China	2	2.535 ± 0.267	A,B,C,D
M	Pu erh	black/China	3	2.062 ± 0.163	A,B,C,D
N	China Huang Jing Cha	black/China	3	1.893 ± 0.292	A,B,C,D
O	Rooibos Origina	herbal/South Africa	10	1.213 ± 0.100	A,B,C,D,E,F,G,H,I
P	Che Nhai Dac Biet Jasmine	green/Vietnam	5	0.514 ± 0.052	A,B,C,D,E,F,G,H,I,J,K

Data is presented as a mean ± SD. Statistical analysis was performed using one-way ANOVA with Tukey *post- hoc* test ($p < 0.05$) *vs.* respective product; each letter corresponds to one product as indicated in column 1.