

REVIEW

OPEN ACCESS

Full open access to this and thousands of other papers at <http://www.la-press.com>.

Species and Cell Types Difference in Tryptophan Metabolism

Yuki Murakami and Kuniaki Saito

Human Health Sciences, Graduate School of Medicine and Faculty of Medicine, Kyoto University, 53 Kawahara-cho, Shogoin, Sakyo-Ku, Kyoto, Japan. Corresponding author email: murakami.yuki.8x@kyoto-u.ac.jp

Abstract: L-Tryptophan (L-TRP) is a nutritionally essential amino acid and the kynurenine (KYN) pathway is the major route of L-TRP catabolism. Besides being synthesized for proteins, L-TRP and its metabolites have critical roles for the functions of nervous and immune systems. Many researches show that optimal amounts of L-TRP in diets depend on species, developmental stages, environmental factors and health status. We have shown that KYN pathway-related enzyme activities vary among species, tissue and cell types in physiological conditions. Furthermore, the response of these enzyme activities to systemic and/or central nervous system immune activation and inflammation depends on species and cell types. Thus, it is very important to choose appropriate animal species and cell types in which to evaluate the physiologic and pathologic effects of increased KYN pathway metabolism. We believe that understanding L-TRP metabolism among species and cell types provides a better idea for analysis of human pathological condition.

Keywords: kynurenine (KYN) pathway, species difference, inflammation, cytokine, indoleamine 2,3-dioxygenase (IDO), kynurenine 3-monooxygenase (KMO), L-tryptophan (L-TRP)

International Journal of Tryptophan Research 2013;6 (Suppl. 1) 47–54

doi: [10.4137/IJTR.S11558](https://doi.org/10.4137/IJTR.S11558)

This article is available from <http://www.la-press.com>.

© the author(s), publisher and licensee Libertas Academica Ltd.

This is an open access article published under the Creative Commons CC-BY-NC 3.0 license.



Introduction

The kynurenine (KYN) pathway is the major route of L-tryptophan (L-TRP) catabolism and an anabolic source of nicotinamide-containing nucleotide.¹ In extrahepatic tissues, the first enzyme of the KYN pathway is indoleamine 2,3-dioxygenase-1 (IDO1), rather than tryptophan 2,3-dioxygenase (TDO).² This enzyme has a much broader substrate profile for indoleamine-containing compounds and its expression is strongly influenced by the state of the immune system. The IDO1 activity is potently induced by several proinflammatory cytokines and endotoxin. In contrast, TDO activity is increased by TRP and its analogues via actions at a distinct allosteric activation site.² Substantial increases in the TRP-KYN pathway metabolites occur in human brain, blood and systemic tissues during immune activation. Many animal model studies on the KYN pathway metabolism during immune activation and normal conditions have used nonhuman primates,^{3–5} gerbils,^{6–9} guinea pigs,^{10,11} mice^{12–14} and rats.⁹ In the field of neuroscience, the rat is one of the most frequently used animal species; however, immunologists generally use mice. It is well known that IDO1 induction in lung following immune stimulation by LPS is substantially less in rats than in mice and other species.¹⁵ On the other hand, in the transient global brain ischemia model, rats and gerbils have been used widely to investigate the molecular mechanism of selective neuronal death because of the vascular anatomy in the brain. Therefore, it is very important to well understand species difference and select animals for analysis of human pathological condition. Furthermore, studies in vitro have shown that not all human cells are capable of directly synthesizing quinolinic acid (QUIN) from L-TRP. The activities of kynurenine pathway enzymes and the production of kynurenine metabolites are depending on cell types.¹⁶ IDO1 is the most important regulatory enzyme for KYN pathway, and kynurenine 3-monooxygenase (KMO), kynureninase and 3-hydroxyanthranic acid oxidase are also important determinates of whether a cell can make QUIN. Indeed, stimulation by proinflammatory cytokines have resulted in large increases of the IDO1 activity in most cell types, although the accumulated amounts of QUIN are very different. It has been shown that blood macrophages and monocyte-derived cells produced the largest amount of QUIN in accordance

with the highest activities of KMO and kynureninase compared to other cell types.¹⁶

L-TRP plays versatile roles in nutrition and physiology, particularly food intake, neurological function and immunity. Optimal amounts of L-TRP in diets likely depend on species, developmental stages, environmental factors and health status. We studied species and cell type differences in IDO1 activity and kynurenine pathway-related enzymes in response to systemic immune activation by LPS or IFN- γ , and central nervous system (CNS) inflammation. Here, we show that KYN pathway enzyme activities and metabolite concentrations vary with cell types and species.

Species Differences in TRP-KYN Pathway Metabolism in Physiological Conditions

In the CNS, several KYN metabolites are neuroactive and may play potential roles in inflammatory neurological diseases.^{17–20} As shown in Figure 1, L-KYN is converted to anthranilic acid (AA), 3-hydroxykynurenine (3-HK), and other metabolites in the systemic tissues. Furthermore, 3-HK is metabolized to 3-hydroxyanthranilic acid (3-HAA), which is a bioprecursor of QUIN. Our group measured serum and cerebrospinal fluid (CSF) L-KYN and AA concentrations in different animal species.²¹ The concentrations of serum L-KYN in rabbits were 4 to 17 times higher than that those in the other species studied. CSF L-KYN concentrations were also the highest in rabbits. There were no large species differences in the ratio of L-KYN concentrations CSF/serum. Compared with L-KYN, serum AA concentrations in rabbits were only slightly higher than those in the other species. AA concentrations in CSF were the highest in rats and lowest in rabbits. Consequently, the ratio of AA/KYN in rat CSF was 198-fold higher than that in rabbit CSF. In addition, the AA ratio in CSF/serum in humans and rats were extremely high compared with those of rabbits and guinea pigs. Thus, it is likely that KYN pathway metabolism in the CNS varies with species, although the cause of this difference remains to be determined. Previous studies have suggested that L-KYN, AA, and 3-HK, at least in rat brain, are derived from the periphery;²² however, it is possible that these species differences depend on the blood-brain permeability and/or transport capacity into the

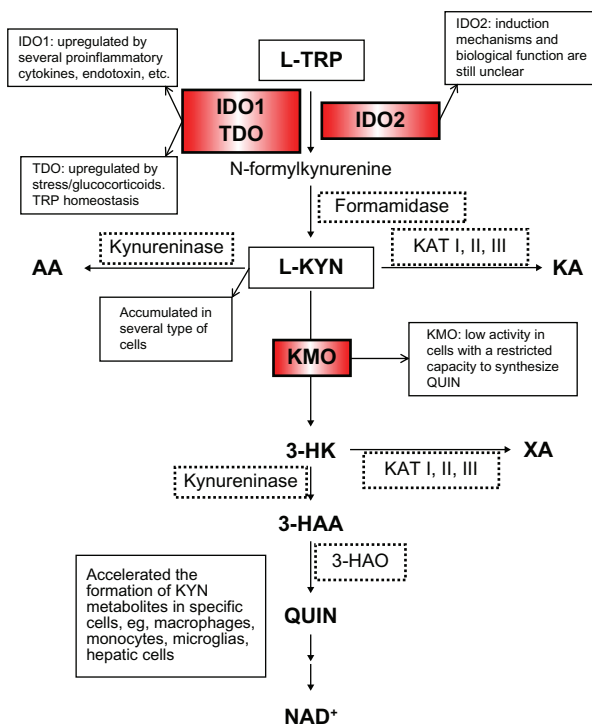


Figure 1. Schematic overview of the kynurenine (KYN) pathway. L-Tryptophan (L-TRP) is the extracellular fluid is transported into cells by a high-affinity tryptophan transporter. The first rate-limiting enzyme indoleamine 2,3-dioxygenase 1 (IDO1) catalyses the initial enzymatic step in the KYN pathway leading to the synthesis of a number of KYN metabolites. IDO1 is induced by several proinflammatory cytokines; therefore, KYN metabolism is increased during many inflammatory conditions. By contrast, glucocorticoid hormones increase transcription of tryptophan 2,3-dioxygenase (TDO) and peripheral degradation of L-TRP via the KYN pathway. The biological function and induction mechanism of IDO2 are still unclear and controversial. IDO1 is an important regulatory enzyme in the production of L-KYN in a broad spectrum of cell types. Low activity of kynurenine 3-monooxygenase (KMO) in some cells restricts the capacity to synthesize QUIN from L-TRP.

Abbreviations: KAT I, II, III, Kynurenine aminotransferase; KA, kynurenine acid; AA, anthranilic acid; 3-HK, 3-hydroxykynurenine; XA, xanthurenic acid; 3-HAA, 3-hydroxyanthranilic acid; 3-HAO, 3-hydroxyanthranic acid oxidase; NAD⁺, nicotinamide adenine dinucleotide⁺.

brain cells for certain KYN pathway metabolites. Our results suggested that blood-brain barrier permeability of KYN metabolites' levels in rats and humans were higher than those of mice, gerbils, and rabbits. The principal bioprecursor of 3-HAA was shown to be AA rather than 3-HK in rat brain;²³ however, it is speculated that L-KYN is mainly metabolized to 3-HAA through 3-HK in the brain except in some species. We also determined tissue KYN pathway enzymes, IDO1, TDO, KMO, and kynureninase in rabbits, rats, gerbils, and mice (Table 1).²¹ The activities of these enzymes vary among species and tissues. Lung IDO1 activity in rabbits was 146–516 times higher than the activity in other species, and rabbit liver KMO activities were lower by one order of magnitude compared to

those of others. Furthermore, brain KMO activities were 12.3–23.2 times higher in gerbil than those on other species. We also showed that brain KMO activities in gerbils were much higher than other species,⁸ although the physiological significance of this difference in gerbil brain is still unclear. KMO is predominantly a mitochondrial enzyme, whereas the other enzymes of the KYN pathway are predominantly located in cytosol extracts of the brain. It is possible that different subcellular as well as cellular localization of these enzymes or substrates is involved in the regulation of KYN pathway metabolism in the brain. Thus, both studies suggested that the different activities of KYN pathway enzymes, IDO1, kynureninase, and KMO, which convert L-TRP to L-KYN, L-KYN to AA, and 3-HK to 3-HAA, resulted in these species differences. In summary, KYN pathway enzyme activities and metabolite concentrations vary by species in normal conditions.

Species Heterogeneity in Response to Brain Injury and Systemic Immune Activation

In certain conditions, the accumulation of neurotoxins within the brain has been implicated in neuronal injury and death. QUIN is a neurotoxic metabolite of the TRP-KYN pathway that activates N-methyl-D-aspartate (NMDA) receptors, which increases neuronal activity, and elevates intracellular calcium concentrations. In humans, large and sustained increases in brain parenchyma and CSF QUIN levels occur during immune activation induced by microglia infections, autoimmune processes, and trauma.²⁰ During brain-localized inflammation, such as following cerebral ischemic injury, QUIN accumulations are attributable to accelerated QUIN synthesis within brain tissue²⁴ and local increases in the activities of IDO1, kynureninase, KMO, and, in some circumstances, 3-HAO.^{4,5,7,8} On the other hand, during systemic immune activation, increases in brain QUIN concentrations involve both de novo synthesis in the brain and QUIN entry as a result of elevated blood levels.²⁴ Our group demonstrated QUIN responses to systemic immune activation (intrapersonal injections of LPS) and CNS inflammation (secondary to cerebral ischemic injury) in rats, gerbils and mice, showing species differences in KYN pathway enzymes activities and production of QUIN (Table 2).^{9,25} LPS administration to gerbils (1 mg/kg, i.p.) significantly

**Table 1.** Activities of tissue kynurenine pathway enzymes in rabbit, rat, gerbil, and mouse.

Tissue	Species	IDO1/TDO* pmol/h/mg protein	KMO pmol/h/mg protein	Kynureninase pmol/h/mg protein
Liver	Rabbit	1810 ± 410*	201 ± 57	1610 ± 290
	Rat	4770 ± 940*	6280 ± 1420	1550 ± 1400
	Gerbil	711 ± 119*	10600 ± 400	1780 ± 190
	Mouse	1120 ± 20*	8740 ± 720	6100 ± 430
Lung	Rabbit	43400 ± 2900	69.9 ± 12.2	131 ± 37
	Rat	84.9 ± 26.0	185 ± 48	81.1 ± 15.3
	Gerbil	296 ± 140	175 ± 9	92.0 ± 3.1
	Mouse	114 ± 50	451 ± 89	69.0 ± 11.8
Brain	Rabbit	19.0 ± 10.0	17.5 ± 3.9	9.34 ± 2.83
	Rat	7.21 ± 1.77	12.4 ± 2.1	11.4 ± 1.8
	Gerbil	27.9 ± 5.6	215 ± 53	6.50 ± 1.87
	Mouse	17.9 ± 0.9	9.26 ± 1.25	3.80 ± 0.67

Note: Values presented are means ± SEM (n = 3 per group).²¹

increased QUIN levels in the brain, serum, lung, liver, and spleen. The magnitude of the increase was identical in several brain regions. In contrast, LPS administration to rats at either 1 or 50 mg/kg had no significant effect on QUIN concentrations in brain, plasma, lung, liver, or spleen. In gerbils, LPS increased IDO1 activity 30-fold in the lung and resulted in a modest 3.3-fold increase in IDO1 activity in the brain.

Table 2. Effects of systemic LPS administration on lung and brain IDO1 activity and hepatic TDO activity in gerbils, Sprague-Dawley rats and C57BL/6 J mice.

	Saline	Enzyme activity	
		LPS	
		1 mg/kg	50 mg/kg
Lung IDO1 (nmol/g/h)			
Gerbil	78 ± 13	2,303 ± 340*	—
Rat	5.9 ± 3.0	15.0 ± 3.2*	6.9 ± 3.0
Mouse	178.5 ± 33.4	3161.2 ± 501.9* [#]	—
Brain IDO1 (nmol/g/h)			
Gerbil	2.1 ± 0.5	6.95 ± 1.1*	—
Rat	<0.1	<0.1	<0.1
Mouse	12.2 ± 1.7	36.9 ± 3.2* [#]	—
TDO (μmol/g/h)			
Gerbil	0.96 ± 0.08	0.71 ± 0.7	—
Rat	2.2 ± 0.2	2.7 ± 0.3	2.2 ± 0.1
Mouse	2.4 ± 0.1	1.6 ± 0.2* [#]	—

Notes: LPS was administered by intraperitoneal injection (i.p.), and samples were collected 24 h later. Control animals received saline vehicle. Data are mean ± SEM values. *Significant differences from respective control by one-way ANOVA and Dunnett's *t* test⁹ or Scheffe's *F* post-hoc test.²⁵ [#]Mice were administered 10 μg LPS in 200 μL saline by i.p. injection. Estimated dose of LPS is much lower than 1 mg/kg (approximately 0.4 to 0.5 mg/kg).

LPS administration also increased IDO1 activity 18-fold in mice lung and resulted in slightly increased activity in the brain. On the other hand, lung IDO1 activity in control rats was less than 10-fold lower than control gerbils and showed only a modest several fold increase after low-dose LPS administration. Thus, the low capacity of rats to accumulate QUIN in response to immune activation is explained by their minimal IDO1 responsiveness in the brain and lung. We also showed that in gerbils, 10 minutes (min) of bilateral carotid artery occlusion resulted in substantial increases in QUIN levels in the hippocampus, with smaller increases in striatum, cerebral cortex, thalamus, and brainstem. Hippocampal QUIN concentrations in post-ischemic gerbils were 65-fold higher than control animal. In gerbils, there were also significant increases in hippocampal IDO1 activity by 16.2-fold. Conversely, transient cerebral ischemia in the rat had no significant effect on QUIN levels in any brain region and IDO1 activity was likewise unchanged. Their presenting data clearly shows that in gerbils, immune activation induced by ischemic brain injury or systemic administration of LPS replicates key features of the increases in QUIN levels in human brain and systemic tissues in response to inflammation. Although their results do not exclude the possibility that there are stimuli or strains of rats that can increase brain and systemic tissue IDO1 activity and QUIN levels, it appears that the rat does not replicate human responses to immune activation. These data emphasize the importance of choosing

an appropriate animal species in which to evaluate the physiologic and neuropathologic effects of an increased KYN pathway metabolism.

Different KYN Pathway Enzymes Activities in Various Human Cell Types

As shown in previous sections, substantial increases in the TRP-KYN pathway metabolites, L-KYN and the neurotoxin QUIN, occur in human brain, blood and systemic tissues during immune activation. Studies in vitro have shown that not all human cells are capable of synthesizing QUIN from L-TRP. To further investigate the mechanisms that limit L-KYN and QUIN production, we demonstrated the activities of KYN pathway enzymes and the ability of different human cells to convert pathway intermediates into QUIN (Tables 3 and 4).¹⁶ Stimulation with interferon γ (IFN- γ) substantially increased IDO1 activity and L-KYN production in primary peripheral blood macrophages and fetal brains (astrocytes and neurons), as well as cell lines derived from macrophage/monocytes (THP-1), astrocytomas (U3736MG), B-lymphocytes (WIL-NS), liver (SK-HEP1) and lungs (MRC-9). LPS also increased L-KYN accumulation in all these cells, but in the case of U373MG, SK-HEP1, WIN-NS and MRC-9 cells, the magnitude of this increase was substantially less than in blood macrophages and THP-1 cells. TNF- α -produced smaller increases in

L-KYN production compared to IFN- γ stimulation in these cells. QUIN was detected in blood macrophages, THP-1 cells and SK-HEP1 cells, but was not detected in U373MG, WIL-NS and MRC-9 cells in response to LPS, IFN- γ or TNF- α . The amount of QUIN formed relative to the amount of L-KYN was the highest in blood macrophages compared with THP-1 and SK-HEK1 cells. IFN- γ stimulation resulted in large increases in the activity of IDO1 in all cell types examined, although the magnitude of the response in blood macrophages was the smallest in the both relative and absolute terms. KMO was detected only in unstimulated blood macrophages, THP-1 and SK-HEK1 cells. No KMO activity was detected in human fetal brain cultures, U373MG, WIL-NS or MRC-9 cells. IFN- γ stimulation produced a small increase in KMO activity in THP-1 cells only. Kynureninase lead to increased activities in all cell types except human fetal brain cultures and WIL-NS cells. IFN- γ slightly increased kynureninase activities in MRC-9 and SK-HEP1 cells. 3-HAO activity was detected in all cell types except U373MG cells, but was unchanged by IFN- γ stimulation. In addition, species heterogeneity might be manifested at the level of the cells that are the sources of QUIN in brain inflammation. Our group demonstrated the abilities of brain microglia, astrocytes, and peripheral monocytes to produce QUIN.⁹ Gerbil brain microglia and peritoneal monocytes spontaneously produced

Table 3. Effects of LPS, IFN- γ and TNF- α on L-KYN and QUIN production in different cell types.

Cell origin	L-KYN (μ M)				QUIN (nM)			
	Control	LPS	IFN- γ	TNF- α	Control	LPS	IFN- γ	TNF- α
Blood macrophages	1.1 \pm 0.1	15 \pm 1*	20 \pm 1*	4.0 \pm 0.6*	215 \pm 25	4450 \pm 90*	4620 \pm 94*	405 \pm 74*
THP-1 (monocyte)	0.2 \pm 0.1	26 \pm 2*	41 \pm 4*	0.3 \pm 0.1*	6 \pm 1	261 \pm 35*	333 \pm 8*	8 \pm 2
U363MG (astrocytoma)	0.3 \pm 0.1	2.1 \pm 0.3*	43 \pm 1*	0.8 \pm 0.1*	<0.2	<0.2	<0.2	<0.2
SK-HEP1 (liver)	0.3 \pm 0.1	0.6 \pm 0.1*	38 \pm 1*	0.6 \pm 0.1*	<0.2	10 \pm 2	523 \pm 22*	0.7 \pm 0.3*
WIL-NS (B-lymphocyte)	<0.05	0.4 \pm 0.1*	0.8 \pm 0.1*	0.1 \pm 0.1*	<0.2	<0.2	<0.2	<0.2
MRC-9 (lung)	<0.05	0.5 \pm 0.1*	41 \pm 1*	0.6 \pm 0.1*	<0.2	<0.2	<0.2	<0.2

Notes: Cells were incubated with or without (control) LPS (1 μ g/mL), IFN- γ (100 unit/mL) or TNF- α (100 unit/mL). L-KYN (μ M) and QUIN (nM) concentrations after 48 h incubation are expressed as means \pm SEM from at least five incubation wells per time. Baseline L-KYN levels in the serum added to the incubation media (approx. 0.05 μ M) have been subtracted. The minimum detectable concentrations were: QUIN < 0.2 nmol/L, L-KYN < 0.05 μ M. For statistical comparisons, non-detected metabolites were set at the minimum detectable values. *Significant differences from respective control by one-way ANOVA and Mann-Whitney test.¹⁶

Table 4. Effects of IFN- γ on KYN pathway enzyme activities in different cell types.

Cell origin	IDO1 pmol/h/mg protein		KMO pmol/h/mg protein		Kynureninase pmol/h/mg protein		3-HAO pmol/h/mg protein	
	Control	IFN- γ	Control	IFN- γ	Control	IFN- γ	Control	IFN- γ
Blood macrophages	651 \pm 134	7537 \pm 864*	3100 \pm 376	2625 \pm 200	949 \pm 133	992 \pm 104	1333 \pm 376	1353 \pm 376
THP-1 (monocyte)	260 \pm 80	(420 \pm 2) \times 10 ³ *	363 \pm 6	574 \pm 27*	11 \pm 0.2	12 \pm 0.2	1600 \pm 60	1200 \pm 20
Human fetal brain (astrocytes and neurons)	<0.1	(260.5 \pm 2.2) \times 10 ³ *	<0.1	<0.1	<0.05	<0.05	37.5 \pm 4.1	43.2 \pm 11
U363MG (astrocytoma)	170 \pm 20	(369 \pm 4.6) \times 10 ³ *	<0.1	<0.1	0.6 \pm 0.1	0.5 \pm 0.39	<0.01	<0.01
SK-HEP1 (liver)	170 \pm 20	(311 \pm 5.2) \times 10 ³ *	85 \pm 17	69 \pm 14	3.0 \pm 0.25	3.9 \pm 0.25	1180 \pm 120	1060 \pm 88
WIL-NS (B-lymphocyte)	150 \pm 20	580 \pm 90	<0.1	<0.1	<0.05	<0.05	1.2 \pm 0.1	1.1 \pm 0.1
MRC-9 (lung)	120 \pm 20	(343 \pm 4.6) \times 10 ³ *	<0.1	<0.1	1.6 \pm 0.1	2.1 \pm 0.39	300 \pm 20	300 \pm 20

Notes: Cells were incubated with or without (control) 100 unit/mL IFN- γ . Values are expressed as means \pm SEM from six incubation wells per time. For statistical comparisons, non-detected metabolites were set at the minimum detectable values. * Significant differences from respective control by one-way ANOVA and Mann-Whitney test.¹⁶

enhanced amounts when stimulated with LPS. Gerbil astrocytes also produced enhanced amount of L-KYN and QUIN when stimulated with LPS, although the amounts were less than those made by microglia and peritoneal monocytes. No QUIN was detected in cultures of rat brain microglia, astrocytes, and peritoneal monocytes even when stimulated with LPS and/or IFN- γ . A small amount of L-KYN was detected from LPS- and IFN- γ -stimulated astrocytes.⁹

A New Enzyme in the Kynurenine Pathway; Indoleamine 2,3-Dioxygenase-2

As shown in previous sections, it has been believed that the first and rate-limiting step in the KYN pathway is performed by either of two enzymes, TDO or IDO1, depending on the tissue and cell type. Recently, a gene with homology to IDO1 was reported²⁶ and then subsequently demonstrated to be an enzyme with the ability to catabolize tryptophan.^{27–29} The enzyme has been referred to as indoleamine 2,3-dioxygenase-like protein, indoleamine 2,3-dioxygenase-2 or proto-indoleamine 2,3-dioxygenase (INDOL1, IDO2, protoIDO) on the basis of its structural similarity to IDO1 and its enzymatic activity. In mice, the IDO2 protein is predominantly expressed in the kidney followed by epididymis, testis, liver and brain.^{27,30} On the other hand, the IDO1 protein is expressed in various organs, like the brain, lung, placenta, epididymis and kidney under normal physiological conditions. Although both IDO proteins are expressed in the epididymis, IDO1 protein is located to the principal and apical cells of the mouse caput epididymis whereas the IDO2 immunoreactivity is in the tail of the spermatozoa.²⁷ Murine IDO1 protein is also located primarily in the renal vasculature of the kidney.²⁷ In contrast, IDO2 is located to the kidney tubules. The different patterns of expression suggest that there is no functional redundant amongst the these enzymes in the epididymis and kidney,^{27,30} but the role of each enzyme remains to be defined. In addition, expression of the proteins in response to stimuli differs. IDO1 is induced systemically dependent or independent on the presence of IFN- γ ²⁵ while IDO2 mRNA expression is unchanged or down-regulated.²⁷ The Km for L-TRP of the human IDO2 protein was approximately 500–1,000 times higher than that of the mammalian IDO1 enzyme.³¹ Furthermore, IDO activity of human

DCs expressing both IDO1 and IDO2 is inhibited by IDO1-specific siRNA.³² These studies indicate that IDO2 is functionally inactive with regard to the TRP metabolism and suggest that IDO2 might be a pseudo-gene for IDO1. However, the biological role of IDO2 is still controversial. We found that depletion of TRP and formation of KYN is detectable at physiological levels of TRP when mouse and human IDO2 enzymes are expressed and assayed in mammalian cells^{27,28} (Saito K, September, 2012). IDO2-like enzymes from lower vertebrates also show significant enzymatic activity when transfected into mammalian cells.³³ These findings might suggest that post-translational modifications of the protein are required for optimal activity. In addition, there is a possibility that different cofactors or conditions may be necessary for measuring IDO2 activity in vitro assay. The lack of an appropriate in vitro assay is a problem for biochemical characterization of IDO2.

Conclusions

We conclude that the KYN pathway enzyme activities and metabolite concentrations in serum and CSF vary with species in normal and pathologic conditions. In addition, immune activation in gerbils, induced by ischemic brain injury or systemic administration of LPS, replicates the increases in QUIN levels in human brain and systemic tissues in response to inflammation. In contrast to gerbils, neither LPS administration nor ischemic brain injury in rats had any significant effects on QUIN concentrations. Although different stimuli or strains of rats can increase brain and systemic tissue IDO1 activity and QUIN levels, it appears that the rat does not replicate human responses to immune activation. Taken together, these findings emphasize the importance of choosing an appropriate animal species in which to evaluate the physiological and pathophysiological effects of increased KYN pathway metabolism. Furthermore, L-KYN accumulated in several cell types; however, production of QUIN from L-TRP is accelerated in specific cell types, eg, macrophages, monocytes, microglia and hepatic cells. KYN pathway of TRP metabolism is involved in a lot of biological processes, including the immune system. We believe that further findings on TRP metabolism and its enzyme regulation in organisms under both physiologic and pathophysiologic conditions might

provoke the implementation of novel therapeutic strategies by targeting the KYN pathway.

Funding

Part of this work was supported by Grant-in-aid for Scientific Research from Smoking Research Foundation. 13th ISTRY Satellite Symposium and the publication fee of this proceeding were supported by The International Council on Amino Acid Science (ICAAS).

Author Contributions

Wrote the first draft of the manuscript: YM. Contributed to the writing of the manuscript: KS. Jointly developed the structure and arguments for paper: YM, KS. Made critical revisions and approved final version: YM, KS. All authors reviewed and approved of the final manuscript.

Competing Interests

Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

References

1. Beadle GW, Mitchell HK, Nyc JF. Kynurenine as an Intermediate in the Formation of Nicotinic Acid from Tryptophan by *Neurospora*. *Proc Natl Acad Sci U S A*. 1947;33:155–8.
2. Ruddick JP, Evans AK, Nutt DJ, Lightman SL, Rook GA, Lowry CA. Tryptophan metabolism in the central nervous system: medical implications. *Expert Rev Mol Med*. 2006;8:1–27.
3. Heyes MP, Lackner A. Increased cerebrospinal fluid quinolinic acid, kynurenic acid, and L-kynurenine in acute septicemia. *J Neurochem*. 1990;55:338–41.
4. Heyes MP, Saito K, Jacobowitz D, Markey SP, Takikawa O, Vickers JH. Poliovirus induces indoleamine-2,3-dioxygenase and quinolinic acid synthesis in macaque brain. *FASEB J*. 1992;6:2977–89.
5. Heyes MP, Saito K, Major EO, Milstien S, Markey SP, Vickers JH. A mechanism of quinolinic acid formation by brain in inflammatory neurological disease. Attenuation of synthesis from L-tryptophan by 6-chlorotryptophan and 4-chloro-3-hydroxyanthranilate. *Brain*. 1993;116(Pt 6):1425–50.



6. Saito K, Crowley JS, Markey SP, Heyes MP. A mechanism for increased quinolinic acid formation following acute systemic immune stimulation. *J Biol Chem.* 1993;268:15496–503.
7. Saito K, Nowak TS Jr, Markey SP, Heyes MP. Mechanism of delayed increases in kynurenine pathway metabolism in damaged brain regions following transient cerebral ischemia. *J Neurochem.* 1993;60:180–92.
8. Saito K, Quearry BJ, Saito M, Nowak TS Jr, Markey SP, Heyes MP. Kynurenine 3-hydroxylase in brain: species activity differences and effect of gerbil cerebral ischemia. *Arch Biochem Biophys.* 1993;307:104–9.
9. Heyes MP, Saito K, Chen CY, et al. Species heterogeneity between gerbils and rats: quinolinic acid production by microglia and astrocytes and accumulations in response to ischemic brain injury and systemic immune activation. *J Neurochem.* 1997;69:1519–29.
10. Blight AR, Saito K, Heyes MP. Increased levels of the excitotoxin quinolinic acid in spinal cord following contusion injury. *Brain Res.* 1993;632:314–6.
11. Blight AR, Cohen TI, Saito K, Heyes MP. Quinolinic acid accumulation and functional deficits following experimental spinal cord injury. *Brain.* 1995;118(Pt 3):735–52.
12. Heyes MP, Quearry BJ, Markey SP. Systemic endotoxin increases L-tryptophan, 5-hydroxyindoleacetic acid, 3-hydroxykynurenine and quinolinic acid content of mouse cerebral cortex. *Brain Res.* 1989;491:173–9.
13. Saito K, Markey SP, Heyes MP. Effects of immune activation on quinolinic acid and neuroactive kynurenines in the mouse. *Neuroscience.* 1992;51:25–39.
14. Saito K, Markey SP, Heyes MP. 6-Chloro-D,L-tryptophan, 4-chloro-3-hydroxyanthranilate and dexamethasone attenuate quinolinic acid accumulation in brain and blood following systemic immune activation. *Neurosci Lett.* 1994;178:211–5.
15. Daley-Yates PT, Powell AP, Smith LL. Pulmonary indoleamine 2,3-dioxygenase activity and its significance in the response of rats, mice, and rabbits to oxidative stress. *Toxicol Appl Pharmacol.* 1988;96:222–32.
16. Heyes MP, Chen CY, Major EO, Saito K. Different kynurenine pathway enzymes limit quinolinic acid formation by various human cell types. *Biochem J.* 1997;326(Pt 2):351–6.
17. Perkins MN, Stone TW. Pharmacology and regional variations of quinolinic acid-evoked excitations in the rat central nervous system. *J Pharmacol Exp Ther.* 1983;226:551–7.
18. Foster AC, Vezzani A, French ED, Schwarcz R. Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid. *Neurosci Lett.* 1984;48:273–8.
19. Stone TW, Connick JH. Quinolinic acid and other kynurenines in the central nervous system. *Neuroscience.* 1985;15:597–617.
20. Heyes MP, Saito K, Crowley JS, et al. Quinolinic acid and kynurenine pathway metabolism in inflammatory and non-inflammatory neurological disease. *Brain.* 1992;115(Pt 5):1249–73.
21. Fujigaki S, Saito K, Takemura M, et al. Species differences in L-tryptophan-kynurenine pathway metabolism: quantification of anthranilic acid and its related enzymes. *Arch Biochem Biophys.* 1998;358:329–35.
22. Fukui S, Schwarcz R, Rapoport SI, Takada Y, Smith QR. Blood-brain barrier transport of kynurenines: implications for brain synthesis and metabolism. *J Neurochem.* 1991;56:2007–17.
23. Baran H, Schwarcz R. Presence of 3-hydroxyanthranilic acid in rat tissues and evidence for its production from anthranilic acid in the brain. *J Neurochem.* 1990;55:738–44.
24. Heyes MP, Morrison PF. Quantification of local de novo synthesis versus blood contributions to quinolinic acid concentrations in brain and systemic tissues. *J Neurochem.* 1997;68:280–8.
25. Fujigaki S, Saito K, Sekikawa K, et al. Lipopolysaccharide induction of indoleamine 2,3-dioxygenase is mediated dominantly by an IFN-gamma-independent mechanism. *Eur J Immunol.* 2001;31:2313–8.
26. Murray MF. The human indoleamine 2,3-dioxygenase gene and related human genes. *Curr Drug Metab.* 2007;8:197–200.
27. Ball HJ, Sanchez-Perez A, Weiser S, et al. Characterization of an indoleamine 2,3-dioxygenase-like protein found in humans and mice. *Gene.* 2007;396:203–13.
28. Metz R, Duhadaway JB, Kamasani U, et al. Novel tryptophan catabolic enzyme IDO2 is the preferred biochemical target of the antitumor indoleamine 2,3-dioxygenase inhibitory compound D-1-methyl-tryptophan. *Cancer Res.* 2007;67:7082–7.
29. Yuasa HJ, Takubo M, Takahashi A, Hasegawa T, Noma H, Suzuki T. Evolution of vertebrate indoleamine 2,3-dioxygenases. *J Mol Evol.* 2007;65:705–14.
30. Fukunaga M, Yamamoto Y, Kawasoe M, et al. Studies on tissue and cellular distribution of indoleamine 2,3-dioxygenase 2: the absence of IDO1 upregulates IDO2 expression in the epididymis. *J Histochem Cytochem.* 2012;60:854–60.
31. Yuasa HJ, Ball HJ, Ho YF, et al. Characterization and evolution of vertebrate indoleamine 2,3-dioxygenases IDOs from monotremes and marsupials. *Comp Biochem Physiol B Biochem Mol Biol.* 2009;153:137–44.
32. Lob S, Konigsrainer A, Schafer R, Rammensee HG, Opelz G, Terness P. Levo- but not dextro-1-methyl tryptophan abrogates the IDO activity of human dendritic cells. *Blood.* 2008;111:2152–4.
33. Ball HJ, Yuasa HJ, Austin CJ, Weiser S, Hunt NH. Indoleamine 2,3-dioxygenase-2; a new enzyme in the kynurenine pathway. *Int J Biochem Cell Biol.* 2009;41:467–71.