

Original Research Article

EFFECTS OF ANTI-INFLAMMATORY MEDICATION ON INDOLEAMINE 2,3 DIOXYGENASE ACTIVITY

Abstract:

Objective:

To study the effects of anti-inflammatory medication on Indoleamine 2,3 dioxygenase activity.

Research Design:

This was an investigational study.

Methodology:

Eighteen fully grown albino rats separated into control and two treated sets, both treated sets were given indomethacin (50mg/1000g). For acute treatment first treated set was sacrificed after 3.5 hrs & for chronic treatment second set was sacrificed after 3 days. However, control set animals were given an equivalent amount of vehicle.

Result:

Outcomes shows that serum IDO enzyme activity was suppressed after acute treatment while serum IDO activity were increased after chronic treatment however no significant effect was seen on brain IDO.

Conclusion:

It is concluded that indomethacin has not shown any significant effect on brain IDO. But inhibits serum IDO activity.

Key words: Tryptophan(TRP), kynurenine(KYN), Indoleamine 2,3 dioxygenase (IDO), Mesenchymal stromal cells (MSCs), tryptophan-2,3-dioxygenase (TDO).

INTRODUCTION

Tryptophan is a precursor of serotonin & kynurenine pathway metabolites, in which metabolization takes place in kidney cells, liver cells and CNS. Tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase metabolized 95% Tryptophan.^{1,2} Kynurenine pathway is triggered by social stress, depression and inflammatory elements.³ At present, numerous factors produced by Mesenchymal stromal cells (MSCs) or as a reaction with target immune cells like indoleamine 2,3-dioxygenase (IDO), PGE2, interleukin10, proinflammatory cytokines secreted by T-cells stimulates IDO & PGE2. Metabolism of TRP activated by pro inflammatory cytokines are the main cause of depression.⁴ Depression appears as a result of ailment in response to severe inflammation due to kynurenine metabolism pathway which produce neurotoxic metabolites such as quinolinic acid.^{5,6} Tryptophan metabolites are triggered due to inflammation and they have immunosuppressive properties.⁷ Inflammation reduces the serotonin accessibility & raises kynurenine synthesis.⁸ Tryptophan with the help of tryptophan-2,3-dioxygenase (TDO) mediates tumor related immunosuppression, which was stimulated by PGE2.⁹ Elevated Kynurenic acid causes psychosis, mental impairments like schizophrenia and bipolar disorders.¹⁰ Kynurenine pathway plays a key role in producing nicotinamide adenine dinucleotide, which means that it regulates immune response & essential constituent of behavioral changes in depression and schizophrenic patients. Kynurenine metabolism is altered by workout, electroconvulsive treatment & NSAIDs.¹¹

NSAIDs modulates IFN- alpha-induced neurochemical variations thus prevents depression. Indomethacin an important NSAIDs therapeutically used as analgesic, anti-inflammatory and

antipyretic agent.¹² Indoleamine 2,3-dioxygenase-1 (IDO-1) speed up the L-tryptophan metabolism, which produces numerous immunosuppressive metabolites, like kynurenine. Thus it causes immunosuppression, T cell negative regulation & contribute in tumor management.¹³ Indoleamine 2,3-dioxygenase (IDO), enhances nitric oxide synthase liable for breakdown of tryptophan and arginine.¹⁴ IDO act as main immunoregulator & transforms tryptophan into kynurenine, which causes cytotoxicity and apoptosis in tumor histology.^{15,16} IDO1 activity is low in normal tissues while raised in cancers due to induction by interferons.^{17,18} Indomethacin cox-2 inhibitor exhibited raised IDO1 inhibitory activity, which is beneficial for the malignant cells immunotherapy by suppressing interleukin-10 & prostaglandin E2.^{19,20} In this investigational study we want to evaluate the effects of indomethacin on IDO activities.

Material & Methods:

This study was take place in biochemistry department & endorsement was taken from ethical committee of KU. 18 Wistar rats of 150-250 gm weight were used in this investigational study & were kept in coops at room temperature, one week earlier the start of experiment. Rats were separated into three groups, six rats per group. Control group get 3 ml (ethanol:saline 1:2 ratio) orally, treated groups get Indomethacin(Adamjee Pharmaceutical) (50mg/1000gm/3ml) orally & sacrificed after 3.5 hr and 3 days correspondingly. Frozen sections of brain were weighed and homogenized in 12% 2ml HClO₄ & 1ml ice-cold water solution per gm of brain tissue for 1 min, them left in ice-cold tubes for 10 min. Then centrifuge for 10 min at 4⁰C, then 0.5 ml portion of filtrate were used to find out IDO enzyme activity by evaluating ratio of KYN/TRP in blood serum and CNS.²¹ L-tryptophan and Kynurenine were purchased from Sigma chemicals. For data

analysis one- way ANOVA followed by Tukey's test used. Variance between the two groups were considered significant when $P < 0.05$.

RESULTS

Acute and chronic influence of indomethacin on blood serum Indoleamine 2,3-dioxygenase activity

Statistical analysis indicates substantial influence of indomethacin on blood serum Tryptophan ($F=12.8$, $P < 0.01$), Kynurenine ($F= 112.2$, $P < 0.001$) & TRP/KYN ratio ($F=25$, $P < 0.001$) correspondingly. Tukey's test showed considerable reduction in blood serum total Tryptophan in acute (37%, $P < 0.01$) & chronic (46%, $P < 0.01$) cases correspondingly in comparison to control. Outcomes revealed a considerable reduction in blood serum Kynurenine in acute case (51.6%, $P < 0.001$) however insignificant effect on blood serum Kynurenine is observed in chronic case in comparison to control. Considerably reduced blood serum KYN/TRP ratio (25.8%, $P < 0.01$) observed in acute cases however ratio was raised in chronic case (40%, $p < 0.001$) in comparison to control. (Table 1)

Acute and Chronic influence of indomethacin on CNS indoleamine 2,3-dioxygenase activity

Statistical analysis indicates substantial influence of indomethacin on CNS Tryptophan ($F=131$, $P < 0.01$), Kynurenine ($F=27.2$, $P < 0.01$) & KYN/TRP ratio ($F=4.9$, $P < 0.01$). Tukey's test showed considerably raised CNS Tryptophan in acute case (71%, $P < 0.01$) & (81%, $P < 0.01$) in chronic cases in comparison to control. Considerably raised CNS Kynurenine in acute case (58%, $P < 0.01$) and (110%, $P < 0.01$) in chronic case in comparison to control. (Table 2)

Table 1

Acute & chronic influence of indomethacin on blood serum Indoleamine 2,3-dioxygenase activity

Parameters	control	Acute	Chronic	Anova (one way)
				df(1,15)
Blood serum (total TRP µg/ml)	14.7±1.5	9.2±0.06* 37%	7.9±.39* 46%	F=12.8 (P<0.01)
Blood serum (Kynurenine ng/ml)	620±4.4	299±31* 51.6%	646±3.8 N.S	F=112.2 (P<0.001)
KYN/TRP	49.9±5.0	37±2.8* 25.8%	69.9±0.07* 40%	F=25 (P<0.001)

values: Mean±SEM

*P<0.01

One way ANOVA was used for statistical analysis.

Table 2

Acute and chronic influence of indomethacin (50mg/kg) on CNS indoleamine 2,3-dioxygenase activity

Parameters	Control	Acute	Chronic	Anova (One way)
				Df(1,15)
CNS TRP $\mu\text{g/g}$	3.2 $\pm$ 0.05	5.5 $\pm$ 0.03* 71%	5.8 $\pm$ 0.1* 81%	F=131.1 P<0.01
CNS Kynurenine ng/g	80 $\pm$ 4.9	127 $\pm$ 13.5* 58%	168 $\pm$ 27.2* 110%	F=27.2 P<0.01
KYN/TRP	24.6	23.0 N.S	30.4 N.S	F=4.9 P<0.01

Values: Mean $\pm$ SEM.

*P<0.01

One way ANOVA was used for statistical analysis.

DISCUSSION

IDO controls the L-Tryptophan levels, and neurotoxic metabolites. Its hyperactivity raised level of kynurenine pathway metabolites especially 3-hydroxykynurenine and quinolinic acid. Enzyme activity can be calculated by (KYN/TRP) proportion. IDO act as main immunoregulator & transforms tryptophan into kynurenine, which causes cytotoxicity and apoptosis in tumor histology.^{15,16} Indomethacin cox-2 inhibitor exhibited raised IDO1 inhibitory activity, which is beneficial for the malignant cells immunotherapy by suppressing interleukin-10 & prostaglandin E2.^{19,20}

Our results showed that acute treatment of Indomethacin inhibits serum IDO but chronic treatment shows induction of IDO. Our outcomes showed the induction of Indoleamine 2,3 dioxygenase by releasing pro-inflammatory cytokines. It had insignificant impact on CNS Indoleamine 2,3 dioxygenase activity but CNS Tryptophan & kynurenine levels become raised after the indomethacin therapy. Similar results are observed by^{19,20} who said that it inhibits IDO activity. Previously it was reported that Diclofenic Sodium prevents hepatic tryptophan-2,3-dioxygenase enzyme activity in chronic therapy, whereas augments CNS Indoleamine 2,3 dioxygenase activity following both acute and chronic Diclofenic Sodium therapy, resulting in raised cerebral kynurenic acid and/or quinolinic acid concentrations.²² Furthermore similar results are also observed by²³ that indomethacin therapy (50mg/kg, intra peritoneally, 3.5hr) raised the concentration of CNS kynurenic acid participates in Schizophrenia.

CONCLUSION

It is concluded that indomethacin has insignificant impact on CNS Indoleamine 2,3 dioxygenase activity but inhibits serum IDO activity.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Tutakhail A, Boulet L, Khabil S, Nazari QA, Hamid H, Coudoré F. Neuropathology of kynurenine pathway of tryptophan metabolism. *Current pharmacology reports*. 2020 Feb;6(1):8-23.
2. Favennec M, Hennart B, Caiazzo R, Leloire A, Yengo L, Verbanck M, Arredouani A, Marre M, Pigeyre M, Bessede A, Guillemin GJ. Erratum: The kynurenine pathway is activated in human obesity and shifted toward kynurenine monooxygenase activation. *Obesity (Silver Spring, Md.)*. 2016 Aug;24(8):1821.
3. Giménez-Gómez P, Ballestín R, de Biedma-Elduayen LG, Vidal R, Ferrer-Pérez C, Reguilón MD, O'Shea E, Miñarro J, Colado MI, Rodríguez-Arias M. Decreased kynurenine pathway potentiate resilience to social defeat effect on cocaine reward. *Neuropharmacology*. 2021 Oct 1; 197:108753.
4. Meesuk L, Tantrawatpan C, Kheolamai P, Manochantr S. The immunosuppressive capacity of human mesenchymal stromal cells derived from amnion and bone marrow. *Biochemistry and biophysics reports*. 2016 Dec 1; 8:34-40.
5. Dantzer R. Role of the kynurenine metabolism pathway in inflammation-induced depression: preclinical approaches. *Inflammation-associated depression: Evidence, mechanisms and implications*. 2016:117-38.

6. Kopra E, Mondelli V, Pariante C, Nikkheslat N. Ketamine's effect on inflammation and kynurenine pathway in depression: A systematic review. *Journal of Psychopharmacology*. 2021 Aug;35(8):934-45.
7. Haruki H, Hovius R, Pedersen MG, Johnsson K. Tetrahydrobiopterin biosynthesis as a potential target of the kynurenine pathway metabolite xanthurenic acid. *Journal of Biological Chemistry*. 2016 Jan 8;291(2):652-7.
8. Regan T, Gill AC, Clohisey SM, Barnett MW, Pariante CM, Harrison NA, MRC Immunopsychiatry Consortium, Hume DA, Bullmore ET, Freeman TC. Effects of anti-inflammatory drugs on the expression of tryptophan-metabolism genes by human macrophages. *Journal of leukocyte biology*. 2018 Apr;103(4):681-92.
9. Ochs K, Ott M, Rauschenbach KJ, Deumelandt K, Sahm F, Opitz CA, Von Deimling A, Wick W, Platten M. Tryptophan-2, 3-dioxygenase is regulated by prostaglandin E2 in malignant glioma via a positive signaling loop involving prostaglandin E receptor-4. *Journal of neurochemistry*. 2016 Mar;136(6):1142-54.
10. Erhardt S, Schwieler L, Imbeault S, Engberg G. The kynurenine pathway in schizophrenia and bipolar disorder. *Neuropharmacology*. 2017 Jan 1;112:297-306.
11. Savitz J. The kynurenine pathway: a finger in every pie. *Molecular psychiatry*. 2020 Jan; 25(1):131-47.
12. Yekta RF, Amiri-Dashatan N, Koushki M, Dadpay M, Goshadrou F. A metabolomic study to identify potential tissue biomarkers for indomethacin-induced gastric ulcer in rats. *Avicenna Journal of Medical Biotechnology*. 2019 Oct; 11(4):299.
13. Costabile M, Bassal NK, Gerber JP, Hughes BP. Inhibition of indoleamine 2, 3-dioxygenase activity by fatty acids and prostaglandins: A structure function analysis. *Prostaglandins, Leukotrienes and Essential Fatty Acids*. 2017 Jul 1;122:7-15.
14. Jesus LB, Santos AB, Jesus EE, Santos RG, Grangeiro MS, Bispo-da-Silva A, Arruda MR, Argolo DS, Pinheiro AM, El-Bachá RS, Costa SL. IDO, COX and iNOS have an important role in the proliferation of *Neospora caninum* in neuron/glia co-cultures. *Veterinary parasitology*. 2019 Feb 1; 266:96-102.
15. Çakır U, Hajdara A, Székely B, Mayer B, Kárpáti S, Mezey É, Silló P, Szakács G, Füredi A, Pós Z, Érsek B. Mesenchymal-Stromal Cell-like Melanoma-Associated Fibroblasts Increase

- IL-10 Production by Macrophages in a Cyclooxygenase/Indoleamine 2, 3-Dioxygenase-Dependent Manner. *Cancers*. 2021 Jan;13(24):6173.
16. Curry JM, Besmer DM, Erick TK, Steuerwald N, Das Roy L, Grover P, Rao S, Nath S, Ferrier JW, Reid RW, Mukherjee P. Indomethacin enhances anti-tumor efficacy of a MUC1 peptide vaccine against breast cancer in MUC1 transgenic mice. *Plos one*. 2019 Nov 6;14(11):e0224309.
 17. Yang C, Ng CT, Li D, Zhang L. Targeting Indoleamine 2, 3-Dioxygenase 1: Fighting Cancers via Dormancy Regulation. *Frontiers in Immunology*. 2021;12.
 18. Yu Y, Yoo SM, Park HH, Baek SY, Kim YJ, Lee S, Kim YL, Seo KW, Kang KS. Preconditioning with interleukin-1 beta and interferon-gamma enhances the efficacy of human umbilical cord blood-derived mesenchymal stem cells-based therapy via enhancing prostaglandin E2 secretion and indoleamine 2, 3-dioxygenase activity in dextran sulfate sodium-induced colitis. *Journal of Tissue Engineering and Regenerative Medicine*. 2019 Oct;13(10):1792-804.
 19. Obata T, Shiratani S, Nada T, Kasaya Y, Arisawa M, Shuto S, Tanaka M. Structure-activity Relationship of Indomethacin Derivatives as IDO1 Inhibitors. *Anticancer Research*. 2021 May 1;41(5):2287-96.
 20. Hermankova B, Zajicova A, Javorkova E, Chudickova M, Trosan P, Hajkova M, Krulova M, Holan V. Suppression of IL-10 production by activated B cells via a cell contact-dependent cyclooxygenase-2 pathway upregulated in IFN- γ -treated mesenchymal stem cells. *Immunobiology*. 2016 Feb 1;221(2):129-36.
 21. Badawy AA, Morgan CJ. Rapid isocratic liquid chromatographic separation and quantification of tryptophan and six kynurenine metabolites in biological samples with ultraviolet and fluorimetric detection. *International Journal of Tryptophan Research*. 2010 Jan;3:IJTR-S6225.
 22. Dawood S, Wambiya EO, Bano S. Diclofenac sodium inhibits hepatic tryptophan 2, 3-dioxygenase but augments brain indoleamine 2, 3-dioxygenase activities in rats. *Journal of Basic and Applied Sciences*. 2016 Mar 2;12:140-5.
 23. Schwieler L, Erhardt S, Nilsson L, Linderholm K, Engberg G. Effects of COX-1 and COX-2 inhibitors on the firing of rat midbrain dopaminergic neurons—Possible involvement of endogenous kynurenic acid. *Synapse*. 2006 Apr;59(5):290-8.

UNDER PEER REVIEW