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Effects of IDO1 and AhR Inhibition on *Trypanosoma musculi co*-cultured with macrophages *in vitro*

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ABSTRACT

During coevolution with their hosts, trypanosomes have developed mechanisms to modulate immune responses. Some of these mechanisms involve deregulating amino acid metabolism, including L-arginine and potentially L-tryptophan metabolism. Then, we wanted to decipher tryptophan (Trp) metabolism via the IDO1 pathway, involving the AhR (Aryl Hydrocarbon Receptor) receptor. The inhibitors 1-M-LT for IDO1 and CH-223191 for AhR were used to study the involvement of IDO1. The method consisted of carrying out a series of trypanosome/macrophage co-cultures with the various inhibitors, assessing the parasite load, and then characterizing the effects of these inhibitors *in vivo*. Our preliminary results demonstrated that *in vitro* inhibitors of IDO1 and AhR distinctly reduced parasite multiplication in co-culture with macrophages. These results show that *in vitro*, IDO1 and AhR are involved in *T. musculi*-induced immunomodulation.

Keywords: *Trypanosoma musculi*; Indoleamine dioxygenase 1; Aryl hydrocarbon receptor; Trpyptophan; Macrophages; Immunomodulation; African trypanosomiasis; CH-223191; *in vitro* study.

1. Introduction

African trypanosomes are extracellular mammalian parasites transmitted by tsetse flies. In addition to human African trypanosomiasis (HAT), caused by *T. b. gambiense* and *T. b. rhodesiense* present in sub-Saharan Africa, animal trypanosomoses are caused by *T. evansi*, responsible for Surra in camels, and *T. b. brucei*, *T. congolense*, and *T. vivax* responsible for Nagana in cattle [1]. Both Surra and Nagana cause significant economic loss in developing countries and are potential threat to developed countries [1]. Despite a decline in the reported cases over the last ten years, HAT remains a danger for populations living in endemic areas, as studies have pointed to possible recombination between human and animal trypanosomes due to potential genetic exchanges [2].

As extracellular parasites, trypanosomes are confronted with host stress states induced by the organism's immune response. Thus, during their coevolution with their mammalian hosts, trypanosomes have developed mechanisms to modulate host immune responses, survive, and multiply. In addition to the sequential change in their surface glycoprotein, trypanosomes have also developed another escape mechanism via the macrophagic arginase pathway. Induction of arginase prevents the synthesis of nitric oxide (NO), which has trypanocidal properties [3],[4]. In addition, the host's arginase hydrolyzes L-arginine into L-ornithine, precursors of the polyamines responsible for trypanothione synthesis, enabling the parasite to resist oxidative stress and multiply [5],[6]. Our previous data have demonstrated the involvement of arginase in the immunomodulation induced in trypanosomiasis through the presence of a kinesin-heavy chain in trypanosomes, responsible for the induction of this arginase [7],[8].

In vivo and *in vitro*, a decrease in serum tryptophan (Trp) levels correlated with an increase in indoleamine 2,3-dioxygenase 1 (IDO1) activity was observed [9]. IDO1 is involved in T-cell immunotolerance and immune-suppression, and its expression is associated with cancer progression and poor vital prognosis [10],[11].

In macrophages, 95% of Trp is catalyzed by IDO1 to kynurenine, whose derivatives activate the Aryl Hydrocarbon Receptor (AhR), a nuclear xenobiotic receptor of interest to the immune system [12],[13]. Activation of AhR by kynurenins controls the adaptive immune response by converting naïve (Th0) T cells into regulatory T cells (T-regs) [14]. IDO1 metabolism of Trp contributes to trypanosome-induced immunopathology [15]. Furthermore, the involvement of AhR was demonstrated during experimental infection with *T. cruzi*, an intracellular parasite [16].

We, therefore, determined the involvement of IDO1 and AhR during experimental trypanosomosis using *T. musculi*, a natural parasite of the mouse and a good model for host/pathogen interaction study. Two inhibitors were used: 1-methyl-L-tryptophan (1-M-LT), a structural analog of Trp as an IDO1 inhibitor, and 2-methyl-2H-pyrazole-3-carboxylic acid (2-methyl-4-*o*-tolylazo-phenyl)-amide (CH-223191), the most potent inhibitor of AhR [17],[18].

1.1. Study objectives

The general objective of this study was to decipher experimentally the involvement of IDO1 and AhR in a murin model of African trypanosomiasis.

The specific objectives were: (i) to assess the effects of the inhibition of IDO1 on *T. musculi in vitro*; (ii) to assess the effects of the inhibition of AhR on *T. musculi in vitro*; (iii) to assess the effect of both inhibitors on *T. musculi* cultured alone; (iv) to determine the effects of serial concentration of IDO1 and AhR inhibitors in the co-culture macrophages/*T. musculi*; and (v) to determine the optimal concentration of IDO1 and AhR inhibitors for further experiments.

2. Methods

2.1. Reagents

Cells were cultured for 72 h at 37 °C (5% CO_2) in RPMI medium enriched with 2-mercaptoethanol (2ME: 10^{-4} mM), HEPES (20 mM), Sodium Pyruvate (2 mM), Penicillin/Streptomycin and fetal calf serum (SVF: 5%). Parasites were purified on a DEAE cellulose column using the ion exchange chromatography technique [19]. 1-M-LT, CH-223191, and DEAE cellulose were obtained from Sigma-Aldrich Chemie (Saint-Quentin Fallavier, France).

2.2. Parasites

The *T. musculi* strain was originally obtained from the London School of Hygiene and Tropical Medicine (Vincendeau *et al.*, 1986). Parasites are maintained in liquid nitrogen or *in vivo* by intraperitoneal injection (5.10^4 parasites per mouse) in naive Swiss mice.

Parasite multiplication was checked by culturing parasites in a macrophage-conditioned medium at different concentration ranges of 2%, 5%, and 10% Fetal Calf Serum (FCS) contained in the culture medium. After enumeration on a malassez slide, the medium containing 5% FCS with the highest yield was selected for the test (Figure 1).

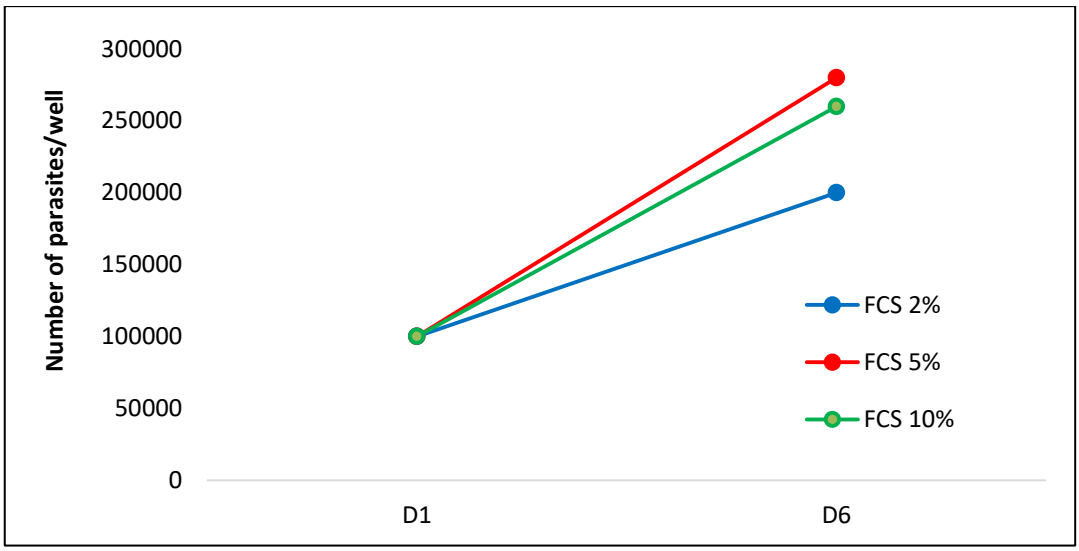

Figure 1. *Trypanosoma musculi* multiplication in culture

2.3. Co-culture of parasites with macrophages

Peritoneal macrophages were cultured with parasites in 96-well plates (10^5 parasites per 10^5 macrophages per well), in RPMI medium enriched as above, at 37 °C and 5% CO_2. The assay was performed in triplicate, and different inhibitor concentration ranges were used: 30 µM, 100 µM and 200 µM for 1-M-LT and 30 nM, 90 nM, 300 nM and 1 µM for CH-223191. Parasites were counted on a malassez slide after 72 h.

3. Results

3.1. No direct effect of inhibitors on parasite viability in the absence of macrophages

To determine parasite viability in the presence of inhibitors, we cultured the parasites alone, without feeder cells [7], but in a conditioned medium in the presence of inhibitors with control wells where the parasites were alone without inhibitors. The result showed that there was no direct effect of 1-M-LT, while CH-223191 had a very slight effect.

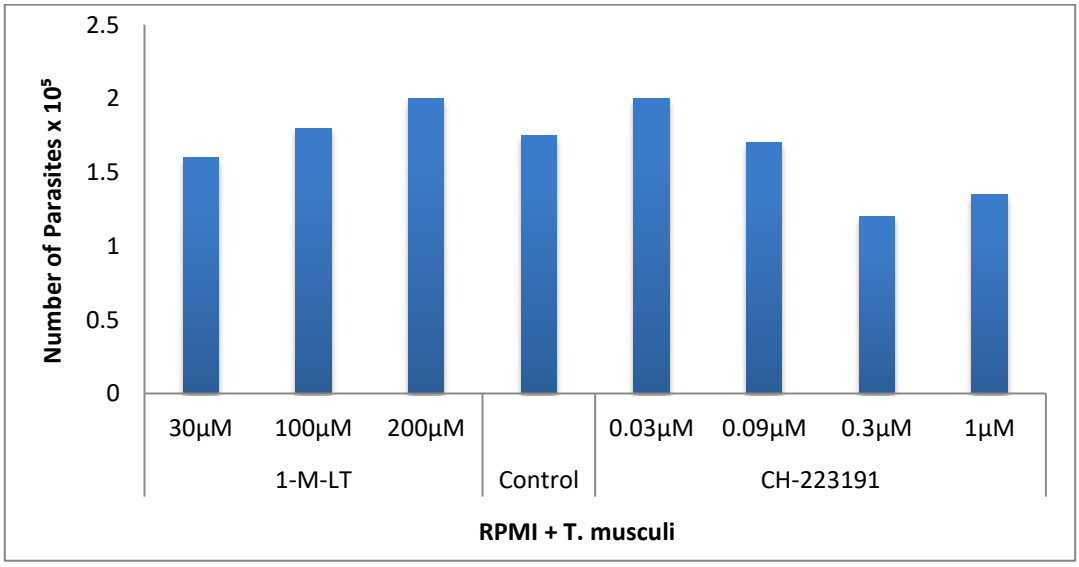

Figure 2. Direct effect of AhR (CH-223191) IDO1 inhibitors (1-M-LT) on *T. musculi* viability

3.2. AhR inhibition slows parasite growth more than IDO1 inhibition in macrophages

Parasites were cultured with macrophages in the presence of inhibitors and counted at D3 on a malassez slide. The following procedure determines the percentage inhibition:

$$Pecentage\ inhibition = \frac{\text{Number of parasites (inhibitor)}}{\text{Number of parasites (control)}} \text{ x } 100 \qquad (1)$$

The result showed that both inhibitors slowed down parasite proliferation.

When parasites were cultured with macrophages, the latter promoted their proliferation. We thus determined the effect of IDO1 inhibition, induced in immune cells, and the subsequent receptor for Trp catabolism, AhR. We found that inhibition of AhR by CH-223191 slowed parasite proliferation to a greater extent than that of IDO1 by 1-M-LT (Figure 3) and that parasite proliferation was lower when the concentration of the inhibitors was increased.

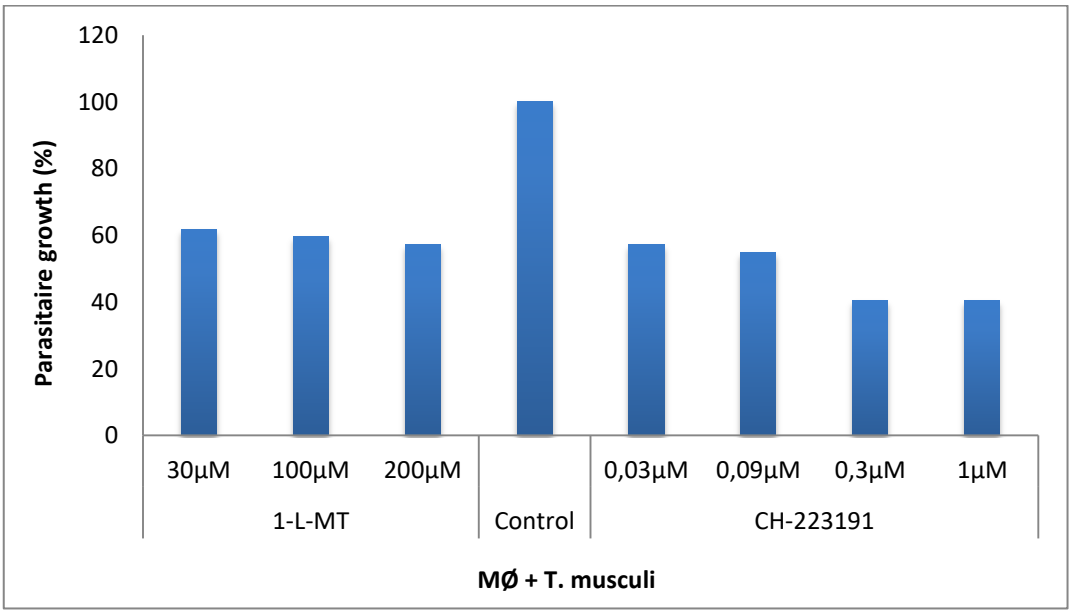

Figure 3. Effect of inhibitors on the *T. musculi* growth co-culturing with des macrophages

4. Discussion

By testing parasite viability in the absence of macrophages and the presence of inhibitors, we have seen that there is no direct effect of the latter on parasites. RPMI medium contains essential elements for cell growth, including Trp, which is essential for trypanosome survival. Studies have shown that trypanosomatidae have a different mechanism from mammals for metabolizing aromatic amino acids to indole derivatives [20]. This also suggests that IDO1 is not present in *T. musculi*. However, this requires further experiments to be confirmed. Nevertheless, the fact that parasite proliferation was slowed when the parasites were co-cultured with macrophages in the presence of the inhibitors shows that trypanosomes also benefit from the results of Trp metabolism through macrophages. A study carried out on the second phase of HAT showed that one of the Trp derivatives (quinolinic acid) metabolized by IDO1 promoted inflammation in the central nervous system, but that inhibition of kynurenine-3-monooxygenase (KMO) improved this inflammation [15]. In addition, kynurenine metabolites have immunosuppressive properties on effector T cells and promote immunotolerance through the proliferation of T-regs. Taken together, these data

suggest that kynurenine derivatives derived from Trp metabolism exert diverse effects on the host immune response and that one or more of these metabolites may promote the survival and/or proliferation of *T. musculi in vitro*.

The study of the effect of IDO1 expression is most developed for cancer therapy, and numerous clinical trials are underway to optimize the best IDO1 inhibitor for this purpose [21],[22]. In a review highlighting the properties of various IDO1 inhibitors, both 1-M-LT and 1-M-DT were cited as pivotal inhibitors that could conjointly with other drugs enhance anti-tumor activity [21]. Thus, we propose to use Epacadostat and LW-106, the best current IDO1 inhibitors [21],[23],[24] in further *in vivo* and *in vitro* experiments.

The immunosuppression of effector T cells and the hyper-activation of T-regs induced by Kynurenine derivatives are mediated by AhR activation. Inhibition of AhR during culture showed a greater slowdown in parasite proliferation than IDO1 inhibition. This could be due to the cytotoxicity of Kynurenine derivatives on parasites, as AhR promotes the production of IL-10 [25]. Previous studies have shown that imidazole compounds have trypanocidal and trypanostatic effects [26].

Structurally, CH-223191 contains an imidazole core which, together with depletion of Trp in the medium through induction of macrophage IDO1, could impair parasite proliferation, as the RPMI 1640 medium used contains little Trp. However, the parasite viability test in the presence of CH-223191 did not show a consequent direct effect of the inhibitor on parasite growth. Thus, the addition of a given amount of Trp, a kynurenine intermediate, indole acetate, or indole pyruvate, could restore *T. musculi* growth. Similarly, supplementation with macrophage growth factors such as GM-CSF or M-CSF would facilitate the macrophages' growth and prevent more Trp consumption. A final possibility would be to condition the medium with macrophage-derived factors, and culture *T. musculi* in this conditioned medium.

5. Conclusion

Work carried out on diseases other than African trypanosomiasis has demonstrated the involvement of IDO1 in the induction of immunomodulation [11],[27],[28],[29],[30], and its expression follows activation by pro-inflammatory cytokines and IFN-γ [12]. Until now, work associating IDO1 with trypanosomiasis has either been carried out on immunopathology induced in the central nervous system [15],[31], or on a model that does not reflect the course of African trypanosomiasis with extracellular parasites [16],[30]. These preliminary data indicate a subsequent involvement of IDO1 and AhR during trypanosomiasis.

Next studies could focus on the effect of IDO1 and Trp derivatives on the immune response during an *in vivo* challenge of African Trypanosomiasis. Thus, we prospect to assess the effects of IDO1 inhibition in an *in vivo* model of African trypanosomiasis with *T. musculi* and *T. b. brucei*. Furthermore, we subsequently prospect to study the IDO1 and AhR genes and proteins expression, to assess the IDO1 activity and finally, the effects of Trp derivatives during macrophages activation and parasites challenge *in vitro*.

Declarations

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Competing Interests Statement

The authors declare having no competing interest with any party concerned during this publication.

Consent for Publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors made full contribution starting from proposal writing, visualization, methodology, data analysis, first draft writing, review and editing.

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References

[1] Radwanska, M., Vereecke, N., Deleeuw, V., Pinto, J., & Magez, S. (2018). Salivarian trypanosomosis: A review of parasites involved, their global distribution and their interaction with the innate and adaptive mammalian host immune system. Front. Immunol., 9: 1–20. doi: 10.3389/fimmu.2018.02253.

[2] Gibson, W. (2015). Liaisons dangereuses: sexual recombination among pathogenic trypanosomes. Res. Microbiol., 166: 459–466. doi: 10.1016/j.resmic.2015.05.005.

[3] Gobert, A.P., Semballa, S., Daulouede, S., et al. (1998). Murine macrophages use oxygen- and nitric oxide-dependent mechanisms to synthesize S-nitroso-albumin and to kill extracellular trypanosomes. Infect. Immun., 66: 4068–4072. doi: 10.1128/.66.9.4068-4072.1998.

[4] Duleu, S., Vincendeau, P., Courtois, P., et al. (2004). Mouse Strain Susceptibility to Trypanosome Infection: An Arginase-Dependent Effect. J. Immunol., 172: 6298–6303. doi: 10.4049/jimmunol.172.10.6298.

[5] Nzoumbou-Boko, R., Dethoua, M., Gabriel, F., et al. (2013). Serum Arginase, a biomarker of treatment efficacy in human African trypanosomiasis. J. Clin. Microbiol., 51: 2379–2381. doi: 10.1128/JCM.03371-12.

[6] Fairlamb, A.H., & Cerami, A. (1992). Metabolism and functions of trypanothione in the Kinetoplastida. Annu. Rev. Microbiol., 46: 695–729. doi: 10.1146/annurev.mi.46.100192.003403.

[7] Nzoumbou-Boko, R., De Muylder, G., Semballa, S., et al. (2017). *Trypanosoma musculi* Infection in Mice Critically Relies on Mannose Receptor–Mediated Arginase Induction by a Tb KHC1 Kinesin H Chain Homolog. J. Immunol., 199: 1762–1771. doi: 10.4049/jimmunol.1700179.

[8] De Muylder, G., Daulouede, S., Lecordier, L., et al. (2013). A *Trypanosoma brucei* Kinesin Heavy Chain Promotes Parasite Growth by Triggering Host Arginase Activity. PLoS Pathog., 9: 1–14. doi: 10.1371/journal.ppat.1003731.

[9] Vincendeau, P., Lesthelle, S., Bertazzo, A., Okomo-Assoumou, M.C., Allegri, G., & Costa, C.V. (1999). Importance of L-tryptophan metabolism in trypanosomiasis. Adv. Exp. Med. Biol., 467: 525–531. doi: 10.1007/978-1-4615-4709-9_65.

[10] Munn, D.H., Zhou, M., Attwood, J.T., et al. (1998). Prevention of allogeneic fetal rejection by tryptophan catabolism. Science., 281: 1191–1193. doi: 10.1126/science.281.5380.1191.

[11] Hornyák, L., Dobos, N., Koncz, G., et al. (2018). The role of indoleamine-2,3-dioxygenase in cancer development, diagnostics, and therapy. Front. Immunol., 9: 1–8. doi: 10.3389/fimmu.2018.00151.

[12] Hubbard, T.D., Murray, I.A., & Perdew, G.H. (2015). Indole and tryptophan metabolism: Endogenous and dietary routes to ah receptor activation. Drug Metab. Dispos., 43: 1522–1535. doi: 10.1124/dmd.115.064246.

[13] Julliard, W., Fechner, J.H., & Mezrich, J.D. (2014). The aryl hydrocarbon receptor meets immunology: Friend or foe? A little of both. Front. Immunol., 5: 1–6. doi: 10.3389/fimmu.2014.00458.

[14] Quintana, F.J., & Sherr, D.H. (2013). Aryl hydrocarbon receptor control of adaptive immunity. Pharmacol. Rev., 65: 1148–1161. doi: 10.1124/pr.113.007823.

[15] Rodgers, J., Stone, T.W., Barrett, M.P., Bradley, B., & Kennedy, P.G.E. (2009). Kynurenine pathway inhibition reduces central nervous system inflammation in a model of human African trypanosomiasis. Brain, 132: 1259–1267. doi: 10.1093/brain/awp074.

[16] Ambrosio, L.F., Insfran, C., Volpini, X., et al. (2019). Role of aryl hydrocarbon receptor (AhR) in the regulation of immunity and immunopathology during *Trypanosoma cruzi* infection. Front. Immunol., 10: 1–17. doi: 10.3389/fimmu.2019.00631.

[17] Zhao, B., De Groot, D.E., Hayashi, A., He, G., & Denison, M.S. (2010). Ch223191 is a ligand-selective antagonist of the Ah (dioxin) receptor. Toxicol. Sci., 117: 393–403. doi: 10.1093/toxsci/kfq217.

[18] Kim, S.H., Henry, E.C., Kim, D.K., et al. (2006). Novel Compound 2-Methyl-2H-pyrazole-3-carboxylic Acid Aryl Hydrocarbon Receptor. Mol. Pharmacol., 69: 1871–1878. doi: 10.1124/mol.105.021832.1997.

[19] Courtois, P., Nabos, P., Nzoumbou-Boko, R., et al. (2019). Purification of Extracellular Trypanosomes, Including African, from Blood by Anion-Exchangers (Diethylaminoethyl-cellulose Columns). JoVE, 146. doi: 10. 3791/58415.

[20] Nowicki, C., & Cazzulo, J.J. (2008). Aromatic amino acid catabolism in trypanosomatids. Comp. Biochem. Physiol., 151: 381–390. doi: 10.1016/j.cbpa.2007.03.010.

[21] Prendergast, G.C., Malachowski, W.P., Du Hadaway, J.B. & Muller, A.J. (2017). Discovery of IDO1 Inhibitors: From bench to bedside. Cancer Res., 77: 6795–6811. doi: 10.1158/0008-5472.CAN-17-2285.

[22] Wang, X.X., Sun, S.Y., Dong, Q.Q., Wu, X.X., Tang, W., & Xing, Y.Q. (2019). Recent advances in the discovery of indoleamine 2,3-dioxygenase 1 (IDO1) inhibitors. Medchemcomm, 10: 1740–1754. doi: 10.1039/c9 md00208a.

[23] Poncelet, L., Ait-belkacem, R., Marillier, R., Gomes, B., & Stauber, J. (2019). Target exposure and pharmacodynamics study of the indoleamine 2, 3-dioxygenase-1 (IDO-1) inhibitor epacadostat in the CT26 mouse tumor model. J. Pharm. Biomed. Anal., 170: 220–227. doi: 10.1016/j.jpba.2019.02.038.

[24] Fu, R., Zhang, Y.W., Li, H.M., et al. (2018). LW106, a novel indoleamine 2,3-dioxygenase 1 inhibitor, suppresses tumour progression by limiting stroma-immune crosstalk and cancer stem cell enrichment in tumour micro-environment. Br. J. Pharmacol., 175: 3034–3049. doi: 10.1111/bph.14351.

[25] Huang, Y., Ogbechi, J., Clanchy, F.I., Williams, R.O., & Stone, T.W. (2020). IDO and Kynurenine Metabolites in Peripheral and CNS Disorders. Front. Immunol., 11: 1–19. doi: 10.3389/fimmu.2020.00388.

[26] Lepesheva, G.I., Hargrove, T.Y., Kleshchenko, Y., Nes, W.D., Villalta, F., & Waterman, M.R. (2008). CYP51: A major drug target in the cytochrome P450 superfamily. Lipids, 43: 1117–25. doi: 10.1007/s11745-008-3225-y.

[27] Metz, R., Smith, C., Du Hadaway, J.B., et al. (2014). IDO2 is critical for IDO1-mediated T-cell regulation and exerts a non-redundant function in inflammation. Int. Immunol., 26: 357–367. doi: 10.1093/intimm/dxt073.

[28] Ye, Z., Yue, L., Shi, J., Shao, M., & Wu, T. (2019). Role of IDO and TDO in cancers and related diseases and the therapeutic implications. J. Cancer, 10: 2771–2782. doi: 10.7150/jca.31727.

[29] Dos Santos, L.M., Commodaro, A.G., Vasquez, A.R.R., et al. (2020). Intestinal microbiota regulates tryptophan metabolism following oral infection with Toxoplasma gondii. Parasite Immunol., 42: 1–2. doi: 10.1111/pim.12720.

[30] Knubel, C.P., Martinez, F.F., Fretes, R.E., et al. (2010). Indoleamine 2,3-dioxigenase (IDO) is critical for host resistance against *Trypanosoma cruzi*. FASEB J., 24: 2689–2701. doi: 10.1096/fj.09-150920.

[31] Sternberg, J.M., Forrest, C.M., Dalto, R.N., et al. (2017). Kynurenine pathway activation in human African trypanosomiasis. J. Infect. Dis., 215: 806–812. doi: 10.1093/infdis/jiw623.