

Characterization of the kynurenine pathway in human oligodendrocytes

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Abstract. It is important to understand the involvement of oligodendrocytes in the kynurenine pathway (KP) and more particularly, their potential ability to produce neuroprotective metabolites such as kynurenic acid (KYNA) or picolinic acid (PIC), and the possibility of taking up and catabolizing the excitotoxin quinolinic acid (QUIN). These mechanisms may play a crucial role in the pathophysiology of neuroinflammatory diseases, especially multiple sclerosis. We used RT-PCR and HPLC to delineate KP enzyme expression and KP metabolite production. We characterized the KP in oligodendrocytes and showed that they lack IDO expression and are unable to catabolize tryptophan. However, the other enzymes in the pathway are present. These results indicate that human oligodendrocytes are more likely to produce neuroprotective KP metabolites such as KYNA and PIC rather than QUIN. However, because of the lack of IDO they are not able to down-regulate the immune response and as such may be more vulnerable to autoimmune phenomena. © 2007 Published by Elsevier B.V.

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1. Introduction

The kynurenine pathway (KP) is a major route of L-tryptophan (TRP) catabolism resulting in the production of several neuroactive metabolites [1] that appear to be important in

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inflammatory diseases of the brain. Knowledge of the cellular expression of the KP is limited especially in relation to oligodendrocytes where little, thus far, has been published. This study aims to characterize the KP in oligodendrocytes in order to better understand the involvement of KP in demyelinating disease such as multiple sclerosis (MS).

We used primary cultures of human fetal oligodendrocytes and compared the expression with that in the oligodendrocytic cell line MO3.13. The characterization of the KP in these cells was based on mRNA expression of eight major KP enzymes using RT-PCR. TRP and kynurenine (KYN) were quantified using HPLC and quinolinic acid (QUIN) and picolinic acid (PIC) using gas chromatography/mass spectrometry (GC/MS).

2. Materials and methods

Human fetal oligodendrocytes were prepared using a protocol adapted from Wilson et al. [2]. The MO3.13 oligodendrocyte cell line was prepared in accordance with the method previously described [3]. Cultured oligodendrocytes were stimulated with and without 100 IU/ml of IFN- γ , the most potent inducer of indoleamine 2–3 dioxygenase (IDO) the first enzyme of the KP.

Purified human primary oligodendrocytes and MO3.13 cell line were characterized for glial and oligodendrocytic markers such as Vimentin, O4, A2B5, GalC, CNPase, and MBP using immunocytochemistry. Controls were also established to determine the purity of the culture. Experiments were performed in triplicate for both the primary cultures and MO3.13.

The eight major KP enzymes, which include, (IDO), TDO, KAT-1, KAT-2, KYNase, KMO, 3HAO and QPRTase were assessed for the mRNA expression using RT-PCR that has been previously described [4].

Quantification of TRP and KYN was performed using HPLC concurrently [5] and QUIN and PIC using GC/MS [6].

3. Results

3.1. Expression of oligodendrocytic markers *in vitro*

Both the primary cultures and MO3.13 cell line, expressed all of the oligodendrocytic markers (Table 1). However, MBP, a specific marker for mature oligodendrocytes was only

Table 1
Immunocytochemical characterization of primary oligodendrocytes and MO3.13 cells

Oligodendrocytic markers	MO3.13 cell line	Human primary oligodendrocytes
A2B5	+	+
CNPase	+	+
O4	+	+
Vimentin (glial marker)	+	+
GalC	+	+
MBP	+ ^a	–

Positive staining, +; negative staining, –.

^a After treatment with PMA to induce differentiation of the cell.

Table 2

Semi-quantification of the expression 8 KP enzymes by RT-PCR in human primary oligodendrocyte and MO3.13 cell line

	Oligodendrocytes		MO3.13		PBMC
	Control	IFN- γ	Control	IFN- γ	IFN- γ
IDO	–	–	–	–	++
TDO	–	–	++	++	++
KAT-I	–	+	–	++	++
KAT-II	–	–	+	+	+
KYNase	+/-	+	+	++	++
KMO	+	++	+	++	++
3-HAO	+	+	+	+	++
QPRtase	+	+	++	++	+

No expression: –; low expression: +/-; moderate expression: +; high expression: ++.

observed in differentiated MO3.13 (after treatment with PMA). Neither the primary cultures nor MO3.13 grown in absence of PMA expressed MBP.

3.2. Expression of KP enzymes in human oligodendrocytes and MO3.13

Interestingly, IDO mRNA was not detected in either primary oligodendrocytes or the MO3.13 cell line, despite stimulation with IFN- γ (Table 2). However, we observed that MO3.13 cells expressed TDO, suggesting that only MO3.13 cells are capable of catabolizing TRP but not primary oligodendrocytes. Expression of KAT-I, KYNase and KMO was increased after treatment with IFN- γ in both primary oligodendrocytes and MO3.13. QPRtase, the catabolic enzyme for QUIN, was found constitutively expressed in both cell types.

3.3. Quantification of KP metabolites in human oligodendrocytes and MO3.13

We did not find a significant variation in the TRP concentrations in cultures of primary oligodendrocytes (Table 3), whereas there was a significant decrease in the oligodendrocytic cell line MO3.13.

The GC/MS results suggest that both primary oligodendrocytes and the MO3.13 cell line do not produce any QUIN; rather they catabolize it, as supported by the RT-PCR results

Table 3

Quantification of neosynthesis or catabolism of TRP, KYN, PIC and QUIN by human primary oligodendrocyte and MO3.13 cell line *in vitro*

	Oligodendrocytes		MO3.13	
	Control	IFN- γ	Control	IFN- γ
[TRP] (μ M)	+3.08 \pm 0.4	+4.69 \pm 0.5	-14.47 \pm 1.5	-15.85 \pm 1.6
[KYN] (μ M)	+1.54 \pm 0.16	+1.8 \pm 0.2	+0.78 \pm 0.8	+0.03 \pm 0.002
[QUIN] (nM)	-5.62 \pm 10.2	-6.85 \pm 11.5	-55.82 \pm 5	-79.89 \pm 2.6
[PIC] (nM)	+44.84 \pm 9.67	+55.55 \pm 7.1	-12.59 \pm 8	-20.59 \pm 1.1

showing QPRTase expression in both cell types (Table 2). We also found that primary oligodendrocytes produce PIC but not the MO3.13 cell line.

4. Discussion

This study provides the first insight into the characterization of the KP in human oligodendrocytes. One important finding from this study is that IDO, the first and regulatory enzyme of the KP is not expressed in human oligodendrocytes, either primary or cell line. Furthermore, we found that MO3.13 has a different profile of KP enzyme expression compared with primary oligodendrocytes. The major difference is that MO3.13 has a strong TDO expression, which gives the cell line the ability to catabolize TRP, as we demonstrated using HPLC, whereas primary oligodendrocytes are unable to catabolize TRP. This is critical in terms of TRP metabolism within the CNS and more particularly in terms of pathophysiology [7] and immuno-tolerance [8] involving oligodendrocytes. Our results are in accordance with a recent study showing that the rat oligodendrocyte cell line, OLN-93, required addition of exogenous kynurenine to be able to produce kynurenic acid (KYNA) [9]. Based on this later study, it appears that MO3.13 and OLN-93 also have different KP profiles. Moreover, OLN-93 seems lacking in IDO/TDO but expresses KAT-I and -II whereas MO3.13 expresses TDO but not KAT-II.

We found that KAT-I expression can be induced by IFN- γ (Table 2), implying that oligodendrocytes are likely to be able to produce KYNA during brain inflammation. Oligodendrocytes express QPRTase, which provides them with the capacity to catabolize QUIN (as shown in Table 3). The above data lead us to believe that oligodendrocytes are more likely to produce neuroprotective KP metabolites such as KYNA and PIC instead of neurotoxic compounds such as 3-hydroxykynurenine or QUIN. However, the absence of IDO means that the oligodendrocyte is unable to down regulate the immune response which in turn may render it more vulnerable to immune attack especially in the context of autoimmune diseases affecting the brain.

Our *in vitro* model is applicable to studies of demyelinating diseases such as MS in which KP has been shown to be involved [7,10–12]. However, data concerning the levels of KP metabolites in the serum or CSF from MS patients are controversial. For example, one study showed that KYNA is elevated in the plasma of MS patients [7] whereas another found a decrease in CSF from MS patients in remission [13]. It is plausible that one of the sources of increased KYNA during MS may be from activated oligodendrocytes. We showed that human primary oligodendrocytes express KAT-I [9], which is the major enzyme for KYNA production during inflammation [14,15]. Activation of KAT-I may lead to the increase of KYNA production from relapsing MS patients. On the other hand, oligodendrocytes do not express KAT-I or -II under normal physiological conditions as we found in this study. This might explain the discrepancy between the two studies described above. Together with the literature, our data demonstrate that the KP is very likely to play a role in MS.

In conclusion, we have optimised a method to obtain highly purified primary cultures of human oligodendrocytes derived from human fetal brain tissue. Further optimisation is necessary to obtain mature oligodendrocytes expressing MBP. We showed that both primary oligodendrocytes and the MO3.13 cell line lack expression of IDO but only

MO3.13 express TDO and is thus able to catabolize TRP. This study provides important new data about the TRP metabolism in human oligodendrocyte, which are likely to be relevant for neurologic disorders including more particularly MS.

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