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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.