

RESEARCH COMMUNICATION**Human macrophages convert L-tryptophan into the neurotoxin quinolinic acid**

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Substantial increases in the concentrations of the excitotoxin and *N*-methyl-D-aspartate-receptor agonist quinolinic acid (QUIN) occur in human patients and non-human primates with inflammatory diseases. Such increases were postulated to be secondary to induction of indoleamine 2,3-dioxygenase in inflammatory cells, particularly macrophages, by interferon- γ . To test this hypothesis, human peripheral-blood macrophages were incubated with L-[$^{13}\text{C}_6$]tryptophan in the absence or presence of interferon- γ . [$^{13}\text{C}_6$]QUIN was quantified by gas chromatography and electron-capture negative-chemical-ionization mass spectrometry. [$^{13}\text{C}_6$]QUIN was detected in the incubation medium of both unstimulated and stimulated cultures. Exposure to interferon- γ substantially increased the accumulation of [$^{13}\text{C}_6$]QUIN in a dose- and time-dependent manner. The QUIN concentrations achieved exceeded those reported in both cerebrospinal fluid and blood of patients and of non-human primates with inflammatory diseases. Macrophages stimulated with interferon- γ may be an important source of accelerated L-tryptophan conversion into QUIN in inflammatory diseases.

INTRODUCTION

Quinolinic acid (QUIN) is a neurotoxic kynurenine-pathway metabolite derived from L-tryptophan, which mediates nerve-cell killing via activation of *N*-methyl-D-aspartate-type excitatory amino acid receptors [1,2]. Substantial elevations of QUIN concentrations have been demonstrated in both cerebrospinal fluid and brain tissue of patients and non-human primates with a broad spectrum of infectious and other inflammatory neurologic diseases, including AIDS (Acquired Immune Deficiency Syndrome) [3,4], simian AIDS [5–7], poliovirus infection of the spinal cord [8], Lyme disease [9] and septicaemia [10]. The clinical relevance of these increases in QUIN are highlighted by the significant correlations between the cerebrospinal-fluid QUIN concentrations and objective measures of neuropsychological impairments in HIV (Human Immunodeficiency Virus)-1-infected patients [3,4]. Further, the measured concentrations of QUIN (low micromolar) in cerebrospinal fluid of some patients exceeded concentrations reported to be neurotoxic to certain neurons *in vitro* by up to two orders of magnitude (100 nM [2,11]). The mechanisms responsible for increasing QUIN production are unclear, but increased activity of indoleamine 2,3-dioxygenase, the first enzyme of the kynurenine pathway, in inflammatory-cell infiltrates after interferon- γ stimulation has been postulated [3,12]. However, L-kynurenine is considered to be the end-product of kynurenine-pathway metabolism in most extrahepatic cells, whereas 3-hydroxyanthranilic acid is the end-product in macrophages [13]. Furthermore, it has been reported that macrophages infected with HIV-1 do not synthesize QUIN [11]. The purpose of the present study was to determine whether human macrophages can synthesize QUIN from L-tryptophan and to investigate the effects of indoleamine 2,3-dioxygenase activation by interferon- γ on QUIN production.

MATERIALS AND METHODS**Chemicals**

L-Tryptophan, QUIN and hexafluoropropan-2-ol were ob-

tained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Trifluoroacetylimidazole was acquired from Pierce Chemical Co. (Rockford, IL, U.S.A.). RPMI-1640 medium, L-glutamine, fetal-bovine serum, gentamicin, penicillin and streptomycin were obtained from Biofluids Inc. (Rockville, MD, U.S.A.). L-[$^{13}\text{C}_6$]Tryptophan was acquired from MSD Isotopes (Montreal, Canada). Human recombinant interferon- γ was obtained from Amgen Biologicals (Thousand Oaks, CA, U.S.A.), and other reagents were obtained from Fisher (Fair Lawn, NJ, U.S.A.).

Preparation of peripheral-blood macrophages

Peripheral-blood macrophages from human volunteers were obtained from Advanced Biotechnologies Inc. (Columbia, MD, U.S.A.). Macrophages were incubated in a 75 cm³ plastic culture flask at a density of 10⁷ cells/flask in 20 ml of tissue-culture medium, consisting of RPMI-1640 supplemented with 10% heat-inactivated fetal-bovine serum, 2 mM-L-glutamine, 25 μg of gentamicin/ml, 100 units of penicillin/ml and 100 μg of streptomycin/ml. Cells were cultured with 50 μM -L-[$^{13}\text{C}_6$]tryptophan and either with or without interferon- γ (1000, 100 or 10 units/ml) at 37 °C in humidified air containing 5% CO₂. After incubation for 24 h or 48 h, the concentrations of [$^{13}\text{C}_6$]QUIN were quantified.

Measurement of QUIN and [$^{13}\text{C}_6$]QUIN

The amounts of [$^{13}\text{C}_6$]QUIN released into the incubation medium were determined by gas chromatography and electron-capture negative-chemical-ionization mass spectrometry [14]. Accuracy was obtained by using [^{18}O]QUIN, rather than structural isomers or chemical analogues of QUIN [14,15]. QUIN, [^{18}O]QUIN and [$^{13}\text{C}_6$]QUIN were esterified to their dihexafluoropropan-2-ol esters with complete retention of isotope and extracted into 500 μl of heptane. Portions of each sample were injected into a 1 m \times 0.53 mm (internal diam.) fused-silica pre-column at 80 °C, which was sealed to a 15 m \times 0.25 mm (internal diam.) DB-5 analytical column (J & W Scientific, Folsom, CA, U.S.A.). Column eluates were passed directly into the ion source

Abbreviation used: QUIN, quinolinic acid.

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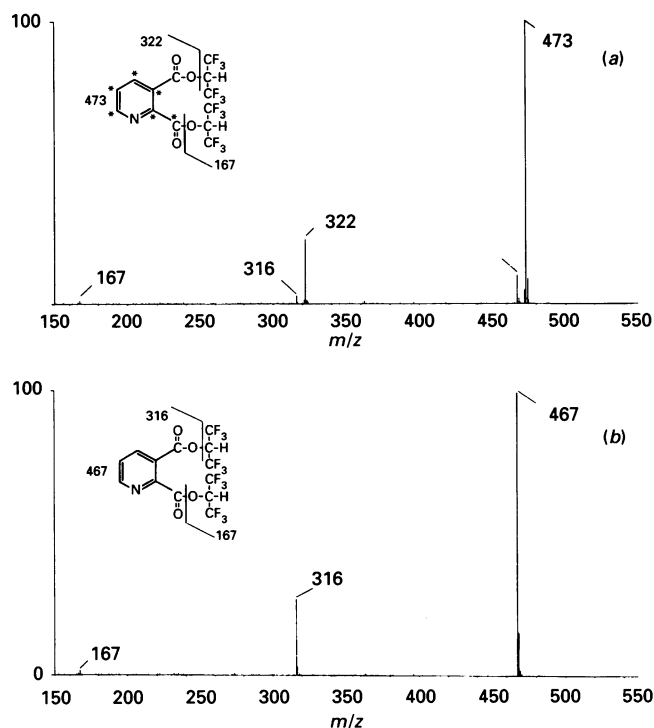


Fig. 1. Identification of $[^{13}\text{C}_6]$ QUIN synthesized from L- $[^{13}\text{C}_6]$ tryptophan by human macrophages *in vitro*

(a) Mass spectrum of $[^{13}\text{C}_6]$ QUIN in the incubation medium from human peripheral-blood macrophages incubated for 48 h in the presence of 1000 units of interferon- γ and 50 μM -L- $[^{13}\text{C}_6]$ tryptophan. The $^{13}\text{C}_6$ atoms are indicated by *. Note the presence of QUIN (m/z 467 and 316), which originated from the serum added to the incubation medium and from unlabelled L-tryptophan in the serum that was metabolized to QUIN. (b) Mass spectrum of QUIN. This culture was treated exactly the same as that used to obtain (a), except that the L-tryptophan was unlabelled. Note the absence of ion peaks corresponding to $[^{13}\text{C}_6]$ QUIN. The mass at 167 represents the loss of the unlabelled $[(\text{CF}_3)_2\text{CHO}]$ from both $[^{13}\text{C}_6]$ QUIN and QUIN. No background ion subtractions were done in either spectrum.

Table 1. Increased accumulation of $[^{13}\text{C}_6]$ QUIN derived from L- $[^{13}\text{C}_6]$ tryptophan (50 μM) in human macrophage cultures *in vitro* stimulated with interferon- γ

QUIN concentrations are means \pm 1 S.E.M. from three incubation wells per time and dose: * $P < 0.01$ versus control; † $P < 0.01$ versus 24 h.

| | [QUIN] (μM) | |
|----------------------|--------------------------|------------------|
| | 24 h | 48 h |
| Control | 2.8 \pm 0.7 | 11.6 \pm 2.3† |
| Interferon- γ | | |
| 10 units/ml | 5.8 \pm 1.3* | 19.2 \pm 0.3*† |
| 100 units/ml | 7.9 \pm 0.3* | 22.1 \pm 0.5*† |
| 1000 units/ml | 8.8 \pm 0.4* | 22.4 \pm 0.5*† |

of a Hewlett-Packard 5988 quadrupole mass spectrometer. Ions corresponding to the intact molecular anions of QUIN (m/z 467), the $[^{18}\text{O}]$ QUIN internal standard (m/z 471) and $[^{13}\text{C}_6]$ QUIN

(m/z 473) were eluted at 2.05 min at a column temperature of 111 $^\circ\text{C}$ and were quantified as peak areas with high sensitivity (minimum detectability of ≤ 50 fg). Characteristic fragments for the loss of $(\text{CF}_3)_2\text{CH}$ from QUIN (m/z 316), $[^{18}\text{O}]$ QUIN (m/z 320) and $[^{13}\text{C}_6]$ QUIN (m/z 322) were detected with the molecular anions and were always of the appropriate relative intensities to the molecular anions [14]. Mass spectra were obtained in samples that did not have $[^{18}\text{O}]$ QUIN added.

Statistical analyses

Results were analysed by one-way analysis of variance with Dunnett's t -test [16]. Regression analysis was done by the method of least squares. A P value of < 0.01 was considered statistically significant.

RESULTS

$[^{13}\text{C}_6]$ QUIN was identified in incubation media from both unstimulated and stimulated cultures at both 24 h and 48 h (Fig. 1a). No ion currents corresponding to $[^{13}\text{C}_6]$ QUIN were found in medium before the addition of L- $[^{13}\text{C}_6]$ tryptophan.

Interferon- γ increased the concentrations of $[^{13}\text{C}_6]$ QUIN in the incubation medium in a dose-dependent and time-dependent manner, compared with unstimulated controls (Table 1). The maximum concentration of $[^{13}\text{C}_6]$ QUIN that accumulated in the incubation medium was equivalent to 46% conversion of L- $[^{13}\text{C}_6]$ tryptophan into $[^{13}\text{C}_6]$ QUIN. When unlabelled L-tryptophan was substituted for L- $[^{13}\text{C}_6]$ tryptophan, no ion currents corresponding to $[^{13}\text{C}_6]$ QUIN (m/z 473 and 322) were observed (Fig. 1b). However, the concentrations of unlabelled QUIN in the incubation medium after interferon- γ stimulation equalled those of $[^{13}\text{C}_6]$ QUIN found in the media containing L- $[^{13}\text{C}_6]$ tryptophan.

DISCUSSION

These results unequivocally demonstrate that human macrophages can synthesize QUIN from L-tryptophan (Fig. 1). The enhanced accumulation of $[^{13}\text{C}_6]$ QUIN after interferon- γ administration (Table 1) is consistent with the induction of indoleamine 2,3-dioxygenase [13]. It is noteworthy that the concentrations of $[^{13}\text{C}_6]$ QUIN achieved in the incubation medium after interferon- γ stimulation actually exceeded those reported in the cerebrospinal fluid, brain tissue and blood of patients and macaques with inflammatory neurological diseases [3,5–7,9,10]. Therefore, QUIN synthesis from macrophages that have infiltrated the central nervous system could account for the QUIN concentrations measured in the cerebrospinal fluid and brain tissue. Localized macrophage infiltrates are a common feature of many inflammatory neurological diseases [17], and so it is likely that local tissue levels substantially exceed cerebrospinal-fluid concentrations and levels in non-infiltrated brain areas [8]. Indeed, after poliovirus infection of macaques, spinal-cord QUIN concentrations exceeded those in cerebrospinal fluid, blood and frontal cortex [8]. We have proposed that QUIN may be an important mediator of neuronal damage and degeneration in responsive brain regions in inflammatory neurological diseases. In this context it is noteworthy that the micromolar concentrations of QUIN achieved in the incubation medium of the present study exceeded the levels reported to be neurotoxic [2,11].

We conclude that QUIN can be synthesized from L-tryptophan in macrophages, particularly when macrophages are stimulated with interferon- γ . From the concentrations achieved in the incubation media, such cells are likely to be an important source of QUIN in patients with inflammatory neurological disease.

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