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Is the L-tryptophan metabolite quinolinic acid responsible for eosinophilic fasciitis?

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Abstract The eosinophilia-myalgia syndrome is accompanied by alterations in L-tryptophan metabolism with elevated levels of L-kynurenine and quinolinic acid having been recorded. It has been suggested that this is due to activation of indoleamine 2,3-dioxygenase by interferon-gamma. It is unknown whether these products of tryptophan metabolism play a role in the pathogenesis of this syndrome and the closely related condition of eosinophilic fasciitis. To explore this possibility, the principal author (RN) received a series of subcutaneous injections of

quinolinic acid. A total of 1200 mg was administered over a 1-month period. Peripheral blood eosinophil counts were monitored and biopsies taken for H&E and immunohistochemical stains. Over the 1-month period the eosinophil count rose from $0.3 \times 10^9/l$ to $0.8 \times 10^9/l$ before falling to $0.4 \times 10^9/l$ approximately 5 weeks later. H&E sections showed a mixed infiltrate of eosinophils and neutrophils extending through the reticular dermis and septa of the panniculus. No deep fascia was obtained on biopsy. The immunohistochemical stain for transforming growth factor beta 1 showed staining of endothelial cells and dendritic cells. The interleukin-5 stain was negative. Our results suggest that quinolinic acid may play a role in cutaneous eosinophilic disorders.

Key words Eosinophilic fasciitis • Quinolinic acid • Indoleamine 2,3-dioxygenase • Interferon-gamma • Transforming growth factor beta-1

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Introduction

The ingestion of L-tryptophan as a dietary supplement has been associated with eosinophilic fasciitis [1, 2] and the eosinophilia-myalgia syndrome (EMS) [3]. An outbreak between October 1988 and June 1989 was blamed on contaminated preparations produced at a single plant but L-tryptophan-associated eosinophilic fasciitis was reported prior to the availability of the contaminated product [4]. Tryptophan is the least common of the essential amino acids. Under the action of tryptophan hydroxylase it is metabolised to serotonin. Alternatively it may be converted to NAD via tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase (IDO) [5].

IDO is induced by interferon-gamma (IFN- γ) [5]. Therefore accelerated tryptophan degradation is seen in diseases and disorders concomitant with cellular immune

activation e.g., infectious, autoimmune and malignant [6]. Alterations in tryptophan metabolism with increased levels of the metabolites L-kynurenine and quinolinic acid have previously been reported in the EMS and the toxic oil syndrome [5, 7] and eosinophilic fasciitis [8]. The proposal investigated is eosinophilic fasciitis and the EMS can best be understood as a result of IFN- γ -induced shunting of available tryptophan through the kynurenine pathway with the metabolite quinolinic acid mediating at least some of the features of these disorders.

Amongst the kynurenine metabolites it has been established that quinolinic acid is capable of being synthesised and stored by many cell types in the immune system [9]. As such it represented the most suitable kynurenine metabolite to investigate.

To explore this possibility the principal author received a series of subcutaneous injections of quinolinic acid.

Materials and methods

Quinolinic acid crystals were obtained from Brainerd Chemical Company and dissolved in sterile water on the day of injection before being injected subcutaneously into the lateral right thigh. The dosing schedule is shown in Table 1. Doses or dosage increments were withheld on the basis of reports of potential neurotoxicity (excessive dysaesthesia, paraesthesia). Peripheral blood eosinophil counts were monitored (Sullivan and Nicolaides Pathology) and the results are graphed in Figure 1. Three-millimetre punch biopsies were taken at baseline and 1200 mg and stained with H&E. Immunohistochemical stains for transforming growth factor beta-1 (TGF- β 1) and interleukin-5 (IL-5) were also performed on the 1200-mg specimen. H&E and immunohistochemical stains were performed at the Queensland Institute of Medical Research. Photomicrographs are shown in Figures 2–6. Saline was used as a control in the lateral thigh of the left leg.

Table 1 Quinolinic acid protocol

Day	Molarity	Quantity, ml	Dose, mg	Progressive (total), mg	Notes
1	0.01	0.1	0.16	0.16	Blood
2	0.01	0.2	0.33	0.5	
3	0.01	0.3	0.50	1.0	
4	0.01	0.4	0.66	1.67	
5	0.01	0.5	0.83	2.5	
6	0.01	0.6	1.00	3.5	
7	0.01	0.7	1.16	4.67	
8	0.01	0.8	1.33	6.01	
9	0.01	1.0	1.67	7.68	
10	Withheld			7.68	Blood
11	0.1	0.2	3.34	11.02	
12	0.1	0.3	5.01	16.03	
13	0.1	0.4	6.68	22.71	
14	Withheld			22.71	Blood
15	0.1	0.6	10.02	32.73	
16	0.1	0.7	11.69	44.42	
17	0.1	0.9	15.03	59.45	
18	0.1	1.1	18.37	77.82	
19	Withheld			77.82	Blood
20	0.1	1.5	25.05	102.87	
21	0.1	1.7	28.39	131.26	
22	0.1	2.0	33.4	164.66	
23	0.1	2.5	41.75	206.41	
24	0.1	2.5	41.75	248.16	
25	Withheld			248.16	Blood
26	Withheld			248.16	
27	0.1	2.5	41.75	289.91	
28	1.0	0.25	41.75	331.66	
29	1.0	0.25	41.75	373.41	
30	1.0	2.5	417.5	790.91	
31	1.0	2.5	417.5	1208.41	
32					Biopsy

Quinolinic acid solution was prepared freshly on the day of injection and injected subcutaneously. The dosage increment is roughly linear but doses were either not increased or withheld based on evidence of neurotoxicity. Two final doses of 417.5 mg were given. Blood indicates the days a full blood count was collected. Biopsy was performed on day 32

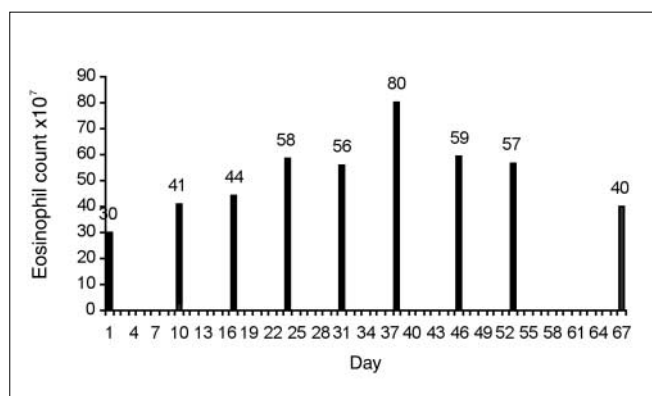


Fig. 1 Eosinophil count vs. time. Quinolinic acid was administered through to day 31. A progressive rise in the peripheral eosinophil count is found through to day 37 after which it falls. By day 67 it has reached similar values to days 10–17



Fig. 2 Baseline biopsy. This was taken prior to quinolinic acid injections. It shows normal skin

Results

The administration protocol is shown in Table 1. The administration dose for each day was incremental but alterations were made based on subjective reports of focal neurotoxicity (dysaesthesia, paraesthesia) and the weekly monitoring of peripheral eosinophil counts.

Mean reported plasma levels of quinolinic acid in 16 patients with confirmed EMS was 5018 ± 1849 nmol/l with a peak value of 15171 nmol/l [5]. This provided the basis for the initial dose concentration of 10 000 nmol/l. The tissue concentration achieved with each injection is unknown as the volume of distribution is indeterminate.

Peripheral blood eosinophil counts are graphed in Figure 1. The eosinophil count rose from $30 \times 10^7/l$ to $80 \times 10^7/l$ before falling to $40 \times 10^7/l$ 5 weeks following the cessation of

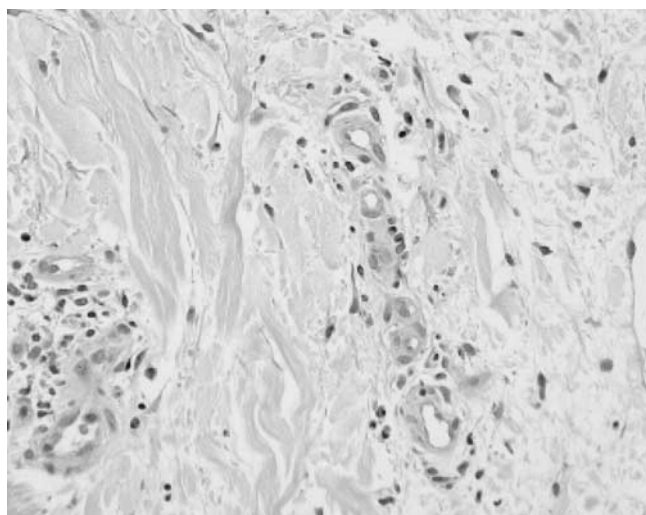


Fig. 3 Photomicrograph H&E stain 1200 mg. An interstitial infiltrate of eosinophils is evident

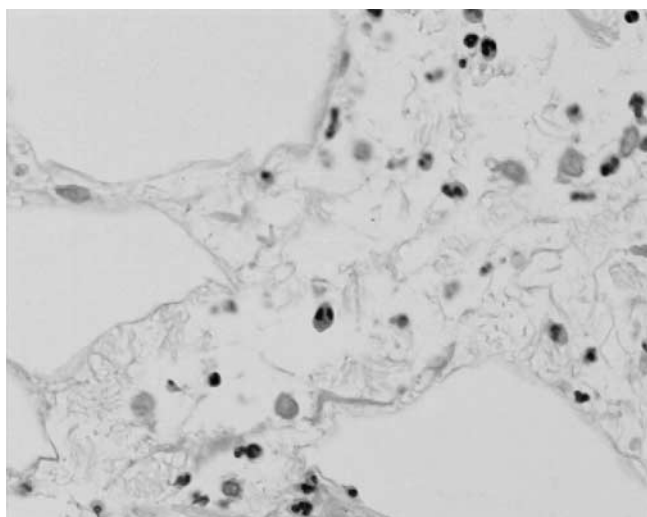


Fig. 4 Photomicrograph. H&E stain of septa of panniculus 1200 mg. Scattered eosinophils are evident throughout the septa of the panniculus. Some neutrophils are present inferiorly

the quinolinic acid injections. Although the peak value was just above normal range ($60 \times 10^7/l$), the results demonstrate a more than doubling of the baseline value and a progressive rise in response to quinolinic acid followed by a fall towards baseline. No reaction was noted at the saline site.

The photomicrographs are shown in Figures 2–6. Figure 2 is a baseline biopsy taken from the injection site prior to the commencement of quinolinic acid injections. Figures 3 and 4 show a mixed neutrophilic and eosinophilic infiltrate. The neutrophilic infiltrate is assumed to be factitial and related to repeated subcutaneous injections into the same site. Excluding neutrophils, the changes in the dermis resemble those seen in dermal hypersensitivity syndrome.

In the panniculus there is a mixed infiltrate comprising predominantly neutrophils and eosinophils extending

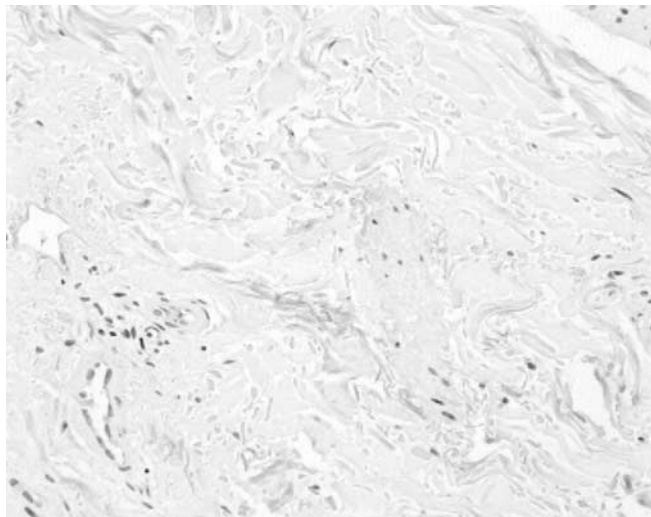


Fig. 5 Photomicrograph immunohistochemical stain for IL-5 1200 mg. There is no evidence of positive staining for IL-5

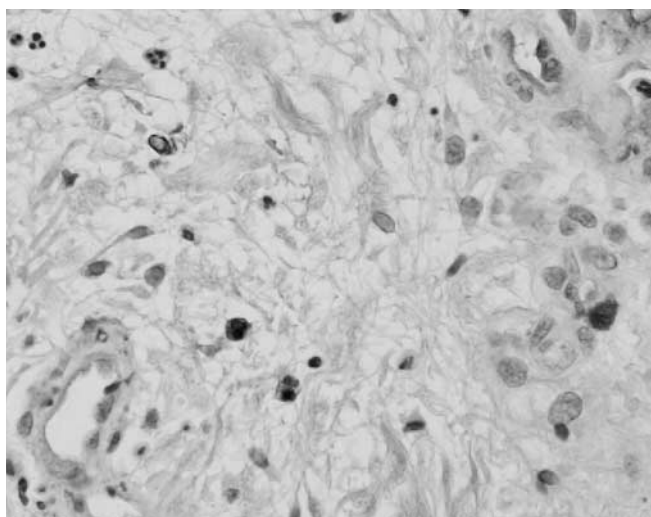


Fig. 6 Photomicrograph immunohistochemical stain for TGF-β1 1200 mg. Both endothelial cells and dendrocytes show evidence of staining

through the septa. No deep fascia was obtained in the biopsy specimen so it is impossible to comment on the presence or absence of eosinophilic fasciitis.

Discussion

In 1981 adulterated rapeseed (canola) oil was responsible for the toxic oil syndrome in Spain. It shared many features in common with the EMS and eosinophilic fasciitis and was associated with altered L-tryptophan metabolism with elevated levels of both L-kynurenine and quinolinic acid being recorded [7]. It established a mindset between these disorders and contamination and when an epidemic

of EMS occurred between 1988 and 1989 attention was directed at a contaminant found in the Showa Denko product, 1,1-ethylidenebis tryptophan, an aberrant amino acid consisting of two tryptophan molecules fused together [10]. Subsequent reports however soon established a link between non-contaminated L-tryptophan and eosinophilic fasciitis [4].

There has been an increased understanding of the physiology of kynurenines in recent years. Tryptophan availability is rate limiting for protein biosynthesis and thus cell growth [11]. Shunting available tryptophan through the kynurenine pathway depletes tryptophan in the local tissue environment. This is postulated to exert a regulatory effect on T cells [12]. Tryptophan degradation via IDO is also postulated to play a role in the ability of tumour cells to evade T-cell-mediated rejection [13] and in the prevention of foetal rejection by the maternal immune system in pregnancy [14]. Tryptophan depletion also functions as an antimicrobial defence mechanism [15]. Munn and his co-workers proposed tryptophan starvation as being central to the effect exerted by IDO on T-cell proliferation [16], however later work has introduced the kynurenine metabolites themselves as immunoregulatory molecules promoting the selective apoptosis of Th1 but not Th2 cells [17].

IDO is expressed in many cell types but is relatively high in the lung, small intestine and placenta [18].

L-tryptophan ingestion is the most frequently reported association of eosinophilic fasciitis however eosinophilic fasciitis may be seen as a para-neoplastic phenomenon [19] and following trichloroethylene exposure [20]. The association with neoplasia is best interpretable in the context of tumour evasion of T-cell immune responses [13]. Trichloroethylene has been demonstrated to increase IFN-γ production from CD-4 T cells in mice [21].

There are additional reasons to imply an aetiological role for the kynurenine metabolites in eosinophilic fasciitis in addition to their role in Th1 apoptosis.

Firstly eosinophilic fasciitis and the EMS are associated with peripheral neuropathy [22, 23] and quinolinic acid is a neurotoxin of the excitotoxin class acting as an agonist at N-methyl-D-aspartate-receptors [24].

Second, the onset of eosinophilic fasciitis often follows strenuous exertion. Induction of IDO with elevated kynurenine levels has been demonstrated to occur following exercise [25].

The aim of this study was to investigate the possibility that the kynurenine metabolite quinolinic acid is capable of mediating some of the features of eosinophilic fasciitis in a human subject. It is proposed that eosinophilic fasciitis and eosinophilia myalgia syndrome arise as a result of immune activation and IFN-γ induction. This is supported by the known associations with neoplasia [19] and trichloroethylene exposure [20]. IFN-γ is a known inducer of IDO [5], which promotes the degradation of tryptophan into kynure-

nine metabolites. It is postulated that at least one of these, quinolinic acid, is capable of mediating some or all of the features of the disease. It has been established that quinolinic acid levels are elevated in eosinophilic fasciitis [8].

A total of 1200 mg of quinolinic acid was injected into the lateral thigh of the principal author over a 1-month period. A biopsy was taken the day following the completion of the protocol. This revealed a dense mixed infiltrate with many eosinophils and neutrophils involving the reticular dermis and septa of the panniculus. The immunohistochemical stains for TGF- β 1 showed staining of endothelial and dendritic cells. TGF- β is known to be synthesised by dendritic cells [26] and endothelial cells [27]. The IL-5 stain was negative, possibly due to technical difficulties. No deep fascia was obtained in the punch biopsies so it was not possible to comment on the presence or absence of eosinophilic fasciitis.

During the protocol the peripheral eosinophil count progressively rose before slowly falling towards baseline following cessation of the quinolinic acid injections.

The data suggest kynurenine metabolites may play a role in cutaneous eosinophilic disorders and further research is warranted. Inhibitors of this pathway may have a role to play in the management of cutaneous eosinophilic disorders.

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