Short Communication

Serum Neopterin Levels and Metal Binding to Transferrin in Parkinson's Disease Patients

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Introduction

Macrophages stimulated by interferon-gamma produce large quantities of neopterin (1). The level of neopterin in body fluids is elevated in viral infections such as hepatitis and HIV, as well as in malignancies, autoimmune disorders and allograft rejection (2). Hence, the level of neopterin can be used as a clinically useful marker for monitoring immune activation in a patient (2). Often associated with increased levels of neopterin in immune activated patients is enhanced excretion of zinc (Zn) (3, 4). Many metals are transported in the body bound to the iron (Fe) transport protein Transferrin (Tf) (5). At any time, 70% of the Fe binding sites are empty and this allows other metals to bind without competition from Fe. Hence, a possible explanation for the increased output of Zn in immune activated patients is a reduction in metal binding to Tf. We have studied the binding of Fe and Gallium (Ga) (an analogue of Al) to Tf in patients with Parkinson's disease (PD). Defects in metal binding and immune factors may be important in the pathogenesis of PD (7, 8). We now report an inverse correlation between the binding of Ga to Tf and the level of neopterin in plasma in PD patients suggesting that immune activation may influence metal binding to Tf.

Materials and Methods

Materials

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Chemicals were supplied by Sigma Chemical Co. Ltd., Poole, UK; Aldrich Chemical Co, Gillingham, UK and BDH Chemicals LTD., Poole, UK. Radio-isotopes were supplied by Amersham International PLC, Aylesbury, UK. ⁶⁷Ga was used as a marker for Al binding in plasma and was supplied as ⁶⁷GaCl₃ in 0.4 M HCl. ⁵⁹Fe was supplied as ⁵⁹FeCl₃ in 0.1 M HCl.

Patients

Ten volunteers living in the community without neurological disease were used as control patients for the metal binding studies. Plasma from 28 PD patients was obtained from Dr. H. Pall (Queen Elizabeth Hospital, Birmingham, UK at the time of clinical diagnosis and prior to drug therapy. In addition, eleven healthy individuals without evidence of infection, malignant or neurological disease were used as control patients for the neopterin study.

Tf binding studies

Binding of ⁶⁷Ga and ⁵⁹Fe to Tf was determined in 900 µl plasma samples, incubated with either 1~5 µC of ⁶⁷Ga or ⁵⁹Fe, by gel filtration chromatography using Sephadex G75 beads in a 60 cm × 1.6 cm glass column (Amicon Ltd, Stonehouse, UK) as described previously (6, 8). Fifty 6 ml fractions were collected from the column and counted for gamma emissions.

Neopterin

Serum neopterin levels were measured in control

Table 1. Serum neopterin and binding of Fe and Ga to Transferrin in samples of control plasma (C) and plasma from Parkinson's disease (PD) patients (n=number of patients examined, SD=Standard deviation).

	C	PD
Fe-Tf binding (%)	100 (n=5)	100 (n=10)
Ga-Tf binding (%)	74.7 (n=10, SD=5.7)	54 (n=28, SD=15.6)
Oxidised serum neopterin (nmol/l)	5.45 (n=11, SD=1.49)	8.85 (n=28, SD=4.2)

Ga-Tf C v PD $t=3.45$ (36 Df, $p<0.001$); Neopterin C v PD $t=2.43$ (37 DF, $p<0.05$).

Figure 1. The relationship between serum neopterin levels and Ga-Tf binding in 28 untreated Parkinson's disease patients; (◆) High binders ($r=0.74$, $p<0.001$), (◻) Low binders ($r=0.61$, $p<0.02$). Comparison between regression lines: Slopes ($F=2.53$, $p>0.05$), Elevations ($F=99.92$, $p<0.001$).

and PD plasma by radioimmunoassay (IMMUTest, Henning-Berlin, Germany).

Results

Binding of Fe to Tf was 100% in all control and PD

Binding of Fe to Tf was 100% in all control and PD patients examined (Table 1). By contrast Ga-Tf binding values varied from 20% to 82%. The mean percent Ga-Tf binding was significantly reduced in the PD population compared with controls ($p<0.001$). Mean serum levels of neopterin were significantly elevated in PD ($p<0.01$) compared with healthy controls. The relationship between Tf binding and neopterin in the PD patients is shown in Fig. 1. It is apparent that two populations of PD patients were present. In both groups, Ga-Tf binding declined with increasing levels of serum neopterin.

Regression lines fitted individually to these two groups had significantly negative slopes confirming that increased neopterin was associated with a dec-

rease in Ga-Tf binding. The two regression lines had significantly different elevations ($p<0.001$) confirming the presence of two PD populations. However, the regression lines did not differ in slope ($p>0.05$) suggesting a similar relationship between neopterin and Ga-Tf binding in the two groups.

Discussion

Two groups of PD patients were identified which may correspond with the high and low binders reported by Hodgkins (6). High binders were defined by Hodgkins as having Ga-Tf binding levels of $>50\%$. However, Ga-Tf binding depends on the level of neopterin and hence, high and low Ga-Tf binding should be defined in relation to the level of neopterin. The present data suggest that at low neopterin ($\leq 5 \text{ nmol l}^{-1}$) high and low binders should have Ga-Tf binding levels of $>65\%$ and $<55\%$ respectively. At high neopterin ($\geq 10 \text{ nmol l}^{-1}$) high and low binders should have Ga-Tf binding of $>50\%$ and $<40\%$ respectively. A negative correlation was found between the levels of neopterin and Ga-Tf binding in both groups of PD patients. This implies either that defects in metal binding to Tf could be responsible for the cellular immune response or that a cellular immune response removes Ga from Tf. Metal ions including mercury and beryllium can induce immune reactions in the body (9, 10). However, before a metal ion initiate an immune response in a patient it has to be presented to the immune system in an antigen presenting cell via the mutual histocompatibility locus system (11). Since, it is likely that metals enter cells via a Tf receptor mediated endocytosis (12), it is not likely that reductions in binding to Tf would be associated with an increased cellular immune response. Hence, it is more plausible that the cellular immune response in PD patients reduces the binding of Ga to Tf. No reduction in Fe-Tf binding was observed which suggests that metals with weaker binding coefficients e.g., Ga and Zn are removed readily from Tf by the immune response. It is possible that increased oxidative activity resulting from the activation of macrophages is responsible for the reduction in Ga-Tf binding in PD patients (13). We suggest that alterations in metal binding to Tf as a consequence of the immune response could explain the elevated urinary Zn levels observed in immune activated patients (3, 4).

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