Original article

Infliximab effect on serum IGF-1 and IGFBP-3 in active inflammatory bowel disease

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SUMMARY

AIM: There are few studies that have investigated the changes of the insulin-like growth factor (IGF) system in inflammatory bowel disease (IBD) and the majority of these studies have examined the alterations of the GH-IGF axis in growth-retarded IBD children. Moreover, the impact of the chimeric monoclonal anti-TNFα antibody (Infliximab) in the IGF system in patients with Crohn's disease (CD) or ulcerative colitis (UC) has not yet been investigated.

PATIENTS-METHOD: Five IBD patients (3 with Crohn's and 2 with ulcerative colitis) and five age and sex matched controls were included in this study. In all patients and controls a baseline serum sample for IFG-1, IGFBP-3 and IL-6 was obtained one week before Infliximab treatment initiation. During the first four times of Infliximab administration morning fasting serum samples were obtained from each patient. At the same time, fasting morning control samples were also obtained.

RESULTS: Short-term effect of infliximab administration (baseline-1st dose comparison) resulted in a statistically significant increase in serum IGFBP-3 levels (p=0.39) while no statistically significant change was noticed in IL-6 and IGF-1 levels. After four doses of Infliximab (long term effect, baseline-4th dose comparison) a statistically significant increase in serum IGF-1 and IGFBP-3 levels was noticed (p=0.02 in both parameters) while IL-6 was decreased to a statistically significant exlent (p=0.01).

DISCUSSION: This study demonstrated that Infliximab can influence the IGF system in IBD patients. This increase of serum IGF-1 and IGFBP-3 with concomitant decrease in IL-6, may represent a probable mechanism through which this drug acts in these patients, in whom there is a evidence of an impaired GH/IGF axis.

Key words: inflammatory bowel disease, ulcerative colitis, Crohn's disease, infliximab, IGF system, interleukin

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INTRODUCTION

There is a rarity of studies that have investigated the changes of the insulin-like growth factor (IGF) system in inflammatory bowel disease (IBD). The majority of these studies have mainly investigated the alterations of the IGF system in growth-retarded IBD children.[1-2] Moreover, the impact of the chimeric monoclonal anti-TNFα antibody (Infliximab) on the IGF system In patients with Crohn's disease (CD) or ulcerative colitis (UC) has not yet been studied.

It has been generally thought that the serum IGF system which includes IGF-1, IGF-2 and their binding proteins (IGFBPs), IGFBP-3 being the most important, is down-regulated during active inflammatory bowel disease through several systemic mechanisms.[3-4] These mechanisms have been proposed to include the acquired growth hormone resistance (AGHR) phenomenon[5] and the induction of a cascade of increased circulating levels of inflammatory cytokines such as interleukins 1a, 1b, 6 and tumor necrosis factor-α (TNFα).[6]

Short and long-term effects of these down-regulating mechanisms on the IGF system are probably reflected in current clinical practice as growth retardation, loss of appetite or weight gain failure.[7-9]

In adult non-steroid dependent patients with Crohn's disease, long term IGF system down-regulation has so far been reported[10] but no reports about the further im-

pact of serum IGF system down-regulation on bowel healing, stricturing or fistulizing have so far been presented in in vivo studies.

The continuously increasing experience of Infliximab use in inflammatory bowel disease patients, especially those with CD, may represent an ideal paradigm to trace and describe in vivo the impact of the TNFa blocking on the serum IGF system in this chronic bowel inflammatory condition.

Thus, in active inflammatory bowel disease, evidence of whether, how, and how much Infliximab administration can stabilize the serum IGF system by blocking TNFα, could be of pathophysiologic, therapeutic and even prognostic interest.

The aim of the present pilot study was to examine the probable short- and long-term changes in the levels of serum IGF-1, IGFBP-3 and IL-6 in adult patients with active inflammatory bowel disease after Infliximab administration.

MATERIALS AND METHODS

Patients

Five patients with inflammatory bowel disease (IBD) were included in the study. Three patients (one female and two male) had Crohn's disease (CD) and two female patients had ulcerative pancolitis (UC). The mean age of the patients was 28.4 years (range 19-42 years).

The diagnosis of inflammatory bowel disease (IBD), Crohn's disease or ulcerative colitis (UC) was made according to clinical, endoscopic, radiologic and histologic criteria. Diagnosis of all these IBD patients was made at least five years ago and all patients are regularly being followed up in the IBD outpatient clinic of our Department. During last year Infliximab use (5mg/kg iv) [*Remicade, Schering Plough Co*] under certain protocol surveillance was decided on in order to achieve IBD remission; it is noteworthy that no patient was diagnosed with another chronic disease or was steroid-dependent in that period. All five patients were prospectively investigated for probable changes of serum levels of IGF-1, interleukin-6 (IL-6) and IGFBP-3 after Infliximab use. For comparison serum samples of five healthy volunteers, matched for sex, age and body mass index (BMI=Kg/m^2), were also examined for IGF-1, IGFBP-3 and IL-6 at the same time.

In all patients and controls a baseline serum sample for IFG-1, IGFBP-3 and IL-6 was obtained one week before Infliximab treatment initiation. During the first four times of Infliximab administration (first, second, sixth and twelfth week) morning fasting serum samples were obtained from each patient the day after each Infliximab administration (4 samples from each patient). At the same time, morning fasting, age and sex matched control samples were also obtained. Serum IGF-1 half time ($t^{1/2}$) is about 16 hours. All samples were stored at -70^0 C until assayed. Our hospital scientific committee approved the study protocol.

Methods

Serum IGF concentrations were determined by RIA after separation from the binding proteins, using a kit from Nichols Institute Diagnostics. The dissociation of IGF-I and proteins was carried out with acid extraction (0.5 N HCl) of the serum samples and differential elution from C-18 sep-pak cartridges (Waters Assoc, MA, USA). The intra- and inter-assay CVs were 3 and 10% respectively.

Competitive protein-binding RIA, directly in serum samples, using the kit from Nichols Institute Diagnostics, measured IGFBP-3. The intra- and inter-assay CVs were 3.5 and 6.5%, respectively.

The cytokine interleukin-6 (IL-6) was measured in serum using the RNT Quantikin kit for human IL-6 (Minneapolis, USA). The intra- and inter-assay CVs were 1.6-4.2% and 3.3-6.4% for the concentration ranges measured.

Statistical analysis

Mean values for IGF-1, IGFBP-3 and IL-6 between the patient and control group were compared. Comparisons were also made between the baseline values (before Infliximab treatment) of IGF-1, IGFBP-3 and IL-6 and the first and twelfth week values (values after one and four doses of Infliximab). Separate analysis of CD and UC patients was also conducted. The Student's t-test was used for these comparisons.

RESULTS

Body mass index values were comparable between patients and controls. Baseline serum concentrations of IGF-1, IGFBP-3 and IL-6 in patients and controls were compared. Serum concentrations of IGF-1, IL-6 and IGFBP-3 after each Infliximab dose (1,2,3,4) for all patients were measured and were compared with the baseline values. Short term effect (baseline values-end of 1st dose values) and long term effect (baseline-end of 4th dose values) of Infliximab administration in serum IGF-1, IL-6 and IGFBP-3 concentrations in all patients was assessed.

The mean serum IGF-1 and IGFBP-3 concentrations in the patient group were significantly lower compared to the control group, while IL-6 serum levels of the patients was significantly higher than that of the control group (data not shown). The short-term effect of infliximab administration (baseline-1st dose comparison) resulted in a statistically significant increase in serum IGFBP-3 levels (p=0.39) while no statistically significant change was noticed in IL-6 and IGF-1 levels.

After four doses of Infliximab (long term effect, baseline-4th dose comparison) a statistically significant increase in serum IGF-1 and IGFBP-3 levels was noticed (p=0.02 in both parameters) while IL-6 was decreased in a statistically significant way (p=0.01) [Table 1]. No differences were noticed between UC and CD patients.

DISCUSSION

The present study emphasizes once again that circulating levels of IGF-1 and its binding protein IGFBP-3 are reduced in adult patients with active IBD in the form of CD and ulcerative colitis.[10]

The reason for the reduced IGF-1 and IGFBP-3 levels in patients with IBD is unclear although high levels of circulating interleukins such as IL-1 and IL-6 and TNFα could be an evident cause. Normally, circulating IGF-1 is derived mainly from the liver under the direct action of GH and mediates its anabolic actions in various peripheral tissues.[11] IGFBP-3, which binds most of the IGF-1, is also produced in the liver under direct regulation by GH.[12-13]Thus, circulating IGF-1 and IGFBP-3 levels are good indicators of peripheral GH action. In addition to GH, IGF-1 is controlled by other factors such as fasting and nutritional status.[14-16] Clinical studies have shown that under nutrition may contribute in part to low IGF-1 and growth retardation in children with CD but this may not be the whole explanation.[17-18]

Recent studies suggest that the low IGF-1 levels may be due to a direct adverse effect of circulating inflammatory cytokines including TNFa and experimental and clinical studies have provided ample evidence of this.[19-20] Thus, animals with experimental colitis have increased plasma concentrations of IL-6 and low concentrations of IGF-1 but not GH. Transgenic mice which overexpress IL-6 have growth impairment and reduced plasma levels of IGF-1[21].The in vivo administration of TNF-a or IL-1 in rats is capable of altering plasma concentration of IGF-1 in a similar way and IL-1 receptor antagonists attenuate this effect.[22-23]

The mechanisms through which inflammatory cytokines cause acquired GH resistance leading to low IGF-1 levels are not clear. In vitro studies, however, have shown that IL-1β and TNFα inhibit GH receptor mRNA, IGF-1 mRNA and IGF-1 protein production in tissue culture.[24] The same cytokines may also blunt the IGF mRNA response to GH in rat hepatocyte primary culture.[25]

In conclusion, adult inflammatory bowel disease is associated with reduced circulating IGF-1 and IGFBP-3 levels, findings suggestive of acquired GH resistance syndrome, probably induced by inflammatory cytokines, as also happens in retarded children with juvenile chronic arthritis.[26]

Infliximab is a recently produced chimeric anti-TNFα monoclonal antibody which is widely used in rheumatoid arthritis in Crohn's disease. Infliximab use in ulcerative colitis is also under investigation in several clinical trials worldwide. These diseases are considered as autoimmune phenomena of gut mucosa and are characterised by high TNFα levels.

The basic Infliximab action of reducing TNFα levels may prove to be only one of its actions. This study clearly showed that Infliximab, either in short or long term use, can also influence serum IGF system in IBD patients. This effect probably starts with an IGFBP-3 increase continued by IGF-1 and IGFBP-3 increase and IL-6 decrease as a late event. This increase of serum IGF-1 and IGFBP-3, in concord with IL-6 decrease, in adult IBD patients after Infliximab use may be another probable mechanism of this drug is action in these groups of patients in whom there is continual supporting evidence of impaired GH/IGF axis. This study also provides direct evidence of the immediate

Table 1. Pre- and post (end of 1st and 4th dose) Infliximab use serum levels of IGF-1, IGFBP-3 and IL-6 in non-steroid dependent patients with active inflammatory bowel disease.

Serum parameters	P (baseline-1st dose)	P (baseline-4th dose)
IGF-1	NS	0.02
IGFBP-3	0.39	0.02
IL-6	NS	0.01

results of Infliximab in modulating several serum levels of markers of inflammation. Whether this is only an indirect effect due to TNFα suppression or an autonomous direct drug action to hepatic tissue has to be further investigated. Another important question that arises is whether efforts to normalize of the IGF system in CD and UC should be considered as a primary or secondary therapeutic target or as a response indicator.

This up-regulating Infliximab effect on the IGF system is an in vivo paradigm of the pathophysiologic action of the drug but also, probably, a quantitative measure of the drug impact on stricturing and fistulizing procedures. This last is stressed because IGF system is considered to be actively included in collagen synthesis and overexpression in mesenchymal and bowel mucosal cells in IBD patients.

Authors note: During this clinical study a female patient (57 years old) with rheumatoid arthritis was also followed up for serum IGF system short and long term changes during Infliximab therapy and showed similar results; the number of Infliximab doses increased in this patient, serum IGF-1 and IGFBP-3 levels showed a clear tendency for stabilization at higher levels compared to those before the initiation of treatment.

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