Physiol. Res. 52: 89-93, 2003

Changes in Acute Phase Proteins after Anti-Tumor Necrosis Factor Antibody (Infliximab) Treatment in Patients with Crohn´s Disease

V. KUPČOVÁ[1], L. TURECKÝ[2], Z. DETKOVÁ[1], M. PRÍKAZSKÁ[3], A. KELEOVÁ[3]

[1]Third Department of Internal Medicine, Dérer´s Hospital, Medical Faculty of Comenius University, [2]Institute of Chemistry, Biochemistry and Clinical Biochemistry, Medical Faculty of Comenius University, and [3]Institute of Preventive and Clinical Medicine, Bratislava, Slovak Republic

Received February 26, 2002
Accepted July 2, 2002

Summary

Acute phase proteins and markers of proteosynthetic activity reflect the clinical activity in Crohn´s disease (CD). The impact of anti-tumor necrosis factor antibody (anti-TNF) therapy on serum levels of acute phase proteins and proteosynthetic markers was studied. Fourteen patients with active CD were treated with 5 mg per kg of anti-TNF in intravenous infusion. Clinical activity (assessed by Crohn´s disease activity index – CDAI), α-1-acid glycoprotein, haptoglobin, cholinesterase and prealbumin were assessed before and in months 1 and 5 after treatment. A sustained decrease in CDAI was observed. This was accompanied by a significant decrease in α-1-acid glycoprotein and haptoglobin in month 1 ($p=0.005$ and $p=0.01$, respectively) while in month 5 the levels of both acute phase proteins rose significantly ($p=0.003$ for α-1-acid glycoprotein and $p=0.02$ for haptoglobin). Cholinesterase and prealbumin significantly increased in month 1 after the treatment ($p=0.02$ and $p=0.0006$, respectively), the increase was sustained in cholinesterase while prealbumin levels diminished in month 5. We conclude that the clinical improvement after anti-TNF therapy for CD is accompanied by changes of acute phase proteins and proteosynthetic markers. The assessment of these laboratory markers may be useful in the management of CD patients treated with anti-TNF.

Key words

Crohn´s disease • Anti-tumor necrosis factor antibody • Acute phase proteins • Cholinesterase • Prealbumin

Introduction

Crohn´s disease (CD) is an inflammation of the intestinal mucosa which is mediated by T helper 1 (Th 1) cytokines (Mullin *et al.* 1992, Pullman *et al.* 1992) that stimulate hepatic synthesis of acute phase proteins, namely α-1-acid glycoprotein (Pous *et al.* 1990) and haptoglobin (Raynes *et al.* 1991).

Clinical studies showed that acute phase proteins rise in clinically active CD and correlate with the clinical activity (Andre *et al.* 1981, Cellier *et al.* 1994). On the other hand, the synthesis of proteosynthetic markers

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

(cholinesterase, albumin, prealbumin) is diminished in active CD (Richter *et al.* 1982, Tromm *et al.* 1992).

A novel therapeutic procedure for CD, chimeric anti-tumor necrosis factor antibody (anti-TNF, Infliximab) down-regulates the Th 1 response (Plevy *et al.* 1997, Baert *et al.* 1999) and seems to have a considerable effect on clinical, histological and endoscopic activity (Targan *et al.* 1997, D´Haens *et al.* 1999). Therefore, the administration of this agent in CD may be followed by changes of acute phase proteins and proteosynthetic markers.

In the study presented here, acute phase proteins and markers of proteosynthetic activity were assessed in CD patients treated with anti-TNF together with the follow-up of the clinical therapeutic effect.

Methods

Fourteen patients (9 women and 5 men, mean age 36 ± 9 years) with active moderate to severe CD were treated with 5 mg per kg of anti-TNF in intravenous infusion. Concomitant medication, 5-aminosalicylic acid-containing drugs (13 patients) and corticosteroids (3 patients), remained unchanged. Before the patients had been included in the study, basic hematological (blood count, parameters of coagulation) and biochemical (urea, creatinine, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, alkaline phosphatase, sodium, potassium) parameters were checked together with the chest X-ray in order to exclude any contraindication against the treatment with anti-TNF. Clinical activity, α-1-acid glycoprotein, haptoglobin, cholinesterase and prealbumin were assessed in all patients before treatment (Sample 1), in month 1 after treatment (Sample 2) and in 12 patients in month 5 after the administration of anti-TNF (Sample 3). At the same time, basic hematological (blood count, parameters of coagulation) and biochemical (urea, creatinine, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, alkaline phosphatase, sodium, potassium) parameters were followed in all patients.

Clinical activity was assessed by Crohn´s disease activity index (CDAI) (Best *et al.* 1979) that represents a composite measure of frequency of loose bowel movements, severity of abdominal pain, general well-being, extraintestinal manifestations, presence of abdominal mass, use of antidiarrheal drugs, hematocrit and body weight.

Serum α-1-acid glycoprotein, haptoglobin, cholinesterase and prealbumin levels were obtained by immunodiffusion method (Laurell 1966) using a monospecific antiserum (USOL Prague).

The study was conducted with the agreement of the Institutional Ethics Board and an informed consent was obtained from each patient.

For statistical analysis non-parametric methods were used. Data are expressed as means and standard deviations and were evaluated by Wilcoxon´s test with a significance limit of $p<0.05$.

Results

Treatment was well tolerated, no side-effects necessitating interruption of the infusion were observed. After the treatment, no important changes in basic hematological and biochemical parameters were noted.

In clinical activity, there was a significant drop in CDAI from 236 ± 54 before treatment (Sample 1) to 132 ± 76 in month 1 after treatment (Sample 2, $p=0.0003$). During the further follow-up a slight non-significant increase in CDAI to 145 ± 93 was observed in month 5 (Sample 3, Fig. 1).

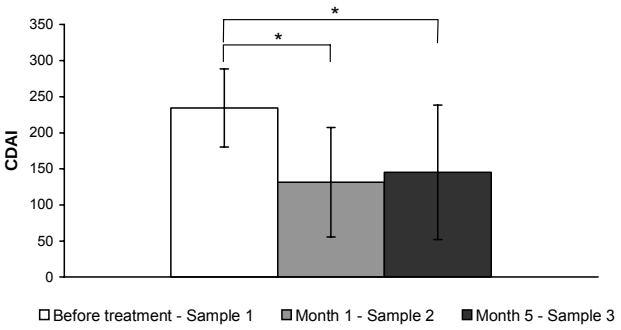

Fig. 1. *Changes in Crohn´s disease activity index (CDAI, means ± SD), *p<0.05.*

In both biochemical markers of inflammatory activity, α-1-acid glycoprotein and haptoglobin, a significant decrease in month 1 (Sample 2) was noticed, followed by an increase in month 5 (Sample 3, Figs 2a,b). α-1-acid glycoprotein levels dropped from 1138 ± 411 mg/l before treatment (Sample 1) to 880 ± 230 mg/l in month 1 (Sample 2, $p=0.005$); this decrease was followed by a significant increase in month 5 (Sample 3) to 1207 ± 376 mg/l ($p=0.003$). Haptoglobin decreased from 1721 ± 894 mg/l (Sample 1) to 1179 ± 613 mg/l (Sample 2),

(p=0.01) with further increase to 1618 ± 790 mg/l (Sample 3, p=0.02).

A sustained, significant increase was observed in cholinesterase and prealbumin levels (Figs 2c,d). Cholinesterase levels rose from 55.9 ± 14.6 μkat/l (Sample 1) to 62.6 ± 16.5 μkat/l (Sample 2, p=0.02), and 63 ± 18.2 μkat/l (Sample 3). Prealbumin increased from 215 ± 47 mg/l (Sample 1) to 283 ± 53 mg/l (Sample 2, p=0.0006); this was followed by a slight decrease to values of 245 ± 54 mg/l (Sample 3) that were still significantly higher than the values obtained before treatment (p=0.01).

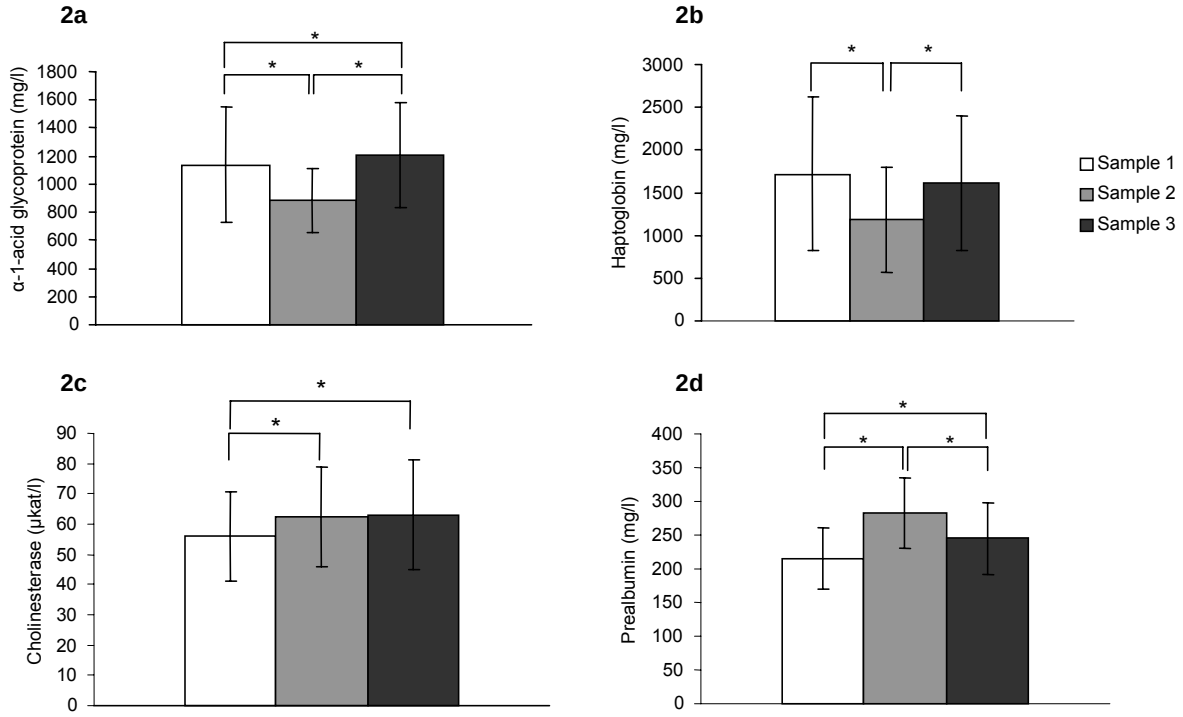

Fig. 2. *Changes in α-1-acid glycoprotein (2a), haptoglobin (2b), cholinesterase (2c), prealbumin (2d), before treatment (Sample 1), in month 1 (Sample 2) and in month 5 (Sample 3), data are presented as means ± SD, * p<0.05.*

Discussion

In the present study we observed that both acute phase proteins studied (α-1-acid glycoprotein and haptoglobin) diminished significantly after anti-TNF therapy for active CD. Together with a decrease in biochemical markers of inflammation, clinical improvement was observed. α-1-acid glycoprotein is considered to be a good laboratory marker of inflammatory activity in CD (Lubega and Davies 1990) whilst haptoglobin, probably due to its polymorphism (Turecký *et al.* 2001), seems to be less reliable (Lubega and Davies 1990). α-1-acid glycoprotein synthesis is enhanced in response to proinflammatory cytokines (interleukin 1, tumor necrosis factor-α – TNF-α) (Pous *et al.* 1990) and these cytokines are thought to mediate the inflammation in CD. Therefore, anti-TNF therapy that is targeting the key proinflammatory cytokine in CD pathogenesis, TNF-α, is expected to be related to changes in acute phase protein levels as observed in the present study.

However, in the further follow-up only a slight, non-significant increase in clinical activity was observed whilst both acute phase proteins raised significantly and, in the case of α-1-acid glycoprotein, exceeded levels observed before treatment. The possibility of a flare of an opportunistic infection was excluded on the basis of the absence of medical history, normal clinical examination, and a physiological leukocyte count. α-1-acid glycoprotein seems to predict a clinical relapse in CD (Brignola *et al.* 1986, Wright *et al.* 1987, Louis *et al.* 1997) that may account for the reported difference

between clinical and laboratory observations. A close further follow-up of these patients is therefore needed with the aim of early therapeutic adjustment.

The two other biochemical parameters studied, cholinesterase and prealbumin, showed a significant increase in response to anti-TNF treatment in active CD. The increase was sustained in cholinesterase whilst prealbumin levels diminished after the initial rise. Prealbumin is a rapid turnover marker of proteosynthetic status (Malejev *et al.* 1979) and the ratio of α-1-acid glycoprotein and prealbumin was found to correlate with clinical (Richter *et al.* 1982) and endoscopic (Simonis *et al.* 1998) activity in CD. The observation of the decreasing level of prealbumin would therefore be in accordance with the above-mentioned increase in α-1-acid glycoprotein.

Cholinesterase is a marker of proteosynthetic activity (Malejev *et al.* 1979). Its serum levels were found to be decreased in active CD and it has been suggested that this decrease might be explained by a suppression of cholinesterase synthesis in severe CD mediated by endotoxins and cytokines rather than by an increased intestinal loss (Tromm *et al.* 1992). Administration of anti-TNF was found to reduce the number of lamina propria mononuclear cells producing TNF and interferon-gamma (Plevy *et al.* 1997) and the observed increase in cholinesterase after anti-TNF therapy might be related to diminished production of CHE synthesis suppressing cytokines.

Based on the above presented results, we can conclude that the clinical improvement after anti-TNF therapy in active CD is accompanied by changes in acute phase proteins, namely α-1-acid glycoprotein and haptoglobin, as well as in proteosynthetic markers, cholinesterase and prealbumin. The assessment of these laboratory markers may be useful in the further management of CD patients treated with anti-TNF.

Acknowledgements

The authors are grateful to Mrs H. Jahodníková for her help in laboratory and secretarial work.

References

ANDRE C, DESCOS L, LANDAIS P, FERMANIAN J: Assessment of appropriate laboratory measurements to supplement the Crohn´s disease activity index. *Gut* **22:** 571-574, 1981.

BAERT FJ, D´HAENS GR, PEETERS M, HIELE MI, SCHAIBLE TF, SHEALY D, GEBOES K, RUTGEERTS PJ: Tumor necrosis factor α antibody (infliximab) therapy profoundly down-regulates the inflammation in Crohn´s ileocolitis. *Gastroenterology* **116**: 22-28, 1999.

BEST WR, BECKTEL JM, SINGLETON JW: Rederived values of the eight coefficients of the Crohn´s disease activity index (CDAI). *Gastroenterology* **77**: 843-846, 1979.

BRIGNOLA C, CAMPIERI M, BAZZOCCHI G, FARRUGGIA P, TRAGNONE A, LANFRANCHI GA: A laboratory index for predicting relapse in asymptomatic patients with Crohn´s disease. *Gastroenterology* **91**: 1490-1494, 1986.

CELLIER C, SAHMOUD T, FROGUEL E, ADENIS A, BELAICHE J, BRETAGNE JF, FLORENT C, BOUVRY M, MARY JY, MODIGLIANI R: Correlations between clinical activity, endoscopic severity, and biological parameters in colonic or ileocolonic Crohn´s disease. A prospective multicentre study of 121 cases. The Groupe d´Etudes Thérapeutiques des Affections Inflammatoires Digestives. *Gut* **35:** 231-235, 1994.

D´HAENS G, VAN DEVENTER S, VAN HOGERAND, CHALMERS D, KOTHE R, BAERT F, BRAAKMAN T, SCHAIBLE T, GEBOES K, RUTGEERTS P: Endoscopic and histological healing with infliximab anti-TNF antibodies in CD: A European multicenter trail. *Gastroenterology* **116**: 1029-1034, 1999.

LAURELL CB: Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal Biochem* **15**: 45-52, 1966.

LOUIS E, BELAICHE J, VAN KEMSEKE C, FRANCHIMONT D, DE GROOTE D, GUEENEN V, MARY JY: A high serum concentration of interleukin-6 is predictive of relapse in quiescent Crohn's disease. *Eur J Gastroenterol Hepatol* **9**: 939-944, 1997.

LUBEGA J, DAVIES TJ: A comparison of serum mucoprotein with serum α1 acid glycoprotein, haptoglobin, α1 antitrypsin assays in monitoring inflammatory bowel disease. *Clin Chim Acta* **188**: 59-70, 1990.

MALEJEV A, KRYSTEV Z, ŠIPKOV T, KOLARSKI V: Individual serum proteins as indicators of dysproteinemia in chronic liver disease (in Czech). *Čs Gastroenterol Výž* **33:** 421-426, 1979.

MULLIN GE, LAZENBY AJ, HARRIS ML, BAYLESS TM, JAMES SP: Increased interleukin-2 messenger RNA in the intestinal mucosal lesions of Crohn´s disease but not ulcerative colitis. *Gastroenterology* **162**: 1620-1627, 1992.

PLEVY SE, LANDERS CJ, PREHN J, CARRAMANZANA NM, DEEM RL, SHEALY D, TARGAN SR: A role for TNF-α and mucosal T helper-1 cytokines in the pathogenesis of Crohn´s disease. *J Immunol* **159**: 6276-6282, 1997.

POUS CH, GIROUD JP, DAMAIS CH, RAICHVARG D, CHAUVELOT-MOACHON L: Effect of recombinant human interleukin-1β and tumor necrosis factor α on liver cytochrome P-450 and serum α-1-acid glycoprotein concentrations in the rat. *Drug Metab Dispos* **18**: 467-470, 1990.

PULLMAN WE, ELSBURY S, KOBAYASHI M, HAPEL AJ, DOE WF: Enhanced mucosal cytokine production in inflammatory bowel disease. *Gastroenterology* **101**: 529-537, 1992.

RAYNES JG, EAGLING S, McADAM KPWJ: Acute-phase protein synthesis in human hepatoma cells: differential regulation of serum amyloid A (SAA) and haptoglobin by interleukin-1 and interleukin-6. *Clin Exp Immunol* **83**: 448-491, 1991.

RICHTER J, BITTER J, KRÁL V, ZUVAČOVÁ J: Use of the ratio of orosomucoid and prealbumin values as an indicator of the activity of Crohn's disease (in Czech). *Čas Lék Česk* **121**: 1493-1494, 1982.

SIMONIS B, HEINE M, HEENE DL, GLADISCH R: Evaluation and validation of a Crohn´s disease inflammatory activity index reflecting pattern of endoscopic severity. *Scand J Gastroenterol* **33:** 283-288, 1998.

TARGAN SR, HANAUER SB, VAN DEVENTER SJH, MAYER L, PRESENT DH, BRAAKMAN T, DEWOODY KL, SCHAIBLE TF, RUTGEERTS PJ: A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor α for Crohn´s disease. *N Engl J Med* **337**: 1029-1035, 1997.

TROMM A, TROMM CD, HUPPE D, SCHWEGLER U, KRIEG M, MAY B: Evaluation of different laboratory tests and activity indices reflecting the inflammatory activity of Crohn´s disease. *Scand J Gastroenterol* **27**: 774-778, 1992.

TURECKÝ L, KUPČOVÁ V, SZÁNTOVÁ M, UHLÍKOVÁ E: Haptoglobin types in patients with chronic liver disease. *Clin Chem Lab Med* **39** (Suppl): S345, 2001.

WRIGHT JP, YOUNG GO, TIGLER-WYBRANDI N: Predictors of acute relapse of Crohn's disease. A laboratory and clinical study. *Dig Dis Sci* **32**: 164-170, 1987.

Reprint requests

Doc. Dr. Kupčová Viera, CSc., Department of Internal Medicine, Dérer´s Hospital, Medical Faculty of Comenius University, Limbova 5, 833 05 Bratislava, Slovak Republic, e-mail: kupcova@cent.sk