Ultrastructural Evidence of Mucosal Healing after Infliximab in Patients with Ulcerative Colitis

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Abstract

Background: Infliximab is a monoclonal anti-TNF-alpha antibody that has been shown to be effective in Crohn’s disease therapy. However, data are scarce about the mechanism of action and its efficacy in ulcerative colitis (UC). Aim: To assess intracellular changes of the colonic mucosa in patients with UC before and after infliximab treatment. Methods: 7 patients (18-65 years, 4 men) with active, refractory, moderate to severe UC (Lichtiger’s Clinical Activity Index >6, Endoscopic Index >4) underwent colonoscopy before and 4 weeks after the initial infusion of infliximab 5mg/kg of body weight. Endoscopically obtained biopsy specimens were processed specifically, stained with uranyl-acetate and lead citrate and examined with a JEOL-1010 transmission electron microscope. Results: Before treatment we noticed severe alterations of the epithelium: microvilli depletion, shattering of the epithelial junctions, cytoplasmic vacuolization, dilatation of the endoplasmic reticulum, pycnotic nuclei, altered structure of mitochondria and Golgi complexes. Rarefaction of the goblet cells, and abnormal mucus formation and secretion were also observed. The corresponding chorion showed structural alteration of component cells, obstructed capillaries, erythrocyte extravasation, and many plasmocytes and neutrophils. After infliximab, improvement in morphology and function of the epithelial organelles, rich mucus secretion and recovery of the chorionic components were noticed. Conclusions: Our study revealed important intracellular alterations of the UC mucosa that were restored after infliximab therapy. These features may contribute to a better understanding of UC pathogenesis and mechanism of action of the anti-TNF-alpha therapies.

Key Words


Introduction

Ulcerative colitis (UC) and Crohn’s disease (CD) are distinct yet somewhat similar idiopathic inflammatory disorders of the gastrointestinal tract. These diseases often require long-term therapy mandating a variety of drugs and/or surgery. Standard medical therapy is classically guided towards anti-inflammatory treatment, antimicrobial treatment and immune modulation [1].

The introduction of steroid therapy in 1955 reduced the mortality of severe ulcerative colitis to 7%, compared with 24% in the placebo group, and it is now below 1% in specialized centres [2]. Although most patients with UC initially respond to corticosteroids, at 1 year approximately 25% become steroid dependent [3]. The remaining options in patients with severe UC include methotrexate, cyclosporine, tacrolimus, azathioprine and 6-mercaptopurine or surgery. Although these agents can be effective in a relatively high proportion of steroid-resistant patients, most of those who do respond initially will eventually need a colectomy [4-6]. Therefore, the ideal therapeutic strategies for patients with Crohn’s disease and UC should induce and maintain long-term remission without steroid exposure and with minimal surgery [7].

The efficacy of infliximab which binds with high affinity and specificity to the soluble form of tumour necrosis factor (TNF)-alpha [8] in treating CD is well established [9-11].

But, whereas CD is classically described as corresponding to a Th1-proinflammatory, TNF-α-mediated response, UC is considered to be mediated by Th2 (humoral) [12]. Considering these two different pathogenetical ways, is infliximab just as suitable in UC as in CD? Several studies have focused on this subject and while some of them supported the use of this agent in UC (especially the Active Ulcerative Colitis Trials - ACT 1 and ACT 2) [5], others considered its use to be controversial and the risk of opportunistic infection was
thought to be high. There are also several case reports with a range of outcomes [13-16].

Mucosal healing is an obvious goal of any luminal disease including UC. Although it is generally accepted that infliximab may induce clinical improvement and remission translated by a decrease in CAI, it is not clearly certified that mucosal healing is also achieved.

Biological activities attributed to TNF-alpha include: induction of proinflammatory cytokines, enhanced leukocyte migration by increased endothelial layer permeability, activation of neutrophil and eosinophil functional activity, induction of acute phase and other liver proteins [17].

A well documented previous study launched two interesting questions which opened the way to new research themes. The unanswered questions referred to whether infliximab had promising effects on the intestinal mucosa and if the UC patients will benefit from this treatment and achieve healing as in CD [1].

Starting from these controversies, the aim of our paper was to assess the intracellular changes of the colonic mucosa in patients with UC, before and after infliximab treatment and to assess not only if Lichtiger’s CAI is improved but also if mucosal healing is achieved.

**Methods**

We studied by ultrastructural examination, after histological control, 7 patients with active, moderate to severe refractory UC who were admitted to the 3rd Medical Clinic – Oradea, Romania. For our research we had the approval of the Ethics Committee of the University of Oradea.

The patients - 4 men and 3 women - were aged 18-65 years. We evaluated previous treatments failing to induce clinical remission. Disease activity was graded according to the Clinical Activity Index (Lichtiger’s CAI) by Lichtiger et al [18], with a highest possible score of 21.

In all patients a colonoscopy had been performed before and after infliximab therapy with an Olympus Exera CLE 145 videoodenscope (Olympus, Japan) for exact evaluation and staging of the disease. The Endoscopic Index was established using a simple grading score which assessed granulation scattering, vascular pattern, vulnerability of mucosa, mucosal damage (mucus, fibrin, exudates, erosions and ulcer) [19].

The Montreal Classification of the extent of UC was also assessed [20]. During endoscopy, we collected biopsies in all 4 quadrants from each 10 cm zone of the macroscopically affected colon, as stated by current protocols [21] - thus resulting in 28-32 biopsy samples as a minimum, for further processing, i.e. light and transmission electron microscopy assessment.

All patients were treated with two IV infusions of infliximab (Remicade® Centocor, Malvern, PA, USA) 5 mg/kg of body weight strictly adhering to the manufacturer’s instructions. The patients received one dose at week 0 and another one two weeks later. Lichtiger’s CAI was assessed before and 4 weeks after the 1st infusion and a follow-up endoscopy with new biopsies was also performed.

We used larger specimens (300-400nm) from which we made semifine sections, later coloured with Epoxy tissue stain and examined with an Olympus BS51 light microscope, thus establishing zones of pathological interest. Further on, the specimens were remodelated and resectioned for electron microscopy study. For ultrastructural examination two biopsy specimens were fixed in 2.5 % phosphate buffered 0.1M glutaraldehyde solution (pH 7.4) and postfixed with 1% osmic acid in phosphate buffer (pH 7.4). After rinsing in 0.15 M phosphate buffer, the samples were dehydrated with acetone and embedded in Epon 812. Thin sections were cut (70nm), stained with uranyl acetate and lead citrate and studied with a JEOL-1010 (Japan) transmission electron microscope (TEM) in “Babes Bolyai” Electron Microscope Centre, Cluj Napoca. We obtained 147 general pictures from semi-fine sections studied with Olympus BS51 and 886 pictures from ultra-fine sections examined with TEM.

**Results**

The demographic and clinical data of the patients, Montreal Classification, Lichtiger’s CAI and Endoscopic Index, before and after infliximab treatment, are recorded in Table I. Before treatment, all patients had a CAI >6 and an Endoscopic Index >4. Six patients had left sided colitis and one patient had extensive colitis according to the Montreal Classification. Inflammatory activity significantly diminished in all patients at the end of the treatment. The disease extent was diminished in two of our patients.

There were no significant adverse events of infliximab observed during the surveillance period.

The initial evaluation of the general pictures obtained from the semi-fine sections showed: the expansion of the mucosa with profound epithelial and chorionic alterations, the reduction and disappearance of the majority of mucus glands and cells, advanced lysis of some mucus cells from the Lieberkühn glands and alteration of the columnar cells structure, rarefying of the chorionic cellular components. These aspects were further confirmed and detailed by electron microscope images.

The ultrastructural assessment performed before the treatment with infliximab showed the following aspects:

- zones with profound epithelial alterations: areas with rarefaction or disappeared microvilli, alteration of the apical membrane and infiltration with neutrophils some migrating through crypt epithelium thus leading to crypt disruption and surface damage; destruction of external surface of the cell, vacuolization of the apical and basal cytoplasm (Fig. 1);
- the rectal mucosa with altered cells at the basal pole, vacuolisation of the cytoplasm, pyknotic nuclei with irregular contour and some areas of cell elimination into the lumen (Fig. 2); nuclei with different aspects - contracted and with irregular shape (not columnar), and dilated mithocondria (Fig. 3);
- rarefaction of the goblet cells with small amount of mucus; the Golgi complexes in the goblet cells elaborate few
and immature mucus granules; dilatation of the endoplasmic reticulum (Fig. 4);

- the chorion with advanced alterations and rarefaction of cellular components, destruction of cells, numerous lysis areas, obstructed capillaries and extravasations of erythrocytes (Fig. 5); presence of many neutrophils (Fig. 6);

The follow-up endoscopy with biopsies showed a clear improvement of the tissue quality after 4 weeks of treatment. Histological images revealed many Lieberkühn glands with tall cylindrical epithelial cells and goblet cells loaded with mucus secreted continuously in the lumen as shown by TEM images (Fig. 7); microvillus border normally developed (Fig. 8). There were no neutrophils infiltrated in the epithelium;

- intense metabolic activity with dense cytoplasm (many ribosomes), normal euchromatic nuclei (Fig. 9) and

### Table 1. Patient characteristics before and after infliximab treatment

<table>
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<td>5</td>
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EI: Endoscopic Index; LCAI: Lichtiger’s Clinical Activity Index

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**Fig 1.** Surface epithelium with altered cell structure (bar=10µm).

**Fig 2.** Altered epithelium with necrotic centers, detached parts (containing a neutrophil) falling into the lumen (bar=10µm).

**Fig 3.** Structural alterations at the base of epithelial cell, vacuolization, deformed nuclei, dilated mitochondria (bar=2µm).

**Fig 4.** Gland with emptied mucous cells (bar=10µm)
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electron-dense normal mitochondria proving the recovery of
the energetic function of cells (Fig. 10);
• the Golgi complex toward the apical pole of the nucleus,
with high activity by elaborating of many normal mucus
granules (Fig. 11);
• chorion with dense populations of cellular components,
normal structured, predominantly lymphocytes and
plasmocytes (Fig. 12). No neutrophils were observed
suggesting inactive stage of UC.

**Discussion**

TNF-α is a key cytokine in the pathogenesis of IBD.

It plays a leading role in inflammatory processes, with
an important contribution to macrophage and neutrophil
activation, increase of capillary permeability, extrinsic
activation of the coagulation pathways and recruitment of
various immunologic cells [1].

It is generally accepted that the excessive production
of either pro- or anti-inflammatory cytokines play a
predominant role in immune and inflammatory reactions and
loss of immune homeostasis in patients with IBD [22]. Anti-
TNF therapy has become an important treatment option in
patients with inflammatory bowel disease. A number of anti-
TNF medications have been investigated for this purpose by
randomized controlled trials. Infliximab, the most studied of
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these agents, has shown impressive efficacy in the treatment of CD, and to a smaller extent in UC [23].

Different authors have described active TNF-α synthesis both locally and systemically, as shown by an increased production in the colonic mucosa [24-26] and an increased detection in the stools, plasma, urine and rectal dyalisates of patients with active UC [27-29]. Moreover, this increase correlates with disease severity as measured by clinical or laboratory parameters. In the study by Ferrante et al (2007), whose objective was to report the outcome of infliximab in UC patients from a single centre and to identify predictors of early clinical response, the authors agreed that infliximab is an efficient therapy in UC, as shown by the 65% early clinical response [30].

All our patients expressed evident clinical improvement of the disease with diminishing morphological inflammatory activity (treatment success). The Lichtiger’s CAI dropped two weeks after infusion, a tendency kept also at week 4. Endoscopic lesions were milder, as confirmed by the biopsies at the end of treatment.

As the research done previously in the aforementioned studies showed, the clinical response observed in all our patients was supported by a descent in Lichtiger’s CAI. Endoscopic severity diminished as well, with 6 out of 7 cases entering remission (EI≤4).

Classically, CD and UC are described as corresponding to a Th1 and a Th2 response, respectively [31-32]. By showing an improvement in the morphology and function of the epithelial organelles after infliximab, our data provide an insight into the pathogenesis of UC. We confirm thus that TNF-α plays a role in the disease process and targeting this cytokine might be an effective choice for UC therapy.

Our study revealed that the intracellular alterations of the UC mucosa can be restored by infliximab. We noticed an improvement in the morphology and function of the epithelial organelles, rich mucus secretion and recovery of the chorionic components. At the end of treatment the new ultrastructural assessment clearly showed signs of epithelial barrier recovery, one of the goals of UC treatment, with appearance of the goblet cells, microvillus border, Golgi complex, mitochondria, resulting in a general aspect of tissue healing. The metabolic activity and the energetic function were reengaged due to numerous vesicles synthesized in the cytoplasm and electrondense normal mitochondria.

IgG-bearing lymphocytes have been found in the mucosal lesions of UC. Isolated mononuclear cells from UC lesions secrete more IgG [33]. Total numbers of macrophages are increased and subpopulations of macrophages - not normally present in the lamina propria of the intestine - appear, indicating ongoing recruitment to the inflamed bowel. Neutrophils are recruited very early and are found within crypts forming crypt abscesses, in the lamina propria and at the basis of ulceration. Eosinophils can cause tissue damage by releasing cytotoxic granule proteins. Activated eosinophils also produce cytokines, chemokines and lipid mediators which modulate the immune response and amplify the immune cascade [34]. Different methods such as electron microscopy [35], determination of eosinophil granule protein levels [36] and immunohistological examination of tissue samples [37] were utilized to show that tissue eosinophils in inflammatory bowel disease are active rather than resting cells.

The Th-2 type cytokine pattern is seen in allergic disorders, and eosinophils are closely associated with Th-2 immune response [38]. All these findings suggest that these types of inflammatory cells contribute to tissue damage and intestinal inflammation in IBD. The aforementioned alteration were found in our pre-treatment biopsy samples as well, expressed by an intense inflammatory infiltrate in the altered chorion. After infliximab, the chorionic components showed signs of improvement with decreasing number of inflammatory cells. The neutrophilic and eosinophilic infiltrate diminished and so did the numerous plasma cells and lymphocytes, suggesting that infliximab binds not only TNF-α freely present in the lamina propria as it was thought initially but also TNF-α expressed on inflammatory cells leading to apoptosis of the activated inflammatory TNF-α bearing cells.

To the best of our knowledge no studies until now have established the beneficial effects of infliximab by ultrastructural assessment. Our study supports the use of this agent in UC not only for the previously established decrease in Lichtiger’s CAI, but especially for its capacity

Fig 11. Intense metabolic activity of Golgi complex by elaboration of secretory mucous granules (bar=1µm).

Fig 12. Chorion infiltrated with lymphocytes and plasmocytes (bar=10µm)
of inducing mucosal healing. Although our study is still ongoing and our data are limited to 7 patients, infliximab may be considered as a remission-inducing agent in patients with refractory moderate to severe UC. This effect is reflected in a regeneration process of the colonic mucosa. All our patients had steroid-refractory disease and some had chronic active colitis refractory to immunosuppressants. Therefore, they required a viable alternative solution for their condition, which was the biological agent infliximab. Our results are promising because despite the fact that only two doses of infliximab were administered, which might have been insufficient for complete healing, we obtained an obvious improvement. Altering the course of UC in the long term requires early and complete suppression of inflammation, therefore early introduction of infliximab and top down therapy might also be carefully investigated, as already suggested for CD [39].

Conclusions

Our study revealed important intracellular alterations of the UC mucosa that were restored after infliximab therapy. These features may contribute to a better understanding of UC pathogenesis and mechanism of action of the anti-TNF-α therapies. This study also supports the achievement by infliximab of the most desirable UC treatment goal, which is mucosal healing.

Conflicts of interest

None to declare.

References

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