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Alimentary Tract

Positive pharmacokinetic effect of azathioprine co-medication on infliximab trough levels is dose-dependent

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ABSTRACT

Background: Thiopurines seem to have positive effect on the pharmacokinetics of anti-tumor necrosis factor biologics. It has been suggested that a reduced dose of thiopurines is sufficient to achieve this synergism.
Aims: To assess the differences of infliximab (IFX) trough levels according to the dose of concomitantly used azathioprine (AZA).
Patients & methods: All IBD patients treated with IFX (Remicade®) in two IBD centres between November 2015 and April 2017 were eligible. Infliximab trough levels were assessed by ELISA (Ridascreen®, R-Biopharm). The differences in IFX trough levels according to AZA dose were analyzed statistically.
Results: In total, 125 patients were included, 42 pts (33.6%) on infliximab monotherapy, 83 pts (66.4%) using combined immune suppression.

The respective median IFX levels according to AZA dose were as follows: group 1 (no concomitant AZA) 2.83 μg/ml (range 0–12); group 2 (AZA dose less than 1 mg/kg) 4.91 μg/ml (range 0.09–15.36); group 3 (AZA dose 1 < 2 mg/kg) 5.67 (range 0.16–16.97); group 4 (AZA dose above 2 mg/kg) 7.53 μg/ml (range 1.15–8.73). The differences in IFX trough levels between the respective groups according to AZA dose were statistically significant ($p = 0.0159$).
Conclusion: The positive effect of azathioprine on infliximab levels seems to be dependent on the dose of concomitantly used azathioprine.

1. Introduction

Combined immune suppression of anti-tumor necrosis factor (antiTNF) biologicals and thiopurines is superior to respective monotherapies in remission induction and maintenance of response in inflammatory bowel disease (IBD) [1]. Anti-TNF therapy is effective in treatment of IBD and leads to an improved quality of life of patients with a reduced number of surgeries and hospitalizations [2]. This therapy has helped to shift the therapeutic goals from symptom control towards sustained control of intestinal inflammation, mucosal healing and prevention of potential complications of the disease as well as side effects of therapies. Despite this advance, around 10–30% of patients do not respond to the initial treatment (primary non-response) and 23–46% of patients lose response over time (secondary loss of response) [3–5]. It has been reported that the annual risk for loss of response to infliximab (IFX) and adalimumab (ADA) in CD patients is 13% and 25%, respectively [6,7]. Immunogenicity with formation of neutralizing anti-drug antibodies is the most common cause of loss of response [8–10]. The available evidence suggests that the combination therapy with infliximab and an immunomodulator such as azathioprine may help to minimize immunogenicity, optimize biologic pharmacokinetics, and improve outcomes [8,11]. One of the putative mechanisms of this clinical benefit is positive pharmacokinetic effect of thiopurines on antiTNF levels. The landmark SONIC trial

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in Crohn's disease and the UC SUCCESS trial in ulcerative colitis demonstrated that combination therapy with infliximab and azathioprine is superior to monotherapy with either agent alone at inducing clinical remission in treatment naïve patients with moderate to severe disease [1,12].

Thiopurines have proven to be effective in maintaining steroid-free remission in both Crohn's disease and ulcerative colitis [13,14] and this efficacy was also confirmed in two recent update meta-analyses [15,16]. There is great inter-individual variation in the metabolism of thiopurines, both in their side effects and efficacy. This is mainly due to the involvement of varying activity of enzymes, which is at least partially genetically determined [17]. The effect of thiopurines monotherapy on disease control is dose-dependent, and the optimal daily dose of azathioprine is 1,5–2,5 mg/kg [18]. This dose-dependent effect, however, is not clearly demonstrated for the combination therapy. Several studies have suggested that the clinical superiority of combination therapy above respective monotherapies is mediated by positive effect of AZA on IFX pharmacokinetics [1,19,20]. In these studies, AZA comedication was associated with increased IFX trough levels and less anti-infliximab antibodies formation. To achieve IFX therapeutic levels, lower threshold of AZA metabolites 6-thioguanines compared with the threshold necessary for clinical efficacy of AZA was shown to be sufficient [19]. In addition, one randomized prospective study following IBD patients stopping AZA comedication compared with patients who had either reduced AZA or had continued the full AZA dose showed that the beneficial clinical effect of thiopurines comedication is also maintained with a reduced dose of azathioprine [21]. In addition, in this study, the proportion of patients with IFX trough levels below 1 μg/ml did not differ between the users of full and reduced AZA dose, respectively. On the other hand, there is an increasing body of evidence supporting the hypothesis that no universal threshold for IFX trough levels is applicable for all disease phenotypes [22] and the proportion of patients with mucosal healing is increasing with increasing IFX trough levels [23]. Considering these new data, the cut-offs used for IFX trough levels in the above mentioned studies were rather low. Thus, the threshold for AZA metabolites determined in these studies might not be sufficient in a real life setting with higher IFX levels necessary for sustainable remission. In addition, it has been shown recently, that stopping AZA leads to higher IFX consumption if the adequate IFX levels are to be maintained [24] which brings forward the question of whether adequate dose regimen of concomitantly used AZA may help to decrease the dose of IFX needed to achieve therapeutic levels of IFX.

Thus, combined immune suppression of IFX and a thiopurine is superior to respective monotherapies in inducing and maintaining remission of IBD. This clinical benefit is thought to be related to improved IFX pharmacokinetics resulting from the thiopurine-induced reduction of anti-infliximab antibody formation [1,19,20]. To achieve therapeutic IFX levels, reduced dose of AZA was considered to be sufficient but the current view on IFX pharmacokinetics favors higher IFX trough levels than originally considered to be adequate. Therefore, in the present study we aimed at determining IFX trough levels in IBD patients stratified according to different AZA doses in order to test whether the effect of AZA on IFX trough levels is dose-dependent.

2. Patients & methods

All IBD patients treated with IFX (Remicade®) in two referral IBD centres between November 2015 and April 2017 were eligible. IFX trough levels were routinely measured in all patients on maintenance IFX therapy using commercially available ELISA kit (Ridascreen®, R-Biopharm).

Table 1
Basic demographic characteristics.

Nr of pts	125
Mean age (yrs, range)	40 (22;84)
Male/female (% males)	78/47 (62,4%/37,6%)
Ulcerative colitis/Crohn's disease	42/83 (33,6%/66,4%)
Ulcerative colitis localization (Nr of pts)	
Proctitis	3
Left-sided	17
Pancolitis	22
Crohn's disease localization (Nr of pts)	
Upper GI	2
Small bowel	34
Large bowel	34
Perianal disease	13
Azathioprine co-medication	
- None	42 (33,6%)
- <1 mg/kg	27 (21,6%)
- 1 < 2 mg/kg	47 (37,6%)
- >2 mg/kg	9 (7,2%)
Surrogates of azathioprine metabolism[a]	
- MCV 92 and more	29
- Leukopenia (less than 4×10^9/l)	4

[a] Data available for 77 out of 83 patients with concomitant AZA therapy.

All patients in clinical remission, defined as Harvey-Bradshaw index 4 and less [25] with stable dose regimen of 5 mg/kg every 8 weeks at the time of the first assessment of IFX trough levels were identified retrospectively from the medical records. All patients were assessed at a steady state of at least six months of a stable dose regimen. Patients with previously intensified dose regimen were excluded.

Concomitant therapy with azathioprine (AZA) was noted and patients were categorized into four categories according to the dose of azathioprine: no concomitant therapy with AZA (group 1), AZA dose less than 1 mg/kg (group 2), AZA dose between 1 and 2 mg/kg (group 3) and AZA dose 2 mg/kg and more (group 4). IFX trough levels of 3 μg/ml and more were considered therapeutic. Statistical analysis was performed using GraphPad Prism 8.0.2. The differences in the mean IFX levels between the four groups of patients stratified according to the AZA dose were analyzed statistically by one-way ANOVA. The differences in the proportion of patients with therapeutic trough levels of IFX between the respective four groups of patients according to AZA dose were analyzed by chi-square test.

The study has been approved by the medical ethical committee of St Michael's Hospital, Bratislava, Slovakia.

Among the patients with concomitant AZA therapy, the surrogate markers of pharmacodynamically acting thiopurines metabolites, 6-thioguanines (6-TGN) as noted at the date of IFX trough levels assessment were analyzed. Mean corpuscular volume of erythrocytes (MCV) 92 fl and more and/or the presence of leukopenia (defined as leukocytes count less than 4×10^9/l) were considered to be markers of metabolic shift towards 6-TGN [26,27]. The differences in the median IFX levels between the group of patients with MCV of 92 fl and more and/or leukopenia vs. the group of patients with MCV below 92 fl and normal leucocytes count were analyzed by Mann Whitney test.

3. Results

Out of a total of 214 IBD patients treated with IFX, there were 154 in remission at the time of the first assessment of infliximab trough levels. After excluding patients with previously intensified dose regimen, 125 patients were further analyzed (demographic characteristics are shown in Table 1). Among these 125 pts, 42 pts (33.6%) were on infliximab monotherapy, 83 pts (66.4%) were using combined immune suppression. Twenty-seven patients (21.6%) were

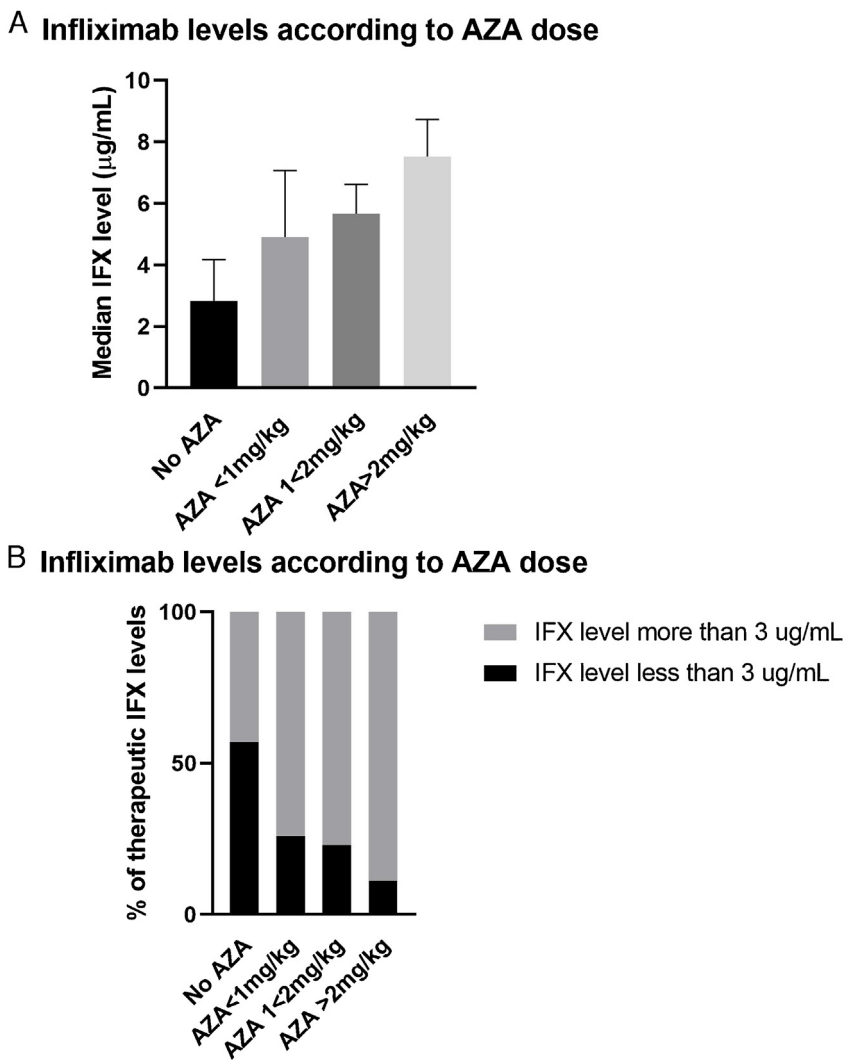

Fig. 1. (A) Infliximab trough levels differ significantly according to azathioprine dose.
Medians of IFX trough levels of respective patients'groups according to AZA dose: group 1 (no concomitant AZA) 2.83 μg/ml (range 0–12); group 2 (AZA dose less than 1 mg/kg) 4.91 μg/ml (range 0.09–15.36); group 3 (AZA dose 1 < 2 mg/kg) 5.67 μg/ml (range 0.16–16.97); group 4 (AZA dose above 2 mg/kg) 7.53 (range 1.15–8.73). The differences in IFX trough levels between the respective groups according to AZA dose were statistically significant (p = 0.0159). (B) Proportion of patients with subtherapeutic levels of infliximab according to azathioprine dose. Significant differences in the proportion of patients with subtherapeutic IFX trough levels among the respective groups of patients with different AZA dose – 24/42 pts (57%), 7/27 (26%), 11/47 (25%) and 1/9 (11%) in the group 1, 2, 3 and 4; respectively; p = 0.0017.

using 1 mg/kg of azathioprine, 47 patients (38%) between 1 and 2 mg/kg and 9 patients (7.2%) had a dose above 2 mg/kg.

Overall, the median IFX level was 4.41 μg/ml (range 0–16.97), with 44 pts (35%) with IFX levels below the cut-off of therapeutic level of 3 μg/ml. The respective median IFX levels in the four groups of patients divided according to the AZA dose were as follows: group 1 (no concomitant AZA) 2.83 μg/ml (range 0–12); group 2 (AZA dose less than 1 mg/kg) 4.91 μg/ml (range 0.09–15.36); group 3 (AZA dose 1 < 2 mg/kg) 5.67 μg/ml (range 0.16–16.97); group 4 (AZA dose above 2 mg/kg) 7.53 (range 1.15–8.73). The differences in IFX trough levels between the respective groups according to AZA dose were statistically significant (p = 0.0159), Fig. 1A. In addition, there were significant differences in the proportion of patients with subtherapeutic IFX trough levels among the respective groups of patients with different AZA dose (57%, 26%, 25% and 11% in the group 1, 2, 3 and 4; respectively; p = 0.0017), Fig. 1B.

To assess whether the synergistic dose-dependent effect of AZA on IFX levels was mediated by pharmacodynamically acting metabolites of AZA, the surrogate markers of AZA metabolism were used to categorized AZA using patients into two categories. Group 1 consisted of patients with MCV of 92 fl and more and/or leukopenia, these patients were considered as having the surrogate markers of metabolic shift towards 6-TGN production. Group 2 consisted of the remaining patients, i.e. AZA users with MCV below 92 fl. These two groups differed significantly in the median IFX trough levels with levels of 6.53 μg/ml (range 0.19–16.97) and 5.06 (range 0–10.24) in the group 1 and 2, respectively, p = 0.0351 (Fig. 2).

4. Discussion

In the present paper, we tested whether the clinically observed synergism of combined immune suppression is dependent on the dose of azathioprine concomitantly used with infliximab. In a cross-sectional study of patients in remission using standard dose regimen of infliximab, we found significant differences in infliximab levels between the respective groups of patients stratified according to AZA dose. The proportion of patients with therapeutic infliximab levels as defined by the cut-off of 3 μg/ml increased significantly with the increasing dose of azathioprine. In addition, using surrogate markers of thiopurines metabolism, we found significantly higher levels of infliximab in the group of patients with high MCV and/or leukopenia, i.e. the supposed markers of the

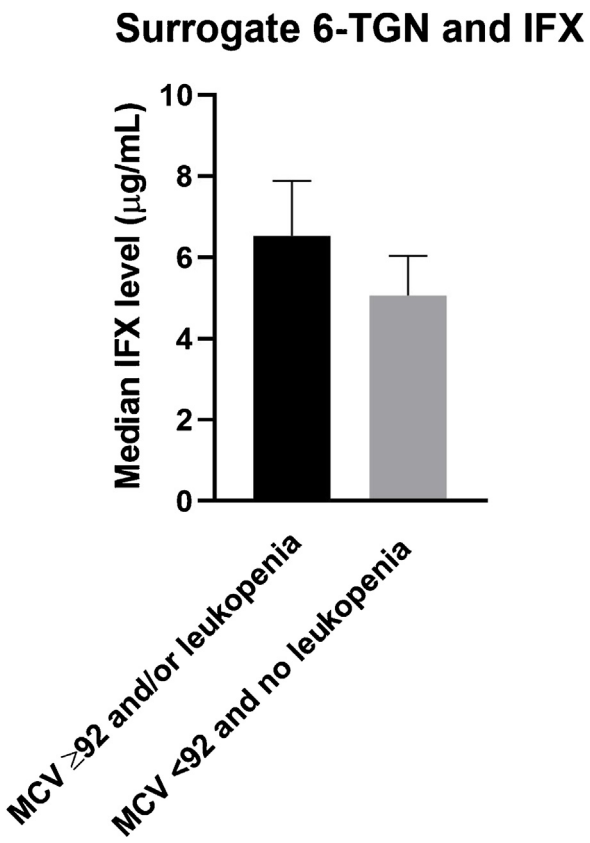

Fig. 2. Median IFX trough levels stratified by surrogate markers of thiopurines metabolism.
Patients were divided into two groups according to the presence of surrogate markers of thiopurines metabolic shift towards 6-thioguanins. The group presenting these markers (MCV 92 fl and more and/or leukopenia) had significantly higher median level of IFX trough levels (6.53 μg/ml; range 0.19–16.97) as compared with the rest of azathioprine users (5.06; range 0–10.24), p = 0.0351.

metabolic shift towards pharmacodynamically active thiopurines metabolites.

The superior clinical effect of combined immune suppression above respective azathioprine and infliximab monotherapies has previously been documented for both, Crohn's disease [1] as well as ulcerative colitis [12]. The mechanism of this synergism has subsequently been attributed to the improved pharmacokinetic profile of infliximab when the antiTNF monoclonal antibody was combined with a thiopurine [19,20]. When used in monotherapy, there is a clear dose-dependent effect of thiopurines with maximal effect reached at the dose of 2–2.5 mg/kg [18]. Thus, the question arose whether the same dose of thiopurine is necessary to achieve the positive effect on infliximab pharmacokinetics. One cross-sectional study addressed this issue by assessing the correlation of 6-TGN and infliximab levels and found the positive correlation of 6-TGN and infliximab levels [19]. In this paper, the authors also reported that a lower level of 6-TGN levels was sufficient to achieve therapeutic levels of infliximab as compared with the 6-TGN levels necessary to improve the outcomes of patients on monotherapy (125 pmol/8 × 10^8 RBCs vs. 230 pmol/8 × 10^8 RBCs, respectively). This suggested that a reduced dose of thiopurines could be used in comedication with infliximab to achieve the positive pharmacokinetic effect of combined immune suppression on infliximab levels. This was supported by one prospective study [21] that showed no difference in the proportion of pts with IFX trough levels below 1 μg/ml between the group of patients who had continued full vs. those who had a reduced dose of AZA. Another study showed association between low 6-TGN levels and anti-infliximab antibody formation with lower threshold of 6-TGN levels when compared to therapeutic threshold of thiopurines in monotherapy [20]. In our study, we were unable to determine the 6-TGN levels but we found that the proportion of patients with infliximab levels above 3 μg/mL increases with increasing dose of azathioprine and that the mean levels of infliximab are significantly higher in the group of patients with surrogate markers of metabolic shift towards 6-TGN levels. Thus, in line with the above mentioned studies using 6-TGN levels, our results support the hypothesis that the positive effect of azathioprine comedication on infliximab pharmacokinetics is mediated by pharmacodynamically active thiopurine metabolites. In contrast to the previously published results, we found a dose-dependent effect of AZA on IFX trough levels which might be explained by different study designs. First, in the above mentioned prospective study, all patients received first combined immune suppression with full AZA dose for at least one year, and this effect on IFX pharmacokinetics might have been maintained with low dose of AZA for longer period of time. Second, the mean IFX trough levels throughout this study were rather low (between 3 and 5 μg/mL) and the currently accepted threshold of adequate levels of 3 μg/ml might not have been achieved in a sufficient proportion of patients. Thus, based on our data, the positive effect of AZA on IFX trough levels seems to be dose-dependent although the exact threshold of 6-TGN levels in this clinical setting and its extrapolation to thiopurine dose needs to be determined in future studies.

The mechanism by which thiopurines improve the PK of IFX is not completely understood. It has been consistently shown that the combination of a thiopurine with IFX reduces the formation of anti-infliximab antibodies [19,20,28]. This effect seems to be associated with metabolic shift towards 6-TGN levels [19,20] and is independent of the previous effect of thiopurines monotherapy on disease activity [29]. In our study, we did not analyze the association of anti-infliximab antibody formation and comedication with azathioprine as only two patients had clinically significant levels of antibody (data not shown). This might be due to the design of our study with inclusion of patients in remission on stable standard dose regimen precluding thus inclusion of patients with immunogenic loss of response. On the other hand, with this design, we still found that patients with higher dose of azathioprine have higher levels of infliximab and this was not mediated by a lower rate of anti-infliximab antibody formation. Thus, other than anti-immunogenic effect can be at the origin of azathioprine related improved pharmacokinetics of infliximab which warrants further research.

Our study has several flaws. First, we included only patients in remission with standard dose regimen of infliximab. This makes it difficult to interpret our results in context of thus far published studies with different inclusion criteria. With this design, however, we pointed out that a significant proportion of patients with azathioprine comedication have subtherapeutic infliximab levels when on low dose of azathioprine reducing thus their chances for long term survival on therapy. Second, as mentioned here above, we were unable to measure 6-TGN levels in order to identify the subpopulation of patients with pharmacodynamically favorable metabolic shift. On the other hand, thiopurine drug monitoring still represents an important methodological challenge [30] and its benefit for clinical practice is a matter of debate [31].

In addition to the pharmacokinetic synergistic effect, there are other beneficial consequences of concomitant thiopurine therapy with IFX. Concomitant use of thiopurines has repeatedly been shown to reduce the formation of anti-infliximab antibodies [1,20,29]. A systematic review and meta-analysis including 8 studies analyzing data with a pooled total of 1351 patients clearly demonstrated that this antibody formation is associated with an increased risk of allergic reactions to IFX, and that this risk is reduced by concomitant use of immunomodulators [32]. Interest-

ingly, the addition of a thiopurine to infliximab monotherapy seems to decrease the level of already developed anti-infliximab antibodies and helps to regain the response to infliximab in patients who lost the response [33]. Thus, there are several mechanisms that account for the clinically observed benefit of combined immune suppression in the treatment of IBD.

Concluding, based on our results, the positive effect of azathioprine comedication on infliximab pharmacokinetics seems to be dose-dependent and the dose of azathioprine monotherapy of 2–2.5 mg/kg of body weight is associated with the highest rate of therapeutic infliximab levels. This effect is probably mediated by 6-thioguanines and might not be exclusively related to reduced anti-infliximab antibody formation. Further studies are needed to determine whether 6-thioguanines monitoring can be useful in this particular clinical setting and to analyze the mechanism of this beneficial pharmacokinetic interaction.

Conflict of interests

ZZ received speakers fee from MSD, Takeda, Abbvie, Janssen and Ferring; other co-authors declare no conflict of interests.

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