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Suppression of infliximab antibody levels by azathioprine in patients with rheumatoid arthritis

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In rheumatoid arthritis (RA) treatment, the concomitant use of methotrexate has been shown to reduce the incidence of antibodies to infliximab (ATI), on the other hand, it is unclear whether azathioprine can reduce ATI production. We enrolled a total of 10 Japanese adult patients with RA who were treated with infliximab concomitantly with methotrexate or azathioprine. Serum concentrations of infliximab and ATI of these patients were measured. The mean serum infliximab concentrations was 1.6±1.3 μg/ml in patients with methotrexate and 1.0±0.5 μg/ml in patients with azathioprine. Serum ATI concentrations were below the limit of quantitation in 4 of 5 patients in each group. The results from the present study suggest that azathioprine suppresses ATI production.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by synovial proliferation and is classified as an autoimmune disease. Drug therapies for RA include disease-modifying anti-rheumatic drugs (DMARDs) using methotrexate as an anchor drug, with the supportive use of non-steroidal anti-inflammatory drugs and low-dose steroids. In Japan, azathioprine has been included under national health insurance coverage for treatment of rheumatic diseases since 2011. Although the first-line treatment for RA is methotrexate, azathioprine is increasingly used as a second-line treatment for patients who are intolerant to methotrexate, such as those with impaired liver/kidney function. If sufficient treatment response is not observed within 3-6 months, the addition of other DMARDs or switching to biological drugs such as infliximab, should be considered as standard practice (Schuna 2011). Infliximab is a chimeric antibody composed of murine variable and human constant regions (Scallon 1995). Immune response to murine proteins produced from the variable regions will lead to the expression of ATI. In RA treatment, as the concomitant use of methotrexate has been shown to reduce the incidence of ATI (Maini et al. 1998; Pascual-Salcsdo et al. 2011). Only few studies have shown a reduced incidence of ATI in patients with Crohn's disease treated with concomitant immunosuppressants, including azathioprine (Vermeire et al. 2007). No association in RA patients has been demonstrated between the concomitant use of DMARDs other than methotrexate and reduced incidence of ATI. Thus, we investigated whether azathioprine co-administered with infliximab can reduce ATI production in RA patients.

2. Investigations, results and discussion

Table 1 summarizes the demographics, serum concentrations of infliximab and ATI in 10 patients included in this study. Patient No. 8 was also treated with prednisolone, in addition to infliximab and azathioprine. The mean serum infliximab concentration was 1.6±1.3 μg/mL in patients who were co-administered with methotrexate and 1.0±0.5 μg/mL in those who were co-administered with azathioprine. Serum infliximab concentrations of < 1.0 μg/

Table 1: Pharmacotherapy of each patients and concentration of infliximab and antibodies to infliximab

Patient		Dose of IFX		Administration interval	Administration period	Concomitant drug		Concentration	
		(mg/body)	(mg/kg)	(week)	(month)			IFX (pg/ml)	ATI (ng/mg)
IFX+MTX	No. 1	200	3.3	9	41	MTX	6 mg/week	0.51	N.D.
	No. 2	200	4.6	9	53	MTX	8 mg/week	0.48	N.D.
	No. 3	300	3.5	8	9	MTX	12 mg/week	2.5	N.D.
	No. 4	300	6.0	7	77	MTX	8 mg/week	3.8	N.D.
	No. 5	200	4.0	8	17	MTX	8 mg/week	0.68	81.6
IFX+AZA	No. 6	200	4.7	8	25	AZA	50 mg/week	1.3	N.D.
	No. 7	300	3.4	8	31	AZA	75 mg/day	1.8	N.D.
	No. 8	200	4.2	9	67	AZA PSL	50 mg/day 6 mg/day	1.1	N.D.
	No. 9	200	3.4	7	28	AZA	50 mg/day	0.80	N.D.
	No. 10	300	7.2	6	7	AZA	75 mg/day	0.20	28.0

IFX: infliximab, MTX: methotrexate, AZA: azathioprine, PSL: prednisolone, ATI: antibody to infliximab, N.D.: not detected

Table 2: Responses to treatment in each patient

Patient		DAS28		3 month change	EULAR
		Baseline	After 3 month	in DAS score	response criteria
IFX+MTX	No. 1	6.18	1.39	-4.79	Good response
	No. 2	4.68	2.06	-2.62	Good response
	No. 3	3.31	2.05	-1.26	Good response
	No. 4	2.30	1.33	-0.97	Moderate response
	No. 5	3.32	1.35	-1.97	Good response
IFX-AZA	No. 6	6.59	1.15	-5.44	Good response
	No. 7	4.26	1.62	-2.64	Good response
	No. 8	4.29	2.23	-2.06	Good response
	No. 9	4.18	2.22	-1.96	Good response
	No. 10	1.13	1.07	-0.06	No response

IFX: infliximab, MTX: methotrexate, AZA: azathioprine

mL were recorded in 3 of 5 patients who were co-administered with methotrexate and 2 of 5 patients who were co-administered with azathioprine. Serum ATI concentrations were below the limit of quantitation in 4 of 5 patients in each group. In the patients with detectable ATI in both groups, the serum infliximab concentrations were less than 1.0 µg/ml (0.68 µg/ml in patient No. 5 who was co-administered with methotrexate and 0.20 µg/ml in patient No. 10 who was co-administered with azathioprine).

The results from the present study suggest that azathioprine also suppresses ATI production, as has been demonstrated with methotrexate. In a previous study involving 174 patients with Crohn's disease, there was no significant difference in the incidence of ATI between patients treated with combined methotrexate and infliximab and those treated with combined azathioprine and infliximab (Vermeire et al. 2007), which supports our results in patients with RA. We noted two patterns of serum trough concentration of infliximab among patients who were co-administered with methotrexate. Two of five patients had higher values (2.5 and 3.8 µg/mL) while the remaining three patients had lower values of 0.51, 0.48 and 0.68 µg/mL, which are below the efficacy cut-off of 1.0 µg/mL (Takeuchi et al 2009). Similarly, three of five patients in the azathioprine group also had values above 1.0 µg/mL while the other two had values less than the cut-off. Of the patients with lower infliximab concentrations, two patients (Nos. 5 and 10) were positive for ATI, which was the most likely reason for the low concentration. For the remaining three patients (Nos. 1, 2 and 9), no clear reason that explains the low serum concentrations could be identified from the information obtained in this study.

In a recent study involving patients with inflammatory bowel disease treated with combined infliximab and thiopurine, no correlation was observed between the trough concentration and the dose of infliximab, while a significant correlation was observed between the trough concentration of infliximab and the concentration of 6-thioguanine nucleosides, an active metabolite of thiopurine (Yarur 2015). All the patients included in this study were concomitantly treated with 6-12 mg/week of methotrexate or 50-75 mg/day of azathioprine.

Table 2 summarizes the results of treatment response-related parameters in 10 patients included in this study. In all patients, DAS28 scores after 3 months of treatment with infliximab were less than 2.6, the cut-off for remission. According to the EULAR response criteria, all patients, except for patient No. 10, had good or moderate response, suggesting acceptable response to treatment, although no correlation was observed between response and infliximab concentration. Patient No. 10 was already in remission at the start of concomitant treatment with infliximab and azathioprine, and maintained it throughout the treatment period, thus was rated as having "no response" according to the EULAR response criteria. Hence, there was no problem with treatment itself.

This study included patients who had been continuously treated with infliximab combined with methotrexate or azathioprine for at least 6 months. This period was based on an estimation derived from the previous observation that after 6 months of treatment, a clear difference would be observed in serum infliximab concentration between patients with ATI and those without ATI (Bartelds et al. 2007; Ben-Horin et al. 2013). Thus, patients who had their treatment changed for some reasons within 6 months were excluded from the study. These excluded patients might have included those who did not respond to infliximab because of emergence of ATI. Therefore, it cannot be concluded from the information obtained in this study alone that azathioprine is as effective as methotrexate in suppressing ATI production.

The present results suggest that azathioprine suppresses the production of infliximab-neutralizing antibody in RA patients. Further studies in larger populations, including patients not responding to treatment, are needed to more clearly demonstrate the suppressive effect of azathioprine on neutralizing antibody production.

3. Experimental

3.1. Patients

This study was approved by the Institutional Review Board of Keio University Hospital. All patients provided written informed consent before participating in this study. We enrolled a total of 10 Japanese adult patients with RA who were treated with infliximab concomitantly with methotrexate or azathioprine (who were contraindicated to methotrexate) for more than 6 months in the rheumatology unit of Keio University Hospital.

3.2. Clinical sample

Before administration of infliximab, 2 mL of blood was obtained from patients. Blood specimens was centrifuged at 3,000 rpm for 5 min. Plasma was aliquoted to polyethylene tubes and stored at –20 °C until assay.

3.3. Quantification of serum concentration of infliximab and ATI

Serum concentrations of infliximab and ATI were measured using SHIKARI® Q-INFLIXI and SHIKARI® Q-ATI (Matriks Biotechnology Co. Ltd., Ankara, Turkey) according to the product manual.

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