Effects of infliximab on sister chromatid exchanges and chromosomal aberration in patients with rheumatoid arthritis

M. Atteritano · S. Mazzaferro · S. Mantuano ·
G. L. Bagnato · G. F. Bagnato

Received: 18 June 2014 / Accepted: 25 August 2014 / Published online: 27 May 2015
© Springer Science+Business Media Dordrecht 2015

Abstract The aim of this study was to evaluate in a 24-weeks the effect of anti-TNF-alpha, infliximab, on cytogenetic biomarkers in peripheral lymphocytes of patients with rheumatoid arthritis (RA). A total of 40 patients with RA met the criteria to be treated with methotrexate (15 mg/week) were evaluated. Twenty patients, randomly selected, were treated with infliximab in addition to methotrexate (group I), whereas the other 20 patients continued with only methotrexate treatment (group M). Twenty healthy volunteers matched for age, gender and smoking habits served as control group (group C). At baseline, sister chromatid exchange rate was 7.20 ± 2.21 in group I, 7.40 ± 1.60 in group M and 4.97 ± 1.32 in group C ($P < 0.01$ vs group I and M). After 24-weeks, sister chromatid exchange rate was 7.87 ± 2.54 in group I and 7.81 ± 1.95 in group M ($P = \text{ns}$). High frequency cells count was 4.9 % and 4.7 % in the groups I and M, respectively, at the end of the study ($P = \text{ns}$). The basal chromosomal aberration frequency was 4.90 % in group I and 5.20 % in groups M; after 24-weeks, this was 5.10 % in group I and 5.10 % in groups M ($P = \text{ns}$). Infliximab treatment, for 24 weeks, did not

increase the cytogenetic biomarkers in patients with RA. Our data show that the use of infliximab has not a genotoxic effect in patients with RA.

Keywords Genomic damage · Biomarkers · Biologic therapy · Rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a long-term disease that leads to inflammation of the joints and surrounding tissues. It can also affect other organs. Anti-tumor necrosis factor (TNF) drugs have proven highly successful in treating RA and have changed the natural history of this disease. The introduction of TNF inhibitors has been accompanied by concerns regarding safety, and in particular the risk of malignancy (NICE 2002). Tumor necrosis factor α (TNF α) therapy exerts biologic effects on carcinogenesis and tumor progression, the impact of which is incompletely understood but is presumably different for different types of cancer and for different time points during carcinogenesis (Balkwill 2006; Van Horssen et al. 2006; Perkins 2004). Several studies show increased risk of cancer in patients with RA (Gridley et al. 1993; Mellemkjaer et al. 1996), whereas other studies have not (Thomas et al. 2000; Esser et al. 2004). Cytogenetic parameters, such a chromosome aberrations, sister chromatid exchanges or micronuclei are detectable by cytogenetic analysis of chromosome in

M. Atteritano (✉) · S. Mazzaferro · S. Mantuano ·
G. L. Bagnato · G. F. Bagnato
Department of Clinical and Experimental Medicine,
University of Messina, Pad. C, 3rd floor, A.O.U.
Policlinico “G. Martino” Via C. Valeria, 98125 Messina,
Italy
e-mail: matteritano@unime.it

metaphase. These endpoints are widely used to assess human exposure to different mutagenic and carcinogenic agents of environmental or internal origin (Albertini et al. 2000). Many human diseases related to chronic inflammation, such as inflammatory bowel diseases, connective's diseases and different carcinomas show cytogenetic alterations (Cottliar et al. 2000; Kang et al. 1997; Palmer et al. 1987). In arthritis cytogenetic endpoints were mainly used to evaluate the genotoxicity of certain anti-rheumatic medications (Rantapää Dahlqvist and Nordenson 1996; McCarthy et al. 1998; Shahin et al. 2001; Ramos-Remus et al. 2002; Kageyama et al. 2008). The aim of this study was to evaluate potential genotoxicity effects of anti-TNF- α therapy, infliximab, in patients with RA.

Materials and methods

Subjects

After the Ethical Committee has approved our study, participants were recruited among those reporting to the Unit of Rheumatology in the Department of Clinical and Experimental Medicine at the University of Messina (Messina, Italy). Forty patients (34 females and 6 males; mean age 60.40 ± 4.70 years; mean disease duration 8.40 ± 4.40 years) with RA, diagnosed in according to the criteria of American College of Rheumatology (Arnett et al. 1988), were involved in this study. Twenty healthy volunteers matched for age, gender and smoking habits served as control group.

All patients were treated with a stable dose of methotrexate (MTX) (15 mg/week) for at least 6 months before and throughout the present study. Twenty patients, randomly selected using a computer-generated randomization sequence, received infliximab in addition to methotrexate (group I), whereas the other 20 patients continued with only methotrexate (group M). Infliximab was administered by intravenous infusion at a dosage of 3 mg/kg at the baseline, then at 2 and 6 weeks, and after that every 8 weeks.

Blood samples and cytogenetic biomarkers assessment

All patients underwent general and physical examinations and routine blood and urinary analysis at the

baseline, and every 12-weeks of treatment. ESR, CRP, the number of swollen joints and tender joints, and the Disease Activity Score 28 (DAS 28) were measured to evaluate the disease activity in RA. Additionally, at baseline and after 24-weeks, the cytogenetic investigation was performed on the chromosomes obtained from peripheral blood lymphocytes. Heparinized venous blood was obtained during the spring season of the same year. Heparinized whole-blood sample (0.5 mL) was added to 8 mL of RPMI medium, supplemented with 20 % heat-inactivated fetal calf serum, penicillin (200 U/mL) and streptomycin (200 μ g/mL). Blood lymphocytes were stimulated with phytohemagglutinin M (final concentration 20 μ g/mL) (all materials were from Sigma-Aldrich, Milano, Italy).

Cells were grown in the presence of 5 μ g/mL 5-bromodeoxyuridine for 2 cycles of DNA replication (48 h) and protected from light. Cultures were harvested by standard procedures after 5 h of exposure to colcemid (Sigma-Aldrich, Milano, Italy) (0.2 μ g/mL). Differential chromatid staining was obtained by a modification of the fluorescence-plus Giemsa procedure methods (Wolff and Perry 1974) using Hoechst 33258 (Sigma). Slides were exposed to light (3 h) and stained in 8 % Giemsa. Sister chromatid exchange frequency was analysed in 50 metaphases of cells randomly selected from each culture. Classic karyotype analyses by G-banding were carried out to identify chromosomal abnormalities. One-hundred metaphase of cells randomly selected spreads per subject were examined for structural and numerical changes. Slides from each culture were randomly numbered and scored "blind" in a numerical fashion. The percentage of high frequency cells for each individual was estimated using the pooled distribution of all sister chromatid exchange cell measurement. High frequency cells are cells which display a number of sister chromatid exchanges per cell that is higher than the 95th percentile of the distribution of sister chromatid exchange per cell in the population (Latt 1981). For each subject, the expected number of high frequency cells (x) was calculated in a binomial distribution to constitute the 95th percentile (Carrano and Moore 1982) by the following equation:

$$x = 1.645[np(1 - p) + n(1 - p) + 0.5]^{1/2}$$
 where n is the number of cells scored and p is equal to 0.95. An individual was considered an outlier if the number of high frequency cells obtained was greater than x .

Statistical analyses

Data are given as mean $\pm$ SD. The value for pre- and post-treatment measurements of sister chromatid exchange, high frequency cell and chromosomal aberrations were compared using Wilcoxon signed rank test. Pearson's correlation coefficient was calculated to evaluate the correlation between two variables. A *P* value of 0.05 or less was considered statistically significant, and 95 % CIs were calculated wherever possible. Statistical analysis was performed using StatSoft, Inc. (1995) STATISTICA for Windows.

Results

Table 1 shows baseline characteristics of the participants. Standard clinical evaluations and laboratory analyses, including hematological, renal and liver function tests were done every 3 months. All unfavorable and unintended clinical effects were considered adverse effects and were evaluated by the investigators. At baseline sister chromatid exchange rate were statistically significantly higher in all patients groups as compared to healthy controls ($P < 0.05$; Table 1), whereas no difference in SCE frequency was detected between infliximab and methotrexate group. Sister chromatid exchange rate in each individual during treatment with infliximab and methotrexate does not show modifications (Table 2). At baseline mean value of sister chromatid exchanges rate was 7.20 ± 2.21 in the infliximab group and 7.40 ± 1.60 in the methotrexate group; after 24-weeks, it was 7.81 ± 1.95 in the methotrexate group and 7.87 ± 2.54 in infliximab group ($P > 0.05$ vs methotrexate and vs baseline). At basal the HFC in infliximab group was 4.6 %, in the methotrexate group was 4.7 % and 1.8 % in the control group; in all groups of patients, the values of HFC were statistically significantly greater than in the controls ($P > 0.05$; Table 1); after 24-weeks, HFC was 4.7 % in the methotrexate group and 4.9 % in the infliximab group ($P > 0.05$ vs methotrexate and vs baseline; Table 2). At baseline chromosomal aberration were statistically significantly higher in all patients groups as compared to the healthy controls ($P < 0.05$; Table 1), whereas no difference in CA value was detect between the infliximab and methotrexate groups. After 24-weeks, CA value does not showed modifications in the

infliximab and methotrexate groups (Table 3). In a single regression analyses no significant correlation between variables was observed (data not shown).

Three patients had side effects of infliximab group and two patients in methotrexate group discontinued protocol for increase of transaminase.

Discussion

There are two principal findings in this study. First the present study clearly demonstrates that there were statistically significant increases of SCE, HFC and CA frequencies in peripheral lymphocytes of the 40 patients with RA as compared to the 20 age and sex-matched controls. In fact in our patients baseline SCE, HFC and CA were significantly higher in patients than in the matched controls. The mechanism of such changes is not known, but we think it may be due to oxidative stress due to systemic inflammatory disease (Ramos-Remus et al. 2002). Second, our study revealed that the number of SCE was not increased in peripheral blood lymphocytes of patients with RA treated with infliximab compared to the group treated with only methotrexate. Sister chromatid exchanges is a reciprocal exchange of DNA segments between sister chromatids at identical loci, it is an excellent parameter for monitoring DNA damage and repair aspects (Latt 1981). It has been used as a test of mutagenicity in order to evaluate cytogenetic responses to chemical exposure and it has been reported to be a powerful method for detecting long-term effects of mutagens (Verma and Babar 1989). Our study showed that infliximab therapy did not increase SCE frequency, these data can exclude cytotoxic damage due infliximab therapy. The significant factors related to the frequency of SCE are age, sex and cigarette smoking. Nevertheless, since there is no difference in age, sex and smoking habits between the two groups, we exclude any interference of these factors. Also, in our study, the increase of cytogenetic biomarkers was independent of disease activity.

The cells with high number of SCE are called “high frequency cells” (HFC), and play an important role in detection of chemical exposure (Carrano and Moore 1982). Therefore, it is recommended that both sister chromatid exchanges and high frequency cell counts should be analyzed. These high frequency cells, which could be due to accumulated persistent damage in

Table 1 Characteristics of patients in groups; results are expressed as mean $\pm$ SD and percentage

Characteristics	Control group (group C, n = 20)	Infliximab group (group I, n = 20)	Methotrexate group (group M, n = 20)
Age (years)	60.28 $\pm$ 3.46	62.30 $\pm$ 5.74	65.64 $\pm$ 5.29
Sex F/M	17/3	17/3	17/3
Duration of disease (years)	0	9.10 $\pm$ 3.40	8.80 $\pm$ 4.60
Smokers (%)	15	20	15
CRP (mg/dL)	0.20 $\pm$ 0.09	5.50 $\pm$ 0.40 ^a	5.10 $\pm$ 0.50 ^a
ESR (mm/h)	7.20 $\pm$ 3.19	56.30 $\pm$ 8.50 ^a	54.35 $\pm$ 8.20 ^a
DAS 28 score	1.30 $\pm$ 0.31	4.23 $\pm$ 0.65 ^a	4.11 $\pm$ 0.79 ^a
Number of swelling joints	0	7.41 $\pm$ 1.35	7.22 $\pm$ 1.42
Number of tender joints	0	4.11 $\pm$ 0.65	4.17 $\pm$ 0.60
Sister chromatid exchange (n)	4.97 $\pm$ 1.32	7.20 $\pm$ 2.21 ^a	7.40 $\pm$ 1.60 ^a
High frequency cells (%)	1.8	4.60 ^a	4.70 ^a
Chromosomal aberration (%)	2.2	4.90 ^a	5.20 ^a

^a $P < 0.05$ compared to control group

Table 2 Mean ($\pm$ SD) sister chromatid exchanges rate and high frequency cell (%) counts in two groups at basal and after 24-weeks treatment

Group	No. of metaphase	Sister chromatid exchanges (mean $\pm$ SD)		High frequency cells (%)	
		Basal	24-weeks	Basal	24-weeks
Infliximab (n = 20)	1,000	7.20 $\pm$ 2.21	7.87 $\pm$ 2.54	4.6	4.9
Methotrexate (n = 20)	1,000	7.40 $\pm$ 1.60	7.81 $\pm$ 1.95	4.7	4.7

Table 3 Percentage of chromosomal aberrations in two groups at basal and after 24-weeks treatment

Group	No. of metaphase	Chromosomal aberration (%)	
		Basal	24-weeks
Infliximab (n = 20)	2,000	4.90	5.10
Methotrexate (n = 20)	2,000	5.20	5.10

certain longlived lymphocytes, might be a valuable indicator of damage from chronic exposure. Furthermore, they might indicate the existence of lymphocyte sub-population with different sensitivities to mutagens. In view of this, Carrano and Moore (1982) suggests that statistical analysis of high frequency cells is more powerful than that based on the sister chromatid exchanges means of individuals. Chromosomal aberrations are changes in normal chromosome structure or number that can occur spontaneously or as

a result of chemical/radiation treatment (Russel 2002). Based on morphological criteria, structural chromosomal aberrations can be divided in two main classes: chromosome type aberrations and chromatid type aberrations. Generations of structural chromosomal aberrations require one or several DNA double-strand breaks, but the mechanism of chromosome type aberrations and chromatid type aberrations formations appear to differ from the mutagen and involve specific DNA repair mechanisms (Hagmar et al. 2004). Structural chromosomal aberrations in peripheral blood lymphocytes as assessed by the chromosomal aberrations assay have been used for over 30 years in occupational and environmental settings as a biomarker of early effects of genotoxic carcinogens (Albertini et al. 2000). Our data showed that infliximab treatment in patients with RA did not increase the sister chromatid exchange mean, high frequency cell counts and chromosome type aberrations and chromatid type aberrations frequency. In fact, in patients treated with

infliximab, we did not observe an increase of sister chromatid exchange rate, high frequency cell counts and chromosomal aberrations frequency after 24-weeks of treatment. The study was designed so that each individual served as his/her own control. A baseline level for each biomarker of genetic damage was established prior to treatment with infliximab and the possible effect of treatment on that biomarker was determined after 24-weeks. In addition, several markers of genotoxicity were utilized for each sample to give a more comprehensive measure of any potential genotoxic effect that this drug may have.

The risk of cancer introduced with the use of anti-TNF drugs is controversial. In the past year an increase in lymphoma and solid cancers has been reported in RA patients receiving anti-TNF treatment (Munshi et al. 2008), although a recent meta-analyses of randomized trials and observational studies have not reported an increased overall cancer risk in arthritis patients treated with anti-TNF drugs (Askling et al. 2011). Thus, we thought that studying DNA damage would reflect a certain aspect of the possible association of the disease and treatment on the risk of malignancy. Recently Demirkaya et al. showed that after an initial improvement at 2 weeks, parameters of genotoxicity worsened, and DNA repair was further impaired 6 months after the addition of anti-TNF agent to treatment (Demirkaya et al. 2010). The mechanism yet remains obscure. Our study has limitations that must be considered in the interpretation of our finding. First, our study included only a small sample size. Another limitation is not having assessed a third population with an only infliximab treatment but in Italy infliximab must be associated with methotrexate in RA patients. In conclusion these data indicate that infliximab, for 24 weeks of treatment, has no genotoxic effects in patients with RA.

Conflict of interest None.

References

- Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, Natarajan AT, Norppa H, Shuker DE, Tice R, Waters MD, Aitio A (2000) IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. International Programme on Chemical Safety. *Mutat Res* 463:111–172
- Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger TA Jr, Mitchell DM, Neustadt DH, Pinals RS, Schaller JG, Sharp JT, Wilder RL, Hunder GG (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 31:315–324
- Askling J, Fahrback K, Nordstrom B, Ross S, Schmid CH, Symmons D (2011) Cancer risk with tumor necrosis factor alpha (TNF) inhibitors: meta-analysis of randomized controlled trials of adalimumab, etanercept, and infliximab using patient level data. *Pharmacoepidemiol Drug Saf* 20:119–130
- Balkwill F (2006) TNF- α in promotion and progression of cancer. *Cancer Metastasis Res* 25:409–416
- Carrano AV, Moore DH (1982) The rationale and methodology for quantifying sister chromatid exchange in humans. In: Heddle JA (ed) *New horizons in genetic toxicology*. Academic Press, New York, pp 267–304
- Cottliar A, Fundia A, Boerr L, Sambuelli A, Negreira S, Gil A, Gómez JC, Chopita N, Bernedo A, Slavutsky I (2000) High frequencies of telomeric associations, chromosome aberrations, and sister chromatid exchanges in ulcerative colitis. *Am J Gastroenterol* 95:2301–2307
- Demirkaya E, Cok I, Durmaz E, Ulutas OK, Ayaz NA, Besbas N, Ozen S (2010) Genotoxicity of anti-tumor necrosis factor therapy in patients with juvenile idiopathic arthritis. *Arthritis Care Res* 62:73–77
- Esser AC, Abril A, Fayne S, Doyle JA (2004) Acute development of multiple keratoacanthomas and squamous cell carcinomas after treatment with infliximab. *J Am Acad Dermatol* 50:S75–S77
- Gridley G, McLaughlin JK, Ekblom A, Klareskog L, Adami HO, Hacker DG, Hoover R, Fraumeni JF Jr (1993) Incidence of cancer among patients with rheumatoid arthritis. *J Natl Cancer Inst* 85:307–311
- National Institute of Clinical Excellence (2002) Guidance on the use of etanercept and infliximab for the treatment of rheumatoid arthritis. Technology appraisal guidance, March 2002, no. 36. National Institute of Clinical Excellence, London
- Hagmar L, Strömberg U, Bonassi S, Hansteen IL, Knudsen LE, Lindholm C, Norppa H (2004) Impact of types of lymphocyte chromosomal aberrations on human cancer risk: results from Nordic and Italian cohorts. *Cancer Res* 64:2258–2263
- Kageyama Y, Takahashi M, Ichikawa T, Torikai E, Nagano A (2008) Reduction of oxidative stress marker levels by anti-TNF- α antibody, infliximab, in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 26:73–80
- Kang MH, Genser D, Elmadfa I (1997) Increased sister chromatid exchanges in peripheral lymphocytes of patients with Crohn's disease. *Mutat Res* 381:141–148
- Latt SA (1981) Sister chromatid exchanges formation. *Annu Rev Genet* 15:11–55
- McCarthy CJ, Sheldon S, Ross CW, McCune WJ (1998) Cytogenetic abnormalities and therapy-related myelodysplastic syndromes in rheumatic disease. *Arthritis Rheum* 41:1493–1496

- Mellemkjaer L, Linet MS, Gridley G, Frisch M, Møller H, Olsen JH (1996) Rheumatoid arthritis and cancer risk. *Eur J Cancer* 32A:1753–1757
- Munshi NC, Diqumarthi S, Rahemtullah A (2008) Case records of the Massachusetts General Hospital: case 13-2008. A 46-year-old man with rheumatoid arthritis and lymphadenopathy. *N Engl J Med* 358:1838–1848
- Palmer RG, Dore CJ, Henderson L, Denman AM (1987) Sister-chromatid exchange frequencies in fibroblasts and lymphocytes of patients with systemic lupus erythematosus. *Mutat Res* 177:125–132
- Perkins ND (2004) NF- κ B: tumor promoter or suppressor? *Trends Cell Biol* 14:64–69
- Ramos-Remus C, Dorazco-Barragan G, Aceves-Avila FJ, Alcaraz-Lopez F, Fuentes-Ramirez F, Michel-Diaz J, Torres-Bugarin O, Ventura-Aguilar A, Zuñiga-González G (2002) Genotoxicity assessment using micronuclei assay in rheumatoid arthritis patients. *Clin Exp Rheumatol* 20:208–212
- Rantapää Dahlqvist S, Nordenson I (1996) Chromosomal changes in rheumatoid arthritis patients treated with CPH82. *Clin Rheumatol* 15:584–589
- Russel PJ (2002) Chromosomal mutations. In: Cummings B (ed) *Genetics*. Pearson Educations Inc, San Francisco, pp 595–621
- Shahin AA, Ismail MM, Saleh AM, Moustafa HA, Aboul-Ella AA, Gabr HM (2001) Protective effect of folinic acid on low-dose methotrexate genotoxicity. *Z Rheumatol* 60:63–68
- Thomas E, Brewster DH, Black RJ, Macfarlane GJ (2000) Risk of malignancy among patients with rheumatic conditions. *Int J Cancer* 88:497–502
- Van Horsen R, Ten Hagen TL, Eggermont AM (2006) TNF- α in cancer treatment: molecular insights, antitumor effects, and clinical utility. *Oncologist* 11:397–408
- Verma RS, Babar A (1989) Sister chromatid differentiation. In: Verma RS, Babar A (eds) *Human chromosomes manual of basic techniques*. Pergamon, New York, pp 124–131
- Wolff S, Perry P (1974) Differential Giemsa staining of sister chromatid and the study of chromatid exchange in human. *Chromosoma* 48:341–353