THE IMPACT OF RITUXIMAB THERAPY ON THE CHROMOSOMES OF PATIENTS WITH RHEUMATOID ARTHRITIS

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Abstract

The open prospective combined cytogenetic and clinical study investigated the impact of biological therapy Rituximab on number and structure of chromosomes in Rheumatoid arthritis patients.

The purpose of this study was to investigate safety of Rituximab on chromosomes as well as cytotoxic therapy Methotrexate.

A total of 8 seropositive Rheumatoid arthritis patients were analyzed for primary end point of eventual cytotoxic effect of Rituximab. Assessment was done before and 1 month later, actually 2 weeks after the administration of full course of Rituximab in infusion. Patients suffering from active Rheumatoid arthritis were randomly assigned according to established protocol to receive infusion of Rituximab in a full dose of 2,0 grams divided in a two doses of 1,0 gram on days 1 and 15. The lymphocytes from peripheral blood were cultured according to Moorhead method.

The results obtained from this investigation showed that normal male and female karyogram was found after the full therapy of Rituximab.

The results from this study, that was done on a rather small number of subjects, indicate that Rituximab does not express either clastogenic or aneugenic effects. But, co-finding of this study was that Methotrexate had a side effect on chromosomal aberration in one female RA patient, and after discontinuation of this treatment the normal karyogram was observed.

KEY WORDS: rituximab, chromosome's aberrations, rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease that affect approximately 0,5 % of the adult population and leads to irreversible joint destruction and permanent disability. The etiology of RA is still unknown, but new bio-tech therapies provide significant efficiacy and safety. The evaluative disease activity requires intensive treatment with disease-modifying anti rheumatic drugs (DMARDs) including biological therapy for rheumatoid arthritis. Rituximab is a genetically engineered human/mouse chimerical novel anti-CD20 monoclonal antibody that selectively binds to C20+ pre-B and mature B lymphocytes (1) causing the profound and prolonged depletion of peripheral-blood B cell subpopulation (2, 3). B lymphocytes play central role in the pathogenesis of Rheumatoid arthritis and in secretion of auto antibodies. The final impact of such depletion is the significant decrease of rheumatoid factor and other disease activity parameters in a patient serum. Methotrexate is standard care for RA, but as well as other cytotoxic drugs have many adverse events and may provoke malignancy, malignant lymphomas and tumor lysis syndrome.

The purpose of this study and primary goal was to investigate the impact of Rituximab on the number and structure of chromosomes in rheumatoid arthritis patients (4). The open-label prospective combined cytogenetic and clinical study was conducted. The secondary aim was to observe the effect of cytotoxic therapy Methotrexate on the same chromosomes.

Materials and Methods

A total of 8 seropositive Rheumatoid arthritis patients, 6 females and 2 males, were analyzed for primary end point of eventual cytogenetic effects of rituximab. Average age of females was 46,5 years and for males 41,5 years with total average of 44 years. The open-label clinical randomized prospective study was conducted. The assessment was done before application of infusion and 1 month later retrospectively, actually 2 weeks after the administration of full course of rituximab in infusion. All patients were randomly assigned to receive infusion of rituximab in a full dose of 2,0 grams divided in two doses of 1,0 gram on Days 1 and 15 (5,6). The lymphocytes from peripheral blood were cultured according to Moorhead method (7). The 5 millilitres of heparinised blood was taken from RA patients and poured into 4 ml of RPMI-60 medium and 1ml of fetal calf sera infiltrate with a certain concentration of Penicillin and Streptomycin. A mytogen stimulator Phittohaemaglutinin in adequate concentration was added. After 48 hours of culture in thermostat at 37 °C, Colcemid was added in adequate concentration for controlling the culture for 90 minutes. Then, the culture was poured into glass containers for centrifugation at 600 spins for 10 minutes. Supernatant was taken out using Pasteur pipette and replaced by 0,75 mol/dm3 KCl as hypotonic for further 45 minutes. Centrifugation at 600 spins and pouring out the supernatant was done again, after that the fixation (methanol 3: glace acetic acid 1) was added to the culture and left for another 10 minutes. This method of fixation was repeated at least twice, the material was fixed on slides and then collared by Giemsa. For each patient 200 mitotic cells were analyzed for structural or numeric changes of chromosomes (8).

The following table shows patient number, sex, average age and number of smokers. All seropositive RA patients had severe deformative and active disease. Disease activity score (DAS) was 6,5 that indicites high disease activity. They were previously treated with DMARDs, including gold, hydroxychloroquine, leflunomide, sulfasalazine that were discontinued due to lack of efficiacy and cytotoxic therapy with methotrexate was prescribed to all.

	N° of patients	Average age	Smokers
Males	2	41,5	2
Females	6	46,5	1
Total	8	44,0	3

TABLE 1. Patient's characteristics

Results

Genetically findings:

No chromosomal abnormalities in terms of their number and structure were found on all Rheumatoid arthritis patients who received the full course of Rituximab therapy.

In one female patient, who was on longstanding Methotrexate therapy, the first analysis done before the rituximab therapy has shown some initiation of chromosomal aberration. After discontinuation of Methotrexate from therapy, and two weeks after application of Rituximab, those chromosomal initial aberrations disappeared. And normal karyogram was observed.

The Figure 1. and 2. shows karyogram of this patient.

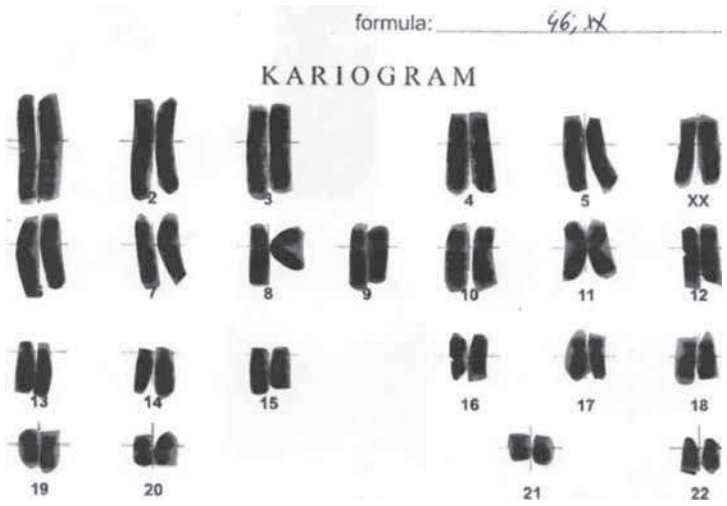

FIGURE 1. 2% of karyogram with structural changes in a patient receiving Methotrexate therapy

FIGURE 2. Normal karyogram of the same patient after discontinuation of Methotrexate. Test done two weeks after receiving full dose of Rituximab therapy.

Discussion

All 8 patients who received biological therapy with Rituximab showed no either numerical or structural changes of their chromosomes. What so ever, one female RA patients who was treated with cytotoxic agent Methotrexate showed initially some chromosomal aberration, but after excluding this drug from treatment and introduction of Rituximab in a therapy, the normal karyogram was found.

Metotrexate

No similar study on chromosomes was done so far, so our data could not be comparable. Other authors evaluated safety profile in many open-label extension studies and also in double-blind prospective randomized ones. The incidence of malignancies after rituximab therapy was 1,6 per 100 patient-years that was within expected range for patients age 60 years and older and they received Repeated courses of rituximab were well tolerated in 1,600 patient-years with no additional safety concerns (9) .

Conclusion

The results obtained from this study, although done on a rather small number of RA patients, indicated that that Rituximab therapy is a safe for the number and structure of human chromosomes.
After this study, we recommend further cytogenetic investigations for cytotoxicity using Brom-deoxiuridin in a blood cultures. Additional tests like micronucleus test (using Cytohalasin B) and analysis of binuclear lymphocytes could provide more information's if needed.
Patients receiving cytotoxic drugs like Methotrexate and others should be screened for eventual chromosomal aberration in order to avoid cumulative drug cell toxicity.

List of Abbreviations

RA	-	Rheumatoid arthritis
(DMARDs)	-	Disease-modifying anti rheumatic drugs

References

(1) Raparon-Schuijt C.C., et al. Presence of a population of CD20+, CD38-B lymphocytes with defective proliferative responsiveness in the synovial compartment of the patients with rheumatoid arthritis. Arthritis Rheum. 2001;44:2029-2037.

(2) Dorner T., Burmaster G.R. The role of B cells in rheumatoid arthritis: mechanisms and therapeutic targets. Curr. Opin. Rheumatol. 2003;15: 246-252.

(3) Emery P. et al., Sustained efficacy at 48 weeks after single treatment course of rituximab in patients with rheumatoid arthritis. Arthritis Rheum. 2003; 48: Suppl: S439.

(4) Cittera E. et al. Rituximab induces different but overlapping sets of genes in human B-lymphoma cell lines. Cancer Immunol. Immunoth. 2005. 54(3): 273-286.

(5) Van Vollenhoven P., Emery P. et al. Long term safety data from Extended Follow-up and repeat use of Rituximab in Rheumatoid arthritis. EULAR 2007, 13-17. June 2007, Barcelona. Abstract book

(6) Gause A., Berek C. The role of B cells in the pathogenesis of rheumatoid arthritis: potential implications for treatment. Bio. Drugs 2001; 15: 73-79.

(7) Moorhead P.S., Nowell P.C., Mellman W.J., Battips D.M., Hungerfor D.A. Chromosome preparations of leucocytes cultured from human peripheral blood. Exp. Cell Res. 1960; 20: 613.

(8) Dutrillaux B., Couturier J. Praktična analiza hromosoma. "Svjetlost" Sarajevo 1989.

(9) Keystone E. et al. Safety and Efficacy of additional courses in patients with active Rheumatoid Arthritis. Arthritis & Rheumatism.2007; 56(12): 3896-3908