PHARMACOLOGY AND TOXICOLOGY

Effect of Afobazole on DNA Damage in Patients with Systemic Lupus Erythematosus

A. K. Zhanataev, T. A. Lisitsyna, A. D. Durnev, E. L. Nasonov*, and S. B. Seredenin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, No. 10, pp. 404-407, October, 2009
Original article submitted March 24, 2009

Using comet assay we studied the effect of anxiolytic afobazole, exhibiting also antioxidant and antimutagenic properties, on spontaneous and *ex-vivo* hydrogen peroxide-induced DNA damage in blood cells from patients with systemic lupus erythematosus. Afobazole treatment (30-60 mg/day for 1 month) in addition to standard therapy decreased spontaneous level of DNA damage in blood cells. The level of *ex vivo* hydrogen peroxide-induced DNA damage decreased by 49% in this group of patients. The number of cells hypersensitive to hydrogen peroxide yielding DNA comets with highly damaged DNA also decreased by 51%. No significant changes in the analyzed parameters were found in the placebo group. Addition of afobazole to complex therapy of patients with systemic lupus erythematosus reduced the level of DNA damage in blood cells and improved cell resistance to oxidative genotoxic exposure.

Key Words: *systemic lupus erythematosus; DNA comets; afobazole; DNA damage*

Excessive production of reactive oxygen species (ROS) and their capacity to induce antigenic DNA modification followed by production of autoantibodies to DNA plays an important role in the pathogenesis of systemic lupus erythematosus (SLE) [6,10,12].

Genotoxic and mutagenic properties of ROS are well known [10,13]; excessive production of ROS can be associated with increased level of spontaneous mutations and DNA damage and impairment of DNA repair observed in some studies in patients with autoimmune diseases, *e.g.* SLE [3,4,7,8].

It was assumed [6] that administration of drugs possessing antioxidant properties produces a positive therapeutic effect and reduces genotoxic effects of ROS in patients with autoimmune diseases. This assumption was supported by investigation of DNA repair and antibody production in patients with SLE and rheumatoid arthritis receiving actoprotector and antimutagen bemithyl in addition to complex therapy [1,5].

The aim of this study was to investigate DNA damage in blood cells from SLE patients and their resistance to genotoxic action of H_2O_2 before and after administration of anxiolytic afobazole exhibiting pronounced cytoprotective, antioxidant, and antimutagenic properties [2].

MATERIALS AND METHODS

The study was conducted in the Clinical Department of Institute of Rheumatology, Russian Academy of Medical Sciences.

Patients were divided into groups, 30 per group. Group 1 patients received afobazole in a dose of 30-

V. V. Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences; *Institute of Rheumatology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** azhanataev@yandex.ru. A. K. Zhanataev

0007-4888/09/1484-0602

60 mg/day for 1 month in addition to basic treatment; group 2 patients received placebo for 1 month.

At the moment of enrolling, the groups did not differ by patient's age and sex, duration and severity of the disease. Pulse therapy with high doses of methylprednisolone was performed in the same number of patients in each group, 14 (47%). Nine patients in each group (30%) received cyclophosphamide. The groups consisted mainly of women (97%), predominantly with chronic SLE (65%). Average age of patients was 36.7±11.4 years (19-57 years), mean SLEDAI score was 7.6 in both groups.

DNA damage in blood cells was estimated using alkaline gel electrophoresis of individual cells (DNA comet assay) according to recommendations [9]. Whole blood (50 µl) was added to the test tube containing 500 µl 1% low-melting agarose and then applied on glass slides precoated with 1% universal agarose. The specimens were covered with coverslips and placed on ice. After gelation of agarose, the slides were incubated in a buffer containing 10 mM Tris-HCl (pH 10), 2.5 M NaCl, 100 mM EDTA-Na_2, 1% sarcosyl, 1% Triton X-100, and 10% DMSO at 4°C for at least 1 h. After completion the lysis procedure, the slides were transferred to a chamber containing electrophoresis buffer (300 mM NaOH, 1 mM EDTA-Na_2, pH>13) and were incubated for 20 min. Electrophoresis was conducted at field voltage 1 V/cm and amperage ~300 mA for 20 min. After electrophoresis, the specimens were fixed in 70% ethanol for 20 min, dried, and kept at room temperature until the analysis.

To estimate the resistance of blood cells to DNA damaging action of H_2O_2, blood samples were settled up to erythrocyte sedimentation, after that 250 µl blood was centrifuged for 10 min at 1000*g*. Blood plasma was removed, the cells were resuspended in 250 µl RPMI-1640 medium, and the washing was repeated. After the second washing, the cells were resuspended in 250 µl RPMI-1640 medium, H_2O_2 was added up to a final concentration of 40 µM, and cells were incubated for 5 min at 37°C. Then the cells were added to agarose gel and specimens were prepared routinely.

Specimens were stained with SYBR Green I (1:10,000 in TE buffer; Invitrogen) and analyzed using a Micmed-2 12T epifluorescent microscope (×200, Lomo) connected to high resolution digital camera (VEC-335, EVS). Images of DNA comets were processed using CASP v.1.2.2 software [11]. At least 100 cells were analyzed in each case. DNA percentage in the tails of DNA comets (% of DNA in the tail) was used as the index of DNA damage. For evaluation of induction of DNA damage, the difference in DNA percent in comet tails before and after H_2O_2 application was calculated (Δ% DNA in the tail). The number of DNA comets from cells with highly damaged DNA (HD-cells) was also calculated and used as the index of the cytotoxic effect of the agent (Fig. 1, *b*). Statistical analysis was performed by Wilcoxon test for paired comparison using Statistica 6.0 software.

RESULTS

The data on the level of DNA damage in blood cells from SLE patients before and after therapy are shown on Figure 2, *a*.

The initial mean values of DNA damage were 4.8±0.9% (from 0.4 to 24.2%) and 5.1±0.9% (from 1.3 to 17.8%) DNA in the tail in the placebo and afobazole groups, respectively. Paired comparison revealed no significant differences between the groups.

After the end of the study, the mean value of DNA damage in the placebo group was 4.8±0.6% DNA in the tail, which did not differ from this index before treatment. The level of DNA damage decreased in 3 patients and considerably increased (by 2-4 times) in 6 patients. In the afobazole group, the mean value of DNA damage decreased to 3.1±0.4% DNA in the tail ($p<0.03$); this parameter decreased in 6 patients and changed insignificantly in other cases.

Thus, the mean level of DNA damage in the afobazole group was significantly lower than before treatment. Moreover, the range of individual values of DNA damage considerably decreased in this group (Fig. 2,

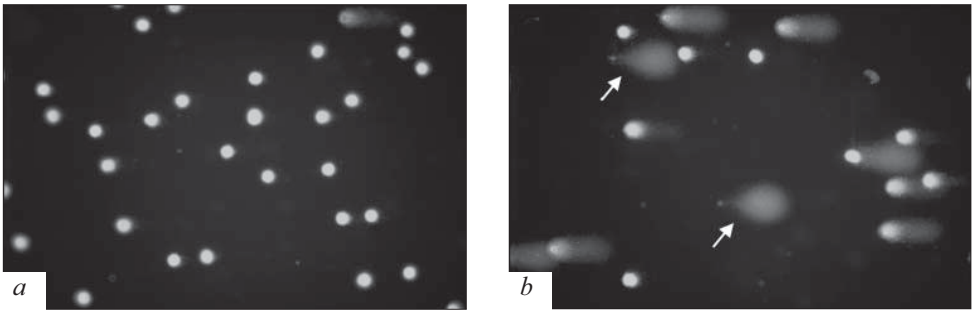

Fig. 1. DNA comets from blood cells of SLE patients before (*a*) and after (*b*) treatment with H_2O_2. Arrows indicate DNA comets of highly damaged cells (HD cells). SYBR Green I staining, ×200.

a). These data suggest that afobazole can reduce DNA damage in blood cells from SLE patients.

This observation was supported in parallel experiments focused on estimation of the resistance of blood cells from SLE patients to the damaging *ex vivo* action of H_2O_2 before and after the corresponding treatment (Fig. 2, *b*, *c*).

Preliminary experiments showed that H_2O_2 in a concentration 100 μM produces a cytotoxic effect on 40% blood cells from SLE patients, which obstructed subsequent analysis. Basing on these data we used H_2O_2 in a concentration of 40 μM; the cytotoxic effect of H_2O_2 does not exceed 25%.

Before therapy, the increase in DNA damage caused by H_2O_2 application was 10.1±1.6 (from 2.5 to 23.8) and 12.9±1.7 (from 1.4 to 20.3)% DNA in the tail in the placebo and afobazole groups, respectively. There were no statistically significant differences between the groups.

After therapy, the mean value of H_2O_2-induced DNA damage in the placebo group was 9.9±1.5% DNA in the tail, which did not differ from the corresponding index before the study. The estimated index decreased in 6 patients and increased in 7 patients. In the afobazole group, Δ% DNA in the tail significantly decreased to 6.5±1.4 (p<0.02), the resistance to DNA damaging action of H_2O_2 increased in 9 of 11 patients in this group.

These findings showed that afobazole improves cell resistance to genotoxic action of oxidizing agent *ex vivo*.

Counting the cells with highly damaged DNA (HD-cells) after application of H_2O_2 confirmed the protective properties of afobazole (Fig 2, *c*). In SLE patients, the percent of H_2O_2-induced HD-cells before treatment was 6.9±1.2 (from 0.8 to 19.5) and 10.5±2.1 (from 0.9 to 20.8)% in the afobazole and placebo groups, respectively. No significant differences between these two values were revealed. By the end of the study, the number of HD cells in the placebo group was 8.0±1.6%, which little differed from the initial value, while in the afobazole group the level of induced HD cells after treatment significantly decreased to 5.1±1.7% (p<0.03).

Thus, addition of afobazole to the complex therapy of SLE patients decreased the level of DNA damage in blood cells. The marked increase of cell resistance to the DNA damaging and cytotoxic action of H_2O_2 *ex vivo* suggests that the protective effect of afobazole is associated with its influence on the efficacy of cellular antioxidant defense system. This conclusion is in line with the known data on the antioxidant effects of afobazole and experimentally shown *in vivo* inhibition of the clastogenic effects of dioxidine, a mutagen with prooxidant action type, by afobazole [2].

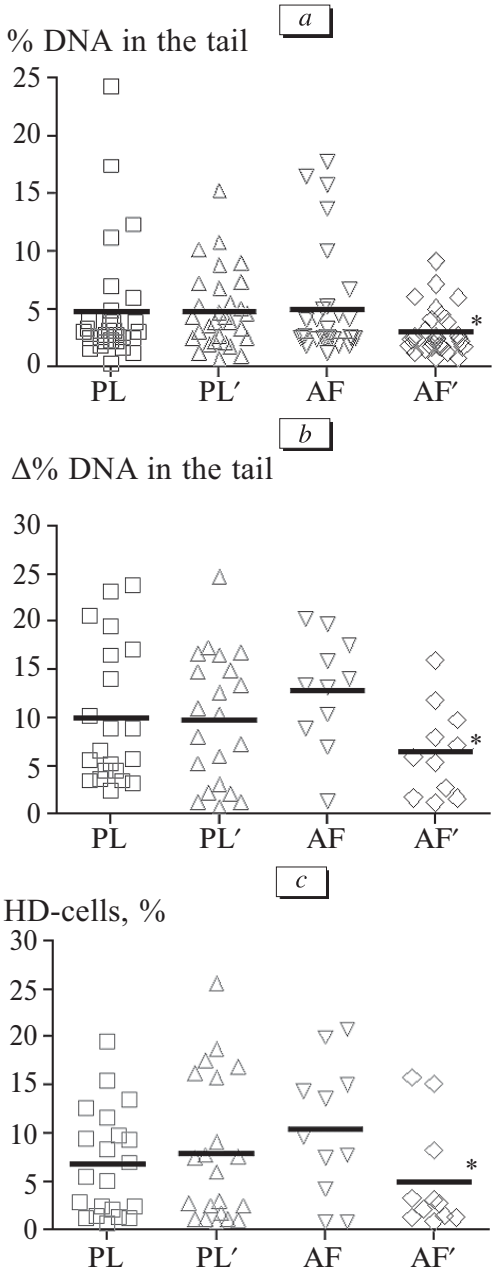

Fig. 2. Level of DNA damage (*a*), *ex vivo* H_2O_2-induced DNA damage (*b*), and HD cells (*c*) in blood cells of SLE patients before and after the corresponding treatment. PL and PL': placebo group before and after the study, respectively; AF and AF': afobazole group before and after the study, respectively. Horizontal line: mean group values for the parameter. *p<0.03 compared to the corresponding parameter before treatment.

REFERENCES

1. I. M. Vasilieva, T. A. Lisitsyna, A. D. Durnev, *et al.*, *Genetika*, **33**, No 12, 1711-1713 (1997).
2. A. D. Durnev, A. K. Zhanataev, O. V. Shreder, S. B. Seredenin, *Eksp. Klin. Farmakol.*, **72**, No. 1, 47-52 (2009).
3. N. A. Iliushina, T. M. Reshetniak, E. Yu. Moskaleva, *et al.*, *Vestn. Ross. Akad. Med. Nauk*, No. 9, 35-38 (1993).

4. N. S. Lipatova, A. L. Alyavi, *Ter. Arkhiv*, No. 5, 35-38 (2002).
5. T. A. Lisitsyna, A. D. Durnev, M. M. Ivanova, *et al.*, *Eksp. Klin. Farmakol.*, **62**, No. 5, 38-41 (1999).
6. T. A. Lisitsyna, M. M. Ivanova, A. D. Durnev, *Vestn. Ross. Akad. Med. Nauk*, No. 12, 15-19 (1996).
7. T. A. Lisitsyna, V. A. Tronov, M. A. Konoplyannikov, *et al*., *Byull. Eksp. Biol. Med.*, **125**, No. 1, 75-78 (1998).
8. S. Bashir, G. Harris, M. A. Denman, *et al*., *Ann. Rheum. Dis.*, **52**, No. 9, 659-665 (1993).
9. A. Hartmann, E. Agurell, and C. Beevers, *Mutagenesis*, **18**, No. 1, 45-51 (2003).
10. F. Khan, F. Khan, R. Ali, *Biotechnol. Appl. Biochem.*, **46**, Pt. 2, 97-103 (2007).
11. K. Konca, A. Lankoff, A. Banasik, *Mutat. Res*, **534**, Nos. 1-2, 15-20 (2003).
12. B. T. Kurien, R. H. Scofield, *Autoimmun. Rev.*, **7**, No. 7, 567-573 (2007).
13. M. Valko, M. Izakovic, M. Mazur, *et al*., *Mol. Cell. Biochem*., **266**, Nos. 1-2, 37-56 (2004).