Acute Myeloid Leukemia

Increased oxidative DNA products in patients with acute promyelocytic leukemia during arsenic therapy

Arsenic trioxide (ATO) has been used to treat acute promyelocytic leukemia (APL), but the oxidative DNA damage occurring in patients has not been fully elucidated. We measured 8-hydroxy-2'-deoxyguanosine (8-OHdG), one of the most abundant oxidative products of DNA, by enzyme-linked immunoassay, and reactive oxidative species (ROS), by luminol- and luminol-H_2O_2 chemiluminescence, in the plasma of four APL patients treated with ATO. After six courses of ATO therapy, the plasma 8-OHdG concentration had increased from 45.6±22.8 ng/mL to 310.2±239.6 ng/mL. The plasma chemiluminescence level did not change significantly. These findings suggest that ATO generates intracellular oxidative DNA damage, but this is not correlated with the plasma ROS level. The clinical significance of 8-OHdG during and after ATO therapy warrants further study.

Haematologica 2006; 91:1571-1572

Arsenic trioxide (ATO) therapy is an established therapy for relapsed or refractory acute promyelocytic leukemia (APL), and induces a second complete remission in more than 80% of such patients.[1] ATO induces of apoptosis and differentiation in several malignant cell lines including APL lines.[2,3] The mechanism of action includes generation of reactive oxygen species (ROS), modulation of signaling pathways, and stimulation of caspase-dependent and –independent apoptosis.[4-7] In addition, arsenite increases the levels of superoxide-driven hydroxyl radicals.[8] Importantly, NADPH oxidase-related genes are reported to be up-regulated in APL cell lines treated with ATO, and this was related to an increase of superoxide anion.[9]

8-hydroxy-2'-deoxyguanosine (8-OHdG) is a well-known oxidative DNA product,[10] and levels of this product have been reported to be increased in the urine from patients with leukemia.[10] In APL cell lines, ROS levels are elevated by *in vitro* ATO treatment, but it has not been clearly determined whether 8-OHdG and ROS levels are increased in clinical samples. Here, we report the oxidative status of APL patients during ATO therapy, assessed by measuring plasma 8-OHdG and ROS.

Between 2001 and 2003, we treated four patients with relapsed APL using ATO at Nagoya University Hospital. For induction therapy, ATO was given intravenously at a dose of 0.15 mg/kg/day until complete remission was achieved or day 60. If complete remission was obtained, two courses of consolidation therapy (0.15 mg/kg/day for 28 days) were given separated by an interval of 3-6 weeks. The level of 8-OHdG were measured using an enzyme linked immunosorbent assay (ELISA) (New 8-OHdG check, Japan Institute for the Control of Aging, Shizuoka, Japan), while the ROS level was determined by an MLA-GOLDS chemiluminescence analyzer (Tohoku Electronic Industry, Sendai Japan) in an air atmosphere at 37°C. A solution of 0.003% H_2O_2 (50 μL) was added to the luminol-plasma measurement, and the light emission was also analyzed for 180 sec (luminol-H_2O_2-plasma measurement). Superoxide dismutase (SOD, Sigma) was added at 500 U/mL as the control for the quenching of

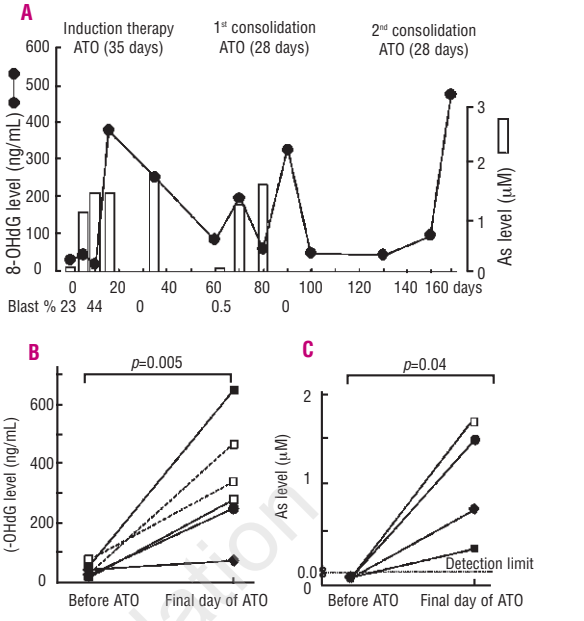

Figure 1. Plasma arsenic and 8- OHdG levels in APL patients who received ATO therapy. (A) Time course change from remission induction therapy (IT) to 2nd consolidation therapy. (B) Plasma 8-OHdG levels in four APL patients before and on the final day of ATO therapy. The levels were significantly increased by the therapy (p=0.005 by the paired t-test, p=0.018 by Wilcoxon's signed-rank test). (C) Plasma arsenic levels in four APL patients before and on the final day of ATO therapy. The levels were significantly increased (p=0.04 by the paired t-test, p=0.067 by Wilcoxon's signed-rank test).

superoxide anions. The plasma 8-OHdG level was increased during remission induction therapy, and two courses of consolidation therapy in one patient (Figure 1A). In the interval between ATO therapy, the 8-OHdG level decreased almost to the normal range. After a total of six courses of ATO in the four patients, the plasma 8-OHdG concentration had increased from 45.6±22.8 ng/mL to 310.2±239.6 ng/mL (p=0.005 by the paired t-test, p=0.018 by Wilcoxon's signed-rank test, Figure 1B). The plasma level of arsenic was below thelimit of detection (0.08 μM) before treatment, and increased to 0.21-1.47 μM after therapy (p=0.04 by the paired t-test, p=0.067 by Wilcoxon's signed-rank test, Figure 1C). Since 8-OHdG has been reported to be generated by arsenic through several chemical reactions involving superoxide anions, hydrogen peroxide, and hydroxyl radicals,[9] plasma level of ROS were measured. The chemiluminescence level per 10 sec in the luminol-plasma assay and in the luminol-H_2O_2-plasma assay was not significantly different before and after remission induction therapy (*data not shown*). Our data suggest that the generation of ROS and peroxidase-dependent was not elevated by ATO *in vivo*. ROS in the plasma might be cleared rapidly by free-radical scavenging enzymes.

These results clearly indicate that ATO therapy is associated with intracellular oxidative DNA damage. Since 8-OHdG levels were similarly increased after remission induction and consolidation therapy, it remains unclear whether the increase is related to anti-leukemia effects. Measurements of plasma 8-OHdG may be important to monitor the long-lasting oxidative damage caused by ATO. A strategy to enhance oxidative stress selectively

may expand the therapeutic spectrum and decrease the toxicity of ATO.

Manabu Ninomiya, Tomohiro Kajiguchi, Kazuhito Yamamoto, Tomohiro Kinoshita, Nobuhiko Emi, Tomoki Naoe

Department of Hematology & Oncology, Nagoya University Graduate School of Medicine, Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

Key words: 8-hydroxy-2'-deoxyguanosine (8-OHdG), acute promyelocytic leukemia (APL), arsenic trioxide, reactive oxygen species (ROS).

Correspondence: Tomoki Naoe, MD, PhD, Department of Hematology & Oncology, Nagoya University Graduate School of Medicine, Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan.
Phone/fax: international +052.7442136.
E-mail: tnaoe@med.nagoya-u.ac.jp

References

1. Douer D, Tallman MS. Arsenic trioxide: new clinical experience with an old medication in hematological malignancies. J Clin Oncol 2005;23:2396-410.
2. Miller WH Jr, Schipper HM, Lee JS, Singer J, Waxman S. Mechanisms of action of arsenic trioxide. Cancer Res 2002; 62:3893-903.
3. Zhang TD, Chen GQ, Wang ZG, Wang ZY, Chen SJ, Chen Z. Arsenic trioxide, a therapeutic agent for APL. Oncogene 2001; 20:7146-53.
4. Chen F, Shi X. Intracellular signal transduction of cells in response to carcinogenic metals. Crit Rev Oncol Hematol 2002; 42:105-21.
5. Kitamura K, Minami Y, Yamamoto K, Akao Y, Kiyoi H, Saito H, et al. Involvement of CD95-independent caspase 8 activation in arsenic trioxide-induced apoptosis. Leukemia 2000; 14: 1743-50.
6. Liu SX, Athar M, Lippai I, Waldren C, Hei TK. Induction of oxyradicals by arsenic: implication for mechanism of genotoxicity. Proc Natl Acad Sci USA 2001;98:1643-8.
7. Chou WC, Jie C, Kenedy AA, Jones RJ, Trush MA, Dang CV. Role of NADPH oxidase in arsenic-induced reactive oxygen species formation and cytotoxicity in myeloid leukemia cells. Proc Natl Acad Sci USA 2004;101:4578-83.
8. Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. Curr Top Med Chem 2001;1:529-39.
9. Qian Y, Castranova V, Shi X. New perspectives in arsenic-induced cell signal transduction. J Inorg Biochem 2003;96:271-8.
10. Honda M, Yamada Y, Tomonaga M, Ichinose H, Kamihira S. Correlation of urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, and clinical features of hematological disorders: a pilot study. Leuk Res 2000;24:461-8.