For induction therapy, ATO was given intravenously at a rate of 0.15 mg/kg/day until complete remission was achieved or day 60. If complete remission was obtained, the dose of ATO was 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. 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.

For relapsed or refractory acute promyelocytic leukemia patients, ATO therapy is an established therapy for APL in which ATO induces apoptosis and differentiation in several malignant cell lines including APL lines. The mechanism of action includes generation of reactive oxygen species (ROS), modulation of signaling pathways, and stimulation of caspase-dependent and –independent apoptosis. 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.

8-hydroxy-2′-deoxyguanosine (8-OHdG) is a well-known oxidative DNA product, and levels of this product have been reported to be increased in the urine from patients with leukemia. In APL cell lines, ROS levels are elevated by in vitro ATO treatment, but it has not been 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 was 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% H2O2 (50 µL) was added to the luminol-plasma measurement, and the light emission was also analyzed for 180 sec (luminol-H2O2-plasma measurement). Superoxide dismutase (SOD, Sigma) was added at 500 U/mL as the control for the quenching of 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). The plasma level of arsenic was below the limit 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). Since 8-OHdG has been reported to be generated by arsenic through several chemical reactions involving superoxide anions, hydrogen peroxide, and hydroxyl radicals, plasma level of ROS was measured. The chemiluminescence level per 10 sec in the luminol-plasma assay and in the luminol-H2O2-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, Kazuhiro 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: naoe@med.nagoya-u.ac.jp

References