Effects of Prolonged Nitrous Oxide Exposure on Hemopoietic Stem Cells in Mice

Mitsuo KONNO, Teruo KIRIKAE*, Kenji SUZUKI, Masao YOSHIDA*,
Kazuhiro J. MORI** and Reiji WAKUSAWA

The effects of prolonged exposure to nitrous oxide on the hemopoietic progenitor cells in bone marrow and spleen in mice were investigated. Fifty percent nitrous oxide caused a marked decrease in the number of pluripotent stem cells (CFU-S) and granulocyte-macrophage progenitor cells (GM-CFC) in the spleen, whereas it caused only a slight decrease in these cells in the bone marrow. These results suggest that prolonged exposure to nitrous oxide induces damage in the splenic hemopoiesis in mice. [Key words: nitrous oxide, pluripotent hemopoietic stem cells (CFU-S), granulocyte-macrophage progenitor cells (GM-CFC)]

(Konno M et al.: Effects of prolonged nitrous oxide exposure on hemopoietic stem cells in mice. J Anesth 1:29–34, 1987)

Nitrous oxide was cited as being toxic to the hemopoietic tissue of man in 1956[1-3]. Since then, there have been a number of reports on the leukocytopenia and acute bone marrow depression, resulting from prolonged administration of nitrous oxide in man[3-5].

In several experiments using rodents, long-term exposure to nitrous oxide induced significant leukocytopenia[6-8]. Histo-cytological studies showed that prolonged exposure of nitrous oxide elicited an injury in the bone marrow[6,9].

In the present study, we have investigated the effects of nitrous oxide on the numbers of pluripotent hemopoietic stem cells (CFU-S) and granulocyte-macrophage progenitor cells (GM-CFC) in murine bone marrow and spleen in vivo.

[1] *Department of Anesthesiology and *Department of Bacteriology, School of Medicine, Iwate Medical University, Morioka, **Department of Microbiology, Faculty of Medicine, Kyoto University, Kyoto, Japan*

Address reprint requests to Dr. Konno: Department of Anesthesiology, School of Medicine, Iwate Medical University, 19-1 Uchimaru, Morioka, 020 Japan

Materials and Methods

Exposure to nitrous oxide

Seven-week-old male mice of C3H/HeSlc strain were exposed to nitrous oxide at the start of experiments. The exposure was carried out in a gas-tight chamber with 50% oxygen and 50% nitrous oxide at 21–24°C and 70% humidity, during experimental periods. The gas-mixture was circulated through the chamber by an anesthetic machine. Carbon dioxide was absorbed by the soda lime placed on the floor of the chamber, and then 50% nitrogen and 50% oxygen were circulated through the chamber in the control group. Both control and nitrous oxide-exposed mice were housed 4 to 5 mice per cage, and fed with standard diets and given water. The chambers were opened every 48 hours for cleaning and the exchange of food and water.

Hematological responses

Blood samples were collected for the counting of numbers of red cells, leukocytes, platelets and the differentiation of leukocytes. Blood smears were stained with May-Giemsa and

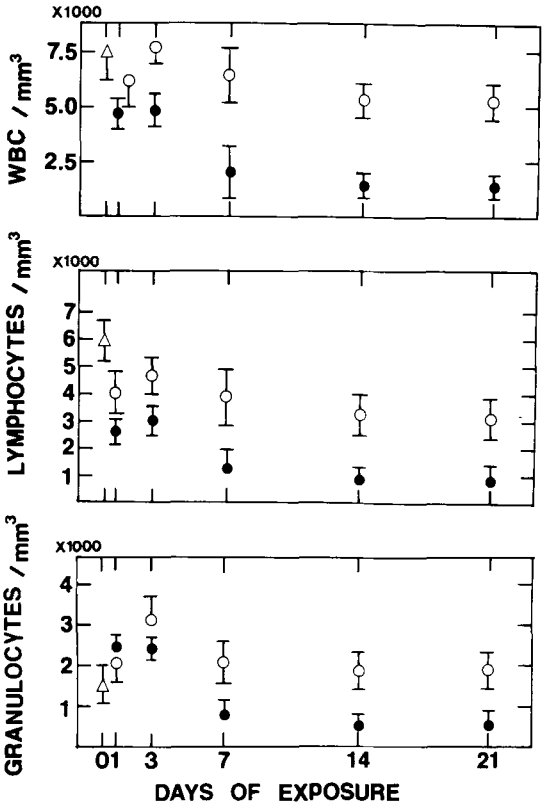

Fig. 1. Changes in number of leukocytes in peripheral blood after nitrous oxide exposure. The upper row shows total white blood cells, the middle shows lymphocytes, and the lower are granulocytes. Open triangle is pre-exposure level in 7-weeks-old male mice. Open circles are levels exposed to 50% nitrogen and 50% oxygen as the control group. Closed circles are those exposed to 50% nitrous oxide and 50% oxygen.

the differential counts were made of the lymphocytes and granulocytes. Spleen cellularity was estimated by measuring wet weight, on the basis that one milligram of the spleen had previously been found to contain about 1.5 x 10^6 nucleated cells[10]. Bone marrow cells were flushed into α-minimal essential medium (α-MEM) from the tibia, using an injection syringe. The cells from 5 mice were pooled and counted.

Assays of CFU-S and GM-CFC

The assays of CFU-S and GM-CFC were similar to those described previously[10]. Briefly, appropriate numbers of spleen cells or bone marrow cells from the tibia were infused i.v. to lethally irradiated mice (850 Rad), and the colonies in the spleen were counted as CFU-S 10 days later[11]. Irradiation was performed using a cobalt-60 radiation source with the rate of exposure about 32 Rad per minute. Spleen and bone marrow cells were also cultured in a semi-solid medium prepared by adding 0.3% agar and 25% horse serum to α-MEM, supplemented with mouse abdominal wall-conditioned medium at 10%, as a source of colony stimulating factor. Four replicate cultures were incubated for 7 days at 37°C in 5% CO_2 in humidified air[12]. Colonies consisting of 50 or more cells were counted as GM-CFC.

Results

General findgins in exposed mice

Mice exposed to nitrous oxide appeared alert and behaved normally, although they consumed slightly less food and water as compared to control. The loss of weight was observed, and body weight of the mice after three weeks of exposure decreased, as compared with control (24.4 ± 1.5 versus 27.9 ± 1.8 g).

Effect of exposure on the peripheral blood cells, spleen size and bone marrow cellularity

As shown in fig. 1, both total leukocyte and lymphocyte counts decreased rapidly within 1 day, and the decrease continued during exposure to nitrous oxide until the third week, as compared to the control group. The decrease of granulocyte counts became apparent on day 7, and lagged behind those of total leukocytes and lymphocytes. There were no significant change in the hematocrit, hemoglobin concentration, and the platelet count in either exposed or control group at any time. These results corresponded well with the previous reports, using rats[6] and mice[13].

Spleen weight and number of nucleated cells in the tibia are shown in fig. 2. In the exposed group, a slight decrease in the spleen size was observed. The decrease continued from day 1 until day 21. There was also a slight diminition in the number of nucleated cells in the tibia after exposure.

Effect of exposure on hemopoietic stem cells in the spleen and the bone marrow

The results are shown in fig. 3–6.

The number of CFU-S in the spleen increased on day 1 after exposure, approximately twice

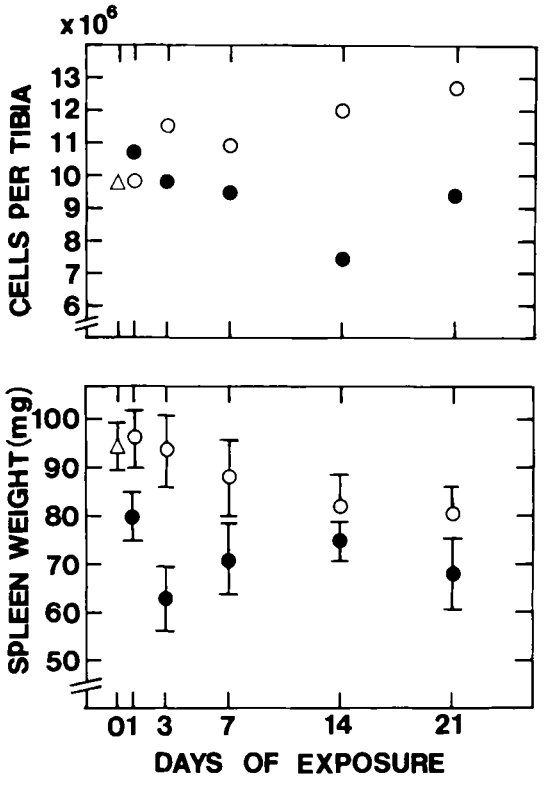

Fig. 2. Changes in number of total nucleated bone marrow cells in the upper row and in spleen weight in the lower during exposure. △ = pre-exposure; ○ = control; ● = nitrous oxide group

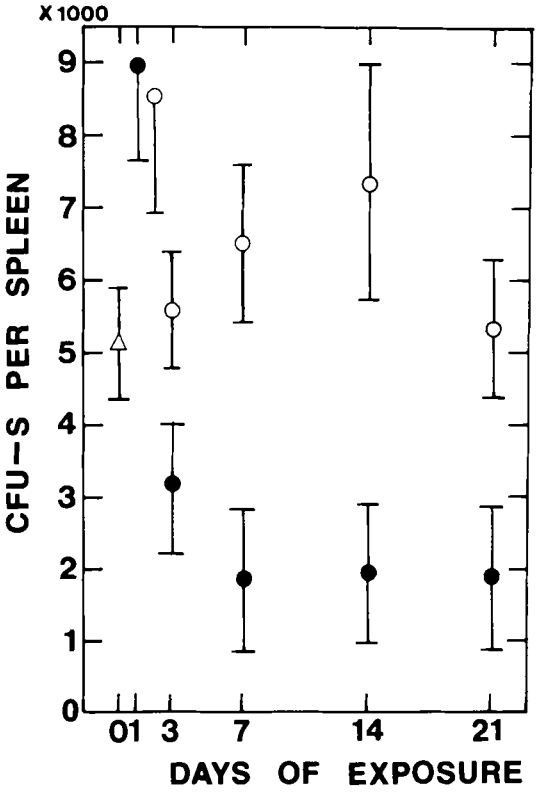

Fig. 3. Changes in number of the pluripotent stem cells (CFU-S) in the spleen. △ = pre-exposure; ○ = control; ● = nitrous oxide group

as many as the pre-exposure level, followed by a significant decrease from day 3 until the end of the experiment (fig. 3). Nitrogen-exposed control mice showed a slight but significant increase in the number of CFU-S throughout the experimental period. The number of the experimental group decreased to about 25 to 35% of those in the control group from day 7 of exposure.

Similar pattern of changes was also observed in the number of GM-CFC in the spleen except for day 1 (fig. 4). The numbers rapidly decreased to about 20 to 35% in the control levels from day 3 of exposure until the end of the experiment.

The number of CFU-S in the bone marrow showed a slight decrease from day 7 until the end of the experiment (fig. 5). On the other hand, the decrease of the numbers of GM-CFC in the bone marrow during nitrous oxide exposure remained at only 50–65% of control levels which were slightly enhanced compared to the pre-exposure level (fig. 6).

Discussion

Since Lassen et al. reported bone marrow depression after prolonged administration of nitrous oxide in tetanus[2], there have been several clinical reports that nitrous oxide induces bone marrow failure (reviewed in 14). Experimental studies in rodents demonstrated that exposure to nitrous oxide induced leukocytopenia and a progressive hypoplasia observed morphologically[6,9].

However, there was no report, as to whether or not nitrous oxide influence hemopoietic stem cells in vivo. The present study has shown that the number of pluripotent stem cells (CFU-S) and granulocyte-macrophage progenitor cells (GM-CFC) in the spleen decreased significantly

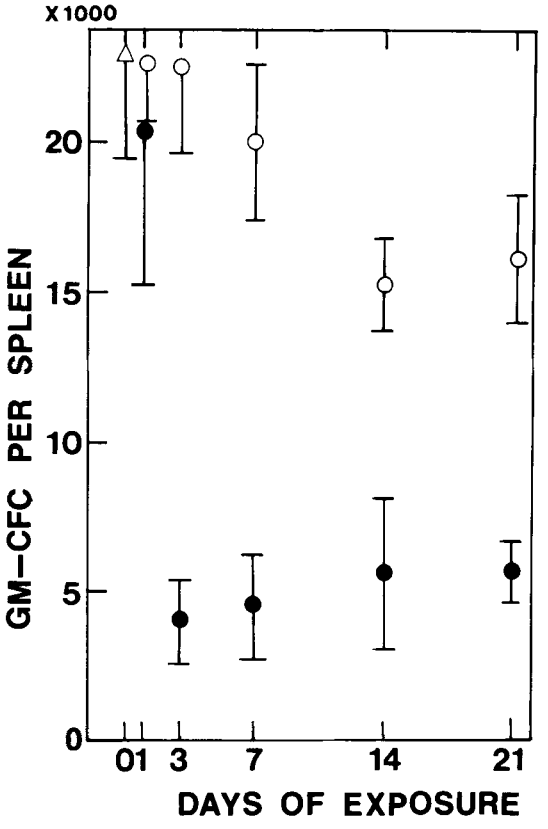

Fig. 4. Changes in number of granulocyte-macrophage progenitor cells (GM-CFC) in the spleen. △ = pre-exposure; ○ = control; ● = nitrous oxide group

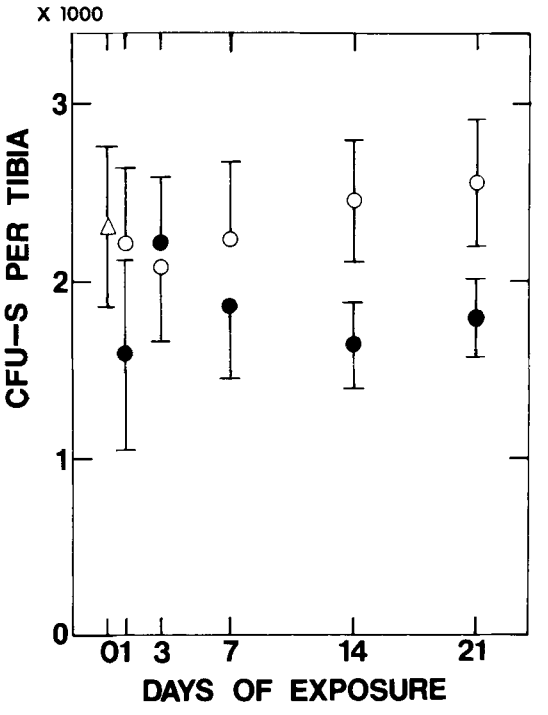

Fig. 5. Changes in number of CFU-S in the tibia. △ = pre-exposure; ○ = control; ● = nitrous oxide group

during exposure to nitrous oxide from day 3 until the end of the experiment. In the bone marrow, such decreases of CFU-S and GM-CFC were, however, significantly less than those in the spleen.

It is well known that spleen is one of the hemopoietic tissues in adult mice and a primary organ for acute hemopoietic response to exogenous stimuli, such as X-irradiation[15], bacterial infection[10,16], and an injection of the bacterial products[17]. We found in the present study that prolonged exposure to nitrous oxide resulted in a more profound inhibition of splenic hemopoiesis than medullary hemopoiesis.

Nitrous oxide has been reported to be an inactivator of an enzyme, methionine synthetase[18]. There are a number of reports that nitrous oxide inhibits the growth and division of many cell lines of plant and animal in vitro[19-21]. Studies on cell cycle and DNA synthesis in bone marrow of man exposed to nitrous oxide revealed the defective DNA synthesis and a significant increase of early S-phase[22,23]. These reports suggest that the cause of the inhibitory effects on hematopoiesis might be the general non-specific depression of cell multiplication.

Nunn et al. tested the direct effect of nitrous oxide on GM-CFC by culturing murine bone marrow cells in 75% nitrous oxide; this caused no significant decrease in growth[24]. Our data shows marked and simultaneous decreases in both CFU-S and GM-CFC in the spleen, in comparison with that in the bone marrow, and that the patterns of kinetics of the decrease are similar to each other. These results, taken together, suggest that nitrous oxide may not directly affect the growth and differentiation of the hemopoietic stem cells but causes deterioration of the splenic microenvironment for hemopoiesis. Although the mechanism causing damage to splenic hemopoiesis is not yet clarified, prolonged exposure of nitrous oxide to mice suppresses splenic hemopoiesis resulting in a leukocytopenia.

The method in the present study in which 50% nitrous oxide and 50% oxygen were used remains the mainstay of routine anaesthetic practice. Lassen et al.[2] and Amess and his colleagues[25] reported that nitrous oxide induced bone marrow depression at the same concentrations as that of the present study. It has been established that oxygen in high concentrations inhibits colony formations of GM-CFC[26] and erythroid progenitor cells[27] in vitro.

However, there is no report as to whether oxygen effect hemopoietic stem cells in vivo. Controls of the present study in which mice were exposed to 50% oxygen in addition to nitrogen showed a slight depression in the number of total leukocytes, especially lymphocytes, and GM-CFC in the spleen, but showed no significant changes in the number of other parameters. Experiments to elucidate this effect of oxygen on hemopoietic tissues are in progress.

Acknowledgements: This work was supported in part by Grants-Aid (61771142) for Scientific Research from The Ministry of Education of Japan. We thank Ms. V.L. Braymer for preparing this manuscript.

(Received Oct. 29, 1986, accepted for publication Nov. 28, 1986)

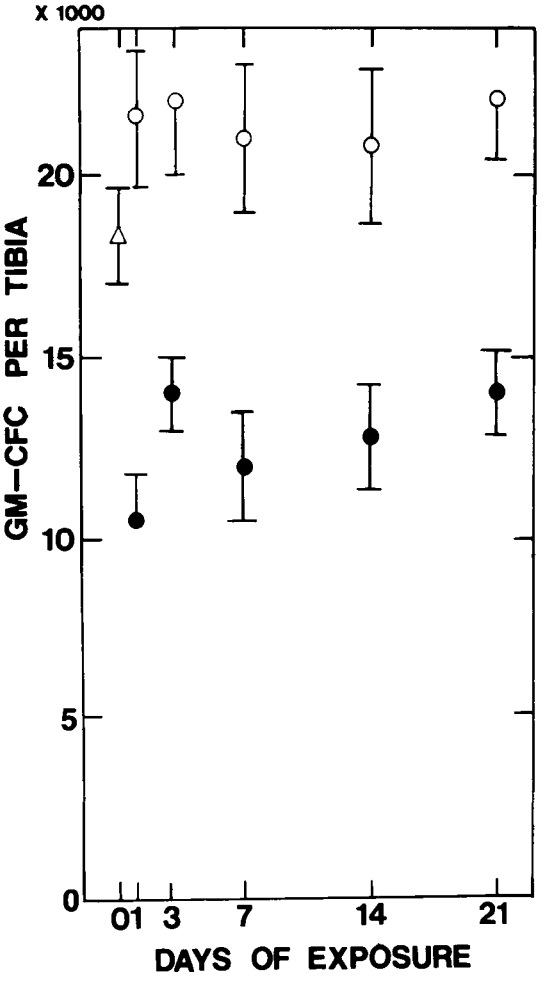

Fig. 6. Changes in number of GM-CFC in the tibia. △ = pre-exposure; ○ = control; ● = nitrous oxide group

References

1. Ablett JJL: Tetanus and the anaesthetist. Brit J Anaesth 28:258–273, 1956
2. Lassen HCA, Henriksen E, Kristensen HS: Treatment of tetanus. Severe bone-marrow depression after prolonged nitrous-oxide anaesthesia. Lancet 1:527–530, 1956
3. Wilson P, Martin FIR, Last PM: Bone marrow depression in tetanus. Report of a fatal case. Lancet 2:442–443, 1956
4. Sando MJW, Lawrence JR: Bone-marrow depression following treatment of tetanus with protracted nitrous-oxide anaesthesia. Lancet 1:588, 1958
5. Stead AL, Bush GH, Roth F: A severe case of tetanus showing interesting features. Br J Anaesth 34:49–56, 1962
6. Green CD, Eastwood DW: Effects of nitrous oxide inhalation on hemopoiesis in rats. Anesthesiology 24:341–345, 1963
7. Johnson MC, Swartz HM, Donati RM: Hematologic alterations produced by nitrous oxide. Anesthesiology 134:42–49, 1971
8. Parbrook GD: Leucopenic effects of prolonged nitrous oxide treatment. Brit J Anaesth 39:119–127, 1967
9. Kripke BJ, Talarico L, Shah NK, Kelman AD: Hematologic reaction to prolonged exposure to nitrous oxide. Anesthesiology 47:342–348, 1977
10. Kirikae T, Yoshida M, Sawada H, Tezuka H, Fujita J, Mori KJ: Effects of splenectomy on the retention of *Salmonella enteritidis* and on the hemopoietic response to *Salmonella* infection. Biomedicine 40:6–10, 1986
11. Till JE, McCulloch EA: A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res 14:213–221, 1961
12. Bradley TR, Metcalf D: The growth of mouse bone marrow cells *in vitro*. Aust J Exp Biol Med Sci 44:287–299, 1966
13. Yefuni SN, Federmesser KM, Smertenko II: Investigation of the peripheral blood and of the

type of cell nucleus under prolonged anaesthesia with nitrous oxide under experimental conditions. Eksp Khir 9:72–77, 1964
14. Editorial: Nitrous oxide and acute marrow failure. Lancet 2:856–857, 1982
15. Mori KJ, Kitamura Y: Role of the spleen in carbon-induced radioprotection. Int J Radiat Biol 30:487–490, 1976
16. Miyanomae T, Okabe T, Mori KJ, Seto A: Effects of *Salmonella* infection on hematopoietic stem cells in mice. Biomedicine 36:348–353, 1982
17. Quesenberry PJ, Moreley A, Ryan M, Stohlmann F: The effect of endotoxin on murine stem cells. J Cell Physiol 82:239–244, 1973
18. Deacon R, Lumb M, Perry J, Chanarin I, Minty B, Halsey MJ, Nunn JF: Selective inactivation of vitamin B in rats by nitrous oxide. Lancet 2: 1023–1024, 1978
19. Fink BR, Kenny GE: Metabolic effects of volatile anaesthetics in cell culture. Anesthesiology 29: 505–516, 1968
20. Jackson SH: The metabolic effects of halothane on mammalian hepatoma cells in vitro. II: Inhibition of DNA synthesis. Anesthesiology 39:405–409, 1973
21. Sturrock JE, Nunn JF: Mitosis in mammalian cells during exposure to anesthetics. Anesthesiology 43:21–33, 1975
22. Sourial NA, Amess JAL, Amos RJ: Role of S-adenosylmethionine in DNA synthesis and haemopoiesis. Scand J Haematol 35:303–307, 1985
23. Cullen MH, Rees GM, Nancekievill DG, Amess JAL: The effect of nitrous oxide on the cell cycle in human bone marrow. Brit J Haematol 42: 527–534, 1979
24. Nunn JF, Sturrock JE, Howell A: Effect of inhalation anaesthetics on division of bone-marrow cells in vitro. Br J Anaesth 48:75–81, 1976
25. Amess JAL, Burman JF, Rees GM, Nancekievill DG: Megaloblastic haemopoiesis in patient receiving nitrous oxide. Lancet 2:339–342, 1978
26. Bradley TR, Hodgson GS, Rosendaal M: The effect of oxygenation on haemopoietic and fibroblast cell proliferation in vitro. J Cell Physiol 97:517–522, 1978
27. Rich IN, Kubanek B: The effect of reduced oxygen tension on colony formation of erythropoietic cells in vitro. Br J Haematol 52:579–588, 1982