INHALED ISOBUTYL NITRITE IMPAIRS T CELL REACTIVITY

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INTRODUCTION

Amyl nitrite has vasodilatory effects and has been used since 1867 for relief from angina pectoris (1). Starting in the 1960s, inhalation of volatile nitrites became a common form of drug abuse, particularly among male homosexuals, but also among adolescents (2). Reports have appeared suggesting that the abuse of nitrite inhalants is a co-factor in AIDS (3) or in Kaposi's sarcoma in AIDS patients (4), but other studies found no such correlation (5). However, no firm conclusions can be drawn based on these conflicting population studies. Animal inhalation studies by Lynch, Lewis, and collaborators (6,7) and by McFadden and Maickel (8) showed that inhalation of isobutyl nitrite at occupational exposure levels (300-400 ppm) had little toxic or immunotoxic consequences. Abusers, however, are exposed to much higher doses of the inhalants (>1500 ppm) for shorter duration (10-20 inhalations over several hours, often daily) (2,9). In the present study, we evaluated immunotoxicity in mice exposed to isobutyl nitrite at levels which mimic abuser exposures.

MATERIALS AND METHODS

Isobutyl Nitrite Treatment

Isobutyl nitrite, obtained at 97% purity from Aldrich Chemical Co., Milwaukee, WI, was stored at 4°C under nitrogen. Isobutyl nitrite was vaporized in a halothane vaporizer (Foregger, Smithtown, NY) and quantitated with a halothane monitor (Puritan-Bennet, model 222, Datex, Tewkesbury, MA), which was calibrated for isobutyl nitrite. Female, 8-10 week old C57BL/6N mice (National Center for Toxicological Research, Jefferson, AR) were exposed to isobutyl nitrite in an inhalation chamber for 45 min per day for 14 days. Mice were exposed to 100 ppm isobutyl nitrite on day 1, 600 ppm on days 2-4, and 900 ppm on days 5-14. Groups of 5 mice were assayed for immune activity 24 hr after the last exposure to isobutyl nitrite.
Immune Assays

For mitogen assays (10), triplicate spleen cell cultures at 2 x 10^5 cells/micro-
culture were stimulated with 3 μg/ml concanavalin A (Con A, Sigma, St. Louis, MO)
or 3 μg/ml lipopolysaccharide (LPS, Salmonella typhimurium, ReG30/C21, Ribi,
Hamilton, MT). Cells were labelled with 1 μCi/ml ^3H-thymidine (^3H-TdR, ICN
Biomed., Costa Mesa, CA) over the final 4 hr of 72 hr cultures. For mixed lympho-
cyte reactions (MLR), responder spleen cells were mixed with irradiated (20 Gy)
allogeneic (BALB/c) stimulator spleen cells at a 1:1 ratio. After 72 hr incubation,
MLR cultures were labeled with ^3H-TdR for 18 hr. Cultures were filtered and
radioactivity was counted. Significance was determined by t-test, using the mean
CPM of 5 individual animals. The data are reported as the mean percent of control
values ± s.e.

For the plaque-forming cell (PFC) assay, mice were immunized intravenously
with 200 μl of a 20% suspension of sheep red blood cells 4 days prior to the day of
assay. A standard slide assay (10) was used to measure specific antibody-forming
cells. Significance was determined by t-test, using the mean PFC of 5 individual
animals. The data are reported as the mean percent of control values ± s.e.

RESULTS

Mice were exposed to isobutyl nitrite in an inhalation chamber for 45 min per
day for 14 days. Twenty-four hr after the final exposure, mice were assayed for
immune responsiveness. As shown in Figure 1, mice exposed to isobutyl nitrite had
a slight, but significant, reduction in spleen cellularity. The number of viable cells
recoverable per spleen was reduced by 15%. The immunotoxicity seemed to
preferentially affect T lymphocyte responsiveness. After correcting for cell numbers,
the T cell mitogen (Con A) responses of spleen cells from mice exposed to isobutyl
nitrite were reduced to half of control responses. In addition, T cell proliferative
responses to allogeneic stimulation in MLR were reduced by a similar magnitude in
cells from mice exposed to isobutyl nitrite. The number of specific T-dependent
antibody-forming cells (PFC) induced in mice exposed to isobutyl nitrite was reduced
by almost 90% compared with mice exposed to air. In contrast, B cell responsive-
ness to LPS was not significantly reduced by the exposure, suggesting that the
immunotoxicity of isobutyl nitrite had target cell specificity. The exposures to
isobutyl nitrite had no effect on hemopoietic activity in the spleen or bone marrow
(data not shown). Histological examination of spleen, thymus, liver, heart, lung and
bone marrow tissues showed no detectable differences due to isobutyl nitrite
treatment. While this data showed immunotoxicity 24 hr after cessation of isobutyl
nitrite exposures, similar results were obtained when mice were assayed for immune
reactivity 48 or 72 hr after the last exposure to isobutyl nitrite.

DISCUSSION

The present studies indicate that habitual inhalation of isobutyl nitrite may
lead to impaired immune reactivity and possibly increased susceptibility to infectious
diseases. Mice intermittently exposed to high doses of isobutyl nitrite by inhalation
had severely impaired T cell reactivity. T cell proliferative responses to mitogenic
and allogeneic stimulation were reduced to about half of control levels and T-
dependent antibody responses were almost totally abolished. The same exposure to
isobutyl nitrite did not significantly affect B cell mitogen responses, suggesting that
the reduced antibody responsiveness was due to toxic effects on T helper function.