The influence of midazolam and nitrous oxide on respiratory depression in laboratory rats

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Abstract

Midazolam in combination with nitrous oxide (N₂O) is a commonly used sedative approach for pediatric dental patients. Respiratory morbidity and mortality have been reported with midazolam administration, particularly when used in combination with other drugs in the absence of supplemental oxygen. Thus, the purpose of this investigation was to determine the effect of midazolam alone and in combination with N₂O on respiration in laboratory rats by measuring arterial blood gas levels. Sixty-four Sprague-Dawley™ rats, weighing 250-320g, were assigned to one of eight groups (eight per group). Groups were allocated based upon the dosage of midazolam administered (0, 1.0, 2.0 or 4.0 mg/kg i.p.) and exposure to N₂O/O₂ (50%/50%) or room air. Arterial blood was obtained from a femoral artery catheter and pH, O₂, CO₂ (mm Hg), and oxygen saturation (%) were determined. Samples were analyzed using a System 1306 pH/Blood Gas Analyzer. Baseline arterial blood gasses were obtained for each animal and at 15-min intervals following midazolam administration throughout the 45-min experiment. Following midazolam administration, animals were placed into a sealed chamber through which flowed either N₂O or room air. Group comparisons demonstrated that: 1) arterial CO₂ levels increased in midazolam-exposed animals breathing room air, but not in those exposed to N₂O (P < 0.05), and 2) as expected, N₂O/O₂-exposed animals showed an increase in arterial O₂ and a % saturation that was not observed in room air groups (P < 0.01). This investigation demonstrated that coadministration of N₂O/O₂ to midazolam-exposed animals did not result in hypercarbia, an early indicator of respiratory depression. (Pediatr Dent 18:281-86, 1996)

Midazolam (Versed®, Roche Laboratories, Nutley, NJ) is a relatively new benzodiazepine approved by the Food and Drug Administration in 1986. Although not approved for pediatric patients, midazolam has become anesthesiology's most frequently used preoperative sedative for children. Its popularity is due to its water solubility and unique anxiolytic, sedative, hypnotic, anticonvulsant, muscle relaxant, and anterograde amnesic properties.¹⁻³ Pharmacologically, midazolam acts at benzodiazepine receptors, resulting in increased glycine and GABA inhibitory activity within the cerebral cortex, hypothalamus, cerebellum, midbrain, hippocampus, as well as in the spinal cord.¹⁻⁵ A significant analgesic effect, through a GABA-ionophore mediated action, also has been reported.²⁻⁷

Midazolam has twice the affinity for benzodiazepine receptors and up to four times the hypnotic potency of diazepam.¹⁻³⁻⁸ Its unique pH-dependent molecular structure accounts for many of midazolam's desirable properties. In the parenteral preparation, midazolam has a pH of 3.5 and is a water soluble nonirritating solution that allows multiple administration approaches. At physiologic pH, midazolam is highly lipophilic. This form greatly facilitates transport across the blood-brain barrier and accounts for the rapid onset of action.²⁻⁵⁻⁸ Because of these properties, midazolam is becoming increasingly more popular as a pharmacologic aid in the behavioral management of selected pediatric dental patients.¹⁻³⁻⁹

As with all sedative/hypnotic agents, midazolam has a respiratory depressant potential. The respiratory influence is variable and thought to be dose related. Studies have demonstrated that hypnotic dosages of midazolam are required to produce depressant effects such as hypventilation, decreased tidal volume, and reduced ventilatory response to elevated arterial CO₂.⁵⁻¹⁰⁻¹¹ These effects are more pronounced when midazolam is administered in combination with narcotics such as meperidine or fentanyl.¹⁰⁻¹¹ In 1989, the Department of Health and Human Services reported epidemiologic data on midazolam-related adverse reactions.¹² At the time of that report, more than 1,600 incidents, ranging from hiccups to death, had been reported. Apnea (defined as no spontaneous respiration for at least 15 sec) and hypoxia (desaturation less than 90% for at least 10 sec) occurred in 12% of the incidents and 86 patients died. Features common to the more ex-
treme adverse reactions included: treatment in an outpatient clinic setting, drug administration by personnel with limited anesthesia training, improper patient monitoring, lack of supplemental oxygen administration, and the simultaneous administration of either meperidine or fentanyl.10,12

The original Guidelines for the E lective Use of Conscious Sedation, Deep Sedation, and General Anesthesia in Pediatric Patients were published largely as a result of outpatient sedation deaths involving pediatric dental patients.13-15 Continued reports of sedation-related adverse reactions, both in medicine and dentistry, are disturbing and prompted the American Academy of Pediatrics (AAP) Committee on Drugs to modify its guidelines.10,12,13,16 Other factors contributing to the AAP update relate to the variety in specialties involved as well as the diverse clinical settings, numerous sedation protocols, and variation in anesthesia training of the different specialists.15 The AAP’s goal was to develop a uniform standard of care regardless of the practice location or specialist involved. It is not surprising that certain subspecialties do not embrace all aspects of such a broad reaching document. Of particular concern for pediatric dentistry is nitrous oxide’s (N2O) deep sedation designation when administered in conjunction with narcotics, sedatives, or other CNS depressants.17 Conscious sedation with agents such as chloral hydrate, meperidine, and midazolam generally employ the simultaneous coadministration of N2O in oxygen.18-22 Although N2O is a CNS depressant and is known to act at opiate receptors, its respiratory depressant action or potentiation of respiratory effects of other agents is considered to be minimal.23-27 The potential for N2O coadministration to alter consciousness to a state of deep sedation is largely unestablished and likely dependent on numerous variables. Objective information in this area is lacking and was the impetus for this investigation.

Our aim was to determine the effect of midazolam and N2O on respiration in laboratory rats. Arterial cannulation and measurement of blood pressure, blood gases, and pH are methods of prospectively evaluating respiratory and cardiovascular influences of sedative combinations. Hypoventilation has been associated with hypoxemia, hypercarbia, and acidosis in laboratory animals and has been studied previously via arterial blood gases.28,29 This investigation’s intention was to evaluate the cardiovascular and respiratory depressant effects of N2O and midazolam, alone and in combination, in laboratory rats using hypercarbia as an indicator of deep sedation.

**Experimental design and methods**

**Animals**

Sixty-four male Sprague-Dawley rats weighing 250-320g (mean 290g) were utilized in this experiment. Animals were allocated to one of eight groups, eight animals per group, based upon the combination of midazolam (0, 1.0, 2.0 or 4.0 mg/kg) and whether animals were exposed to N2O/O2 or room air. Rats were housed in a standard laboratory animal facility with free access to food and drinking water prior to and during the study.

**Femoral artery cannulation**

Rats were anesthetized by inhalation of methoxyflurane. The animals underwent sterile surgical cannulation of the left femoral artery under a binocular microscope (Sterio Star, American Optical, Los Angeles, CA) using a surgical procedure previously reported.30,31 A heparinized polyethylene cannula was inserted into the femoral artery with the pulse confirmed by use of a polygraph (Model 7; Grass Instrument Co, Quincy, MA). The tubing was sutured to the femoral artery and then tunneled subcutaneously to exit from a sterile incision on the back of the animal’s neck. This prevented chewing and allowed easy access for collection of arterial blood and blood pressure measurement. Surgical incisions were stapled and the animal was allowed to recover for 24 hr.

**Drugs administration**

Midazolam and N2O were used in this study. Midazolam was diluted with normal saline to a concentration of 2.5 mg/ml and administered intraperitoneally (i.p.) at a dose of either 1.0-, 2.0- or 4.0-mg/kg, depending on group assignment. Two groups of animals received no midazolam and served as controls. Previous information was utilized to establish the 1.0, 2.0 or 4.0 mg/kg dosages selected for this investigation.32,33 Midazolam was administered and each animal was placed into a sealed Plexiglas™ container and exposed to either N2O or room air. Gases were delivered to the container via a length of polyethylene tubing at a total flow rate of 6 L/min using a standard N2O/O2 anesthesia machine (Matrix Medical Inc, Orchard Park, NY). Half of the animals were exposed to 50% N2O (3 L/min N2O and 3 L/min oxygen) and the other half were exposed to room air. Exhausted gas was vented from the Plexiglas cage to a nearby fumehood via a second length of polyethylene tubing.

**Blood gas and blood pressure analysis**

The study protocol called for baseline blood pressure and arterial blood sampling prior to and every 15 min (up to 45 min) following i.p. administration of midazolam. Arterial blood samples (0.2 cc) were collected in a heparinized syringe and analyzed immediately using a blood gas analyzer (System 1306 pH, Instrumentation Laboratory, Lexington, MA). Arterial blood pressure was measured continuously by use of a polygraph and recorded just prior to the sampling of arterial blood.

**Statistical analysis of data**

Baseline arterial pH, PO2, PCO2 (mm Hg), oxygen saturation (%), and blood pressure were obtained for each animal. Data were expressed as changes from
TABLE 1. COMPARISON OF GROUP MEAN CHANGE (±SD) IN ARTERIAL PCO₂ LEVELS (mm Hg) OVER THE EXPERIMENTAL PERIOD

<table>
<thead>
<tr>
<th>Room Air</th>
<th>Nitrous Oxide (50%)</th>
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<tbody>
<tr>
<td></td>
<td>Midazolam 0 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Midazolam 1 mg/kg</td>
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<td></td>
<td>Midazolam 2 mg/kg</td>
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<tr>
<td></td>
<td>Midazolam 4 mg/kg</td>
</tr>
<tr>
<td>Baseline (mm Hg)</td>
<td>31.0 ± 1.6</td>
</tr>
<tr>
<td>15 min (change from baseline)</td>
<td>1.3 ± 1.3*</td>
</tr>
<tr>
<td>30 min (change from baseline)</td>
<td>1.4 ± 1.4*</td>
</tr>
<tr>
<td>45 min (change from baseline)</td>
<td>1.6 ± 1.6*</td>
</tr>
</tbody>
</table>

- Signifies significant difference in the group mean change from pre-exposure baseline levels, P < 0.05.
-† Signifies significant difference in the group mean change from pre-exposure baseline levels, P < 0.01.
-‡ Signifies significant difference from control group animals not receiving midazolam at corresponding time interval, P < 0.05.
-§ Signifies significant difference in mean change from baseline between N₂O and room air exposed animals at corresponding midazolam dosage and time interval, P < 0.01.

Results

Table 1 shows mean baseline PCO₂ values and intra- and intergroup changes following i.p. midazolam administration in rats exposed to the various experimental conditions. Arterial CO₂ initially increased from pre-exposure baseline levels in all animals breathing room air (paired t-test, P < 0.01). Dose response differences with 2- and 4-mg/kg groups breathing room air were significant. Similar animals exposed to N₂O did not demonstrate increased PCO₂ levels. In fact, PCO₂ levels in N₂O-exposed animals pretreated with 2 or 4 mg/kg of midazolam were significantly lower than corresponding animals exposed to room air (two-sample t-test, P < 0.01). These differences became non-significant over time.
mals not receiving midazolam (P < 0.05). N2O-exposed animals did not demonstrate increased PCO2 levels. Intergroup differences were evident among the 2- and 4-mg/kg groups (P < 0.01).

Apnea and hypoxemia have been shown to occur early after midazolam administration. Table 2 demonstrates early changes in oxygenation, mean PO2, and % saturation in rats exposed to the various experimental conditions 15 min after i.p. midazolam administration. Other than the 4-mg/kg group, arterial O2 levels and % saturation in animals exposed to room air did not change significantly from baseline values. The lower PO2 levels in that group became nonsignificant over time. N2O-exposed animals showed highly significant increases in arterial O2 and % saturation when compared with baseline (paired t-test, P < 0.01) and to corresponding room air exposed groups (two-sample t-test, P < 0.01).

Examination of arterial pH and hemodynamic data (blood pressure and heart rate) found no significant differences in any parameter from pre-exposure baseline over time or between any of the eight groups studied.

**Discussion**

The 1992 revision of the AAP guidelines for management of pediatric patients during and after sedation stemmed from continuing reports of life-threatening complications involving respiratory morbidity and mortality during outpatient sedation of pediatric patients. Commonalities of morbid incidents included: multiple drug protocols, outpatient clinic environment, anesthesia administration by untrained personnel, inadequate monitoring, and lack of supplemental oxygen administration. The intent of the guideline change was to ensure patient safety by establishing minimal standards for patient assessment, dietary restrictions, and electronic monitoring, irrespective of the clinical setting. The child’s responsiveness and ability to maintain protective reflexes (as opposed to the drug protocol, drug combination, or administration route) are fundamental in distinguishing the conscious state from deep sedation. Under current guidelines, the deep sedation designation that N2O coadministration constitutes, restricts use without consideration of potential dose reduction benefits that N2O allows when used in multidrug protocols. Guidelines may also preclude the possible beneficial influences of enriched oxygen received during N2O administration should practitioners abandon this approach.

The objective of this investigation was to further the understanding of respiratory influences of a common sedative combination in laboratory rats.

A review of respiratory physiology indicates that respiration is regulated by two basic mechanisms: the central involuntary control and the backup for involuntary respiration. The central involuntary control is influenced by arterial PCO2 levels, which, when sufficiently elevated (hypercarbia), initiate spontaneous respiration. The backup for involuntary respiration are chemoreceptors located in the carotid and aortic bodies that are sensitive to hypoxia and trigger respiration when PO2 levels fall below 80mmHg. Agents that depress the central nervous system decrease the ventilatory response to elevated arterial PCO2, and / or suppress the apneic threshold so that greater arterial PCO2 levels would be required to stimulate spontaneous respiration. Depressant medications also may influence the hypoxic drive so that significantly lower levels of PO2 would be required to activate respiratory backup mechanisms.

The depressant effects of agents such as the opioids, benzodiazepines, and N2O are dose related and reduce both CO2 sensitivity and the respiratory drive. For example, opioids produce a reduction of the ventilatory response to elevated arterial CO2, an increase of the apneic threshold, and also suppression of the hypoxic drive. Benzodiazepines alter respiration through hypoventilation, decreased tidal volume, and reduced responsiveness to PCO2 — but without alteration of the apneic threshold. The influence of N2O on respiration is limited. It produces a decreased tidal volume that is offset by a corresponding increase in the respiratory rate. N2O has little effect on the ventilatory response to CO2 but does depress the ventilatory response to hypoxemia. Although N2O’s ability to potentiate...
respiratory depressant effects of other sedative agents is unestablished, some data suggest that its combined administration may adversely influence respiration.10

Midazolam/narcotic combinations in sufficient dosages are known to produce significant respiratory depression. Although analgesia produced by N2O is comparable to opioids, the respiratory depressant properties of N2O are not fully established. This animal investigation evaluated respiratory and cardiovascular influences of midazolam both alone and in combination with N2O. Our results show that blood pressure and heart rate effects were not significant and confirm previous reports stating that midazolam and N2O have limited cardiovascular influences.8,38 This animal investigation demonstrated that the coadministration of N2O and midazolam did not result in hypercarbia. Midazolam-treated animals breathing room air did however retain CO2, an early sign of respiratory depression. Some beneficial influence apparently was provided the animals receiving N2O, either from the enriched oxygen administration or the N2O itself.

In a pilot investigation, we attempted to ascertain the influence of N2O on PCO2 levels by studying animals pretreated with 4 mg/kg of midazolam and exposed to 50% nitrogen (N2) mixed with 50% O2. At the 15-min time period, arterial CO2 levels in the N2/O2-exposed animals (37.7 ± 1.8 mmHg) were not significantly different from corresponding animals exposed to room air (36.5 ± 1.7 mmHg). Thus we theorize that N2O, not the supplemental O2, somehow prevented hypercarbia. Determination of the mechanism for this effect was beyond the scope of our investigation. However, N2O's sympathomimetic action of the reticular activating system25,46 may stimulate involuntary respiration and explain the CO2 stability that was not apparent with either room air or nitrogen-exposed animals. N2O's later parasympathetic activation also may explain why mild hypercarbia was evident over time.

The safety benefits of enriched oxygen administration after conscious sedation or general anesthesia have been reported.35,36,41,42 Earlier incidents of midazolam-related morbidity and mortality have been associated with a lack of supplemental oxygen administration.18,12 Thus, the increased arterial PO2 levels observed during supplemental oxygen administration would seem desirable during pediatric sedation. The possibility of similar benefits from supplemental oxygen received during N2O administration has not been established. However, reports suggest that N2O does not cause hypoxia and that the high oxygen content received during N2O/O2 administration may, in fact, reduce the likelihood of hypoxemia.31,43-45 This animal investigation confirms that possibility. Arterial PO2 levels in rats exposed to N2O increased from 90 to approximately 180 mmHg. Although the increased arterial oxygen tension is not surprising, the N2O/midazolam-exposed animals did not demonstrate hypercarbia or hypoxemia — regardless of the midazolam dose.

Readers should be cautious in the interpretation and clinical application of these animal data. The average changes reported here do not reflect individual variation displayed by hyper-responders. This was evident in our investigation where sedated animals, breathing N2O or room air, demonstrated PCO2 increases of up to 22% and 32% respectively. The clinical significance of individual potential for development of this degree of hypercarbia is obvious and is why our results are not directly related to conscious sedation practices. Moreover, the possibility exists that the supplemental oxygen the animals received during N2O administration might have masked hypoventilation and respiratory depression.26,35 This would be of particular concern for pediatric patients where pulse oximetry is the sole method of physiologic monitoring. So, until further information involving human subjects is available, one should adhere strictly to AAPD guidelines and monitor patients cautiously for signs and symptoms of respiratory depression.

Conclusions

1. Arterial PCO2 levels increased in midazolam-exposed animals breathing room air, but not in those exposed to N2O/O2. The hypercarbia detected was significant and was an early indicator of respiratory depression.

2. Arterial PO2 levels increased by approximately 100% in midazolam-exposed animals breathing N2O/O2 but no significant change was observed in animals exposed to room air. The coadministration of N2O to sedated animals did not result in hypoxemia, regardless of the midazolam dose received — in fact oxygen saturation increased significantly.

3. Blood pressure, heart rate, and arterial pH values were not significantly different among groups or over time.

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12. Department of Health and Human Services, Office of Epidemiology and Biostatistics, Center for Drug Evaluation and Research, Data Retrieval Unit HFD-737, June 27, 1989.