Evaluation of plasma levels of melatonin after midazolam or sodium thiopental anesthesia in children


Abstract: Midazolam and sodium thiopental are two commonly used drugs in anesthesia for minor surgical procedures in children. A relationship exists between benzodiazepines (BNZ), barbiturates and melatonin. Whereas these drugs increase pineal melatonin production, the indoleamine amplifies the effects of both BNZ and barbiturates on the central nervous system (CNS). Our purpose was thus to analyze the plasma levels of melatonin before and during midazolam or sodium thiopental anesthesia in children subjected to ambulatory surgical procedures. Midazolam (0.4 mg/kg) or sodium thiopental (5 mg/kg) were administered i.v. to 33 and 32 children (aged between 2 and 14 yr), respectively, and blood samples were taken before and 5, 10 and 20 min after the drugs were administered. Melatonin was measured in plasma by a commercial radioimmunoassay kit previously standardized in our laboratory. The results showed that neither midazolam nor sodium thiopental anesthesia significantly affected the levels of melatonin studied at anytime. Significant correlations were found comparing the levels of melatonin between the different times studied. These results suggest that midazolam or sodium thiopental did not affect melatonin production by the pineal gland, thus avoiding a possible potentiation effect of the indoleamine on the central effects of these drugs during anesthesia. However, the possibility that changes in melatonin had been masked by the antioxidant role of the neurohormone are discussed.

Introduction
Midazolam and sodium thiopental are largely used drugs in pediatric clinic because of their pharmacological properties. Midazolam is better than diazepam for i.v. sedation because of its faster onset of action, more consistent anterograde amnesia, and virtual lack of venous complications [1]. Sodium thiopental is a barbiturate also used for i.v. anesthesia during brief surgical procedures [2].

Melatonin is an indoleamine produced in several tissues including the pineal gland. Pineal production melatonin is mainly controlled by norepinephrine action on β-adrenergic receptors in the pinealocyte [3]. As the pineal gland is outside the blood–brain barrier, any compound may reach the gland with the circulation and may alter its production of melatonin. Melatonin is a benzodiazepine (BNZ)-like drug with hypnotic, anxiolytic, sedative and anticonvulsant properties [4, 5] and its production is suppressed by γ-aminobutyric acid (GABA) and BNZ administration in humans [6–8]. In turn, melatonin administration increased brain GABA production [9] and modulated the circadian rhythms of brain GABA_A and BNZ receptors [10, 11]. These psychopharmacological effects of melatonin suggest an interaction between the indoleamine with brain GABA–BNZ receptor complex [12]. A double-blind, placebo-controlled study comparing melatonin with midazolam showed that melatonin can be used effectively for premedication of adult patients [13]. Nevertheless, no clinical studies delineate the consequences of BNZ and/or barbiturate administration on melatonin levels in children, although melatonin potentiates the effects of BNZ and barbiturates in children [4, 8, 14]. Thus, the present study was designed to examine the effects of two common anesthetics used in ambulatory surgery, i.e. midazolam and sodium thiopental, on the plasma melatonin levels in children under ambulatory surgery.

Methods
A total of 65 children, submitted for the University Granada hospital for minor surgical procedures, were studied. The children have normal psychomotor development, normal clinical and routine biochemical findings, absence of obstetrics and/or perinatal antecedents that
might represent neurological risk factors, and absence of familial, neurological or endocrine illness. Information was
given and authorization obtained from the parents and from the hospital’s Ethical Committee, and the Code of
Ethics of the World Medical Association was observed. The children were randomly divided into two groups: (a)
midazolam group, comprising 33 children aged between 2 and 13.5 yr, who received an i.v. injection of 0.4 mg/kg of
midazolam, and (b) sodium thiopental group, contained 32 children aged between 2 and 14 yr receiving i.v. injection of
5 mg/kg of sodium thiopental. All children of both groups were classified as class 1 according to the American Society
for Anesthesiology classification for physical status.

The following anesthetic protocol was performed in all children: (1) atropine, 0.01 mg/kg; (2) sodium thiopental
[2,4,6 (1H,3H,5H)-pyrimidinetrione, 5-ethyl-5-(l-methylbutyl), Abbot Laboratories, Madrid, Spain], 5 mg/kg (thio-
portal group) or midazolam (4H-imidazol [1,5-a]
[1,4]benzodiazepine, 8-chloro-6-(2-fluorophenyl)-1-Methyl,
Roche, Barcelona, Spain), 0.4 mg/kg (midazolam group);
(3) vecuronium, 0.1 mg/kg; (4) intubation; (5) alfentanil,
starting with 40 μg/kg and successive doses of 15 μg/kg; (6)
maintenance with 35% O₂N₂O; (7) artificial ventilation with
Vₜ of 10 mL/kg and a respiratory rate of 16–20; (8)
liquid support, between 11 and 20 kg, 4 mL/kg/h; between
21 and 40 kg, 3 mL/kg/h, and above 41 kg, 2.5–3 mL/kg/
h). All drugs were i.v. injected, and the studies were
performed between 09:00 and 12:00 hr.

Peripheral blood samples were collected from the ant-
cubital vein (contralateral to that used for drug injection)
in groups before (basal) and 5, 10 and 20 min after the drug
administration. Blood was centrifuged at 3000 g for
10 min, and plasma aliquots were separated and frozen at
–20°C until assays. Melatonin was determined by a
commercial radioimmunoassay (DLD, Hamburg, Ger-
many) and a quality control [15] was performed showing
an intra- and interassay coefficients of variation of 11.3 and
6.3%, respectively. The recovery of added melatonin was
84.4% and the sensitivity of the assay was 0.02 nmol/L.

Data are expressed as mean ± S.E.M. of melatonin
values in plasma. Statistics included one-way analysis of
variance (ANOVA), followed by a Newman–Keuls test, and
Pearson’s regression and correlation analysis.

Results

The plasma levels of melatonin before and after midazolam
administration did not show significant changes (Fig. 1A).
Before the administration of this BNZ, the melatonin
concentration was 0.064 ± 0.005 nmol/L. At 5, 10 and
20 min after anesthesia, the values of melatonin were
0.073 ± 0.007, 0.063 ± 0.005, and 0.068 ± 0.005 nmol/L,
respectively. The concentrations of plasma melatonin
concerning to the thiopental group were also without
significant changes during anesthesia (Fig. 1B). In fact,
melatonin levels were 0.0719 ± 0.006 before anesthesia,
and 0.068 ± 0.006, 0.079 ± 0.007, and 0.073 ± 0.00
nmol/L at 5, 10 and 20 min, respectively, after thiopental
administration.

The results of the correlation analyses are shown in
Table 1. In both groups there was a significant correlation
for the levels of melatonin between each time of extraction
except the correlation for melatonin levels between basal
and 10 min and between 10 and 20 min in the thiopental
group. The coefficient of determination was only significant
in the relation 10–20 min of the midazolam group.

Discussion

Our results show an interesting finding as neither midazo-
lam nor sodium thiopental modified the plasma levels of
melatonin. Because of the relationships between BNZ,
barbiturates and melatonin, these results were surprising.
The first consideration is that as we have measured
melatonin levels up to 20 min after drug administration,
we cannot know if the levels of the neurohormone may
change after this time. Circulating melatonin decreases
during development, but both prepubertal and pubertal
children have similar patterns of melatonin production [16,
17], with a half-life about 0.67–0.78 hr [18]. These data,
with the fact that melatonin produced by the pineal is not
stored in the gland but it is quickly released to the
circulation after its synthesis, made it unlikely that changes
in melatonin levels were not detected during the time of the
study.

The relationships between melatonin, GABA and BNZ
are largely known. Melatonin regulates both brain GABA
and BNZ binding sites and increases brain GABA levels,
which may explain its BNZ-like actions [5, 10, 11]. In turn,
BNZ inhibit pineal production of melatonin and decrease
brain melatonin binding sites, an effect that may be counteracted by administration of the indoleamine [19]. These interactions between melatonin and BNZ have been used for reversal BNZ tolerance. Human pineal gland has both central and mitochondrial BNZ receptors [20], and it was reported that BNZ decrease melatonin production [7] inhibiting N-acetyl-5-methoxytryptamine activity (NAT), the key enzyme for its synthesis.

A comparative study with melatonin and midazolam in adults showed a preoperative anxiolysis and sedation by the former compatible with its use for premedication [21]. It seems that at the dose used in our study, midazolam does not induce an increase in melatonin synthesis by the pineal gland, which has two important consequences for the children: (a) there is no more melatonin that could potentiate the effect of the BNZ on the CNS, delaying the awake time for the children, and (b) there are no changes in the circadian rhythm of the neurohormone that could alter the synchronization of the melatonin-dependent rhythms. Barbiturates act on the GABA_A–BNZ receptor complex increasing GABAergic neurotransmission [22]. Some studies suggest that barbiturates increase pineal NAT levels and activity, thus stimulating melatonin synthesis [23]. In turn, the indoleamine potentiates the effects of barbiturates in brain [4] and thus, a feedback regulation between melatonin and barbiturates can be proposed. The lack of changes in melatonin following thiopental anesthesia may account also for the dose of the drug used, and yield the same considerations as for midazolam anesthesia.

A last consideration should be done in the light of the surgical-induced oxidative stress. Surgical trauma intensifies the oxidative stress by generating reactive oxygen species (ROS) and diminishing the endogenous defense against ROS attack. Thus, antioxidant protection during the perioperative period may be of great importance. Midazolam and sodium thiopental are not good antioxidants. Midazolam is a weak antioxidant because it only suppresses superoxide anion and reduces mitochondrial ROS at concentrations far beyond clinical relevance [24, 25]. Sodium thiopental protects the neurons from oxidative stress, but it spontaneously generates hydroxyl radical inducing lipid peroxidation [26]. Melatonin scavenges ROS including superoxide anion, hydroxyl radical and hydrogen peroxide [27–29]. Melatonin also increases glutathione levels and the activity of enzymes involved in redox homeostasis, protecting mitochondria and cell against oxidative attack [30, 31]. A consequence of the antioxidant ability of melatonin is that during anesthesia increasing levels of the neurohormone could be not detected because they are masked by the rapid oxidative metabolism of the indoleamine. Thus, the possibility that during midazolam or sodium thiopental anesthesia significant changes in melatonin production as a protective mechanism against oxidative damage should be further examined.

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**References**


