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Case report

The importance of the toxicological analysis in newborns: clearing the case of a tetralogy of Fallot with a paradoxical reaction to 1-hydroxymidazolam

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Abstract

Short half-life benzodiazepines like midazolam are used as premedication to relieve intra-partum pain, and are known to have no adverse effects to the newborn. However, such short-acting drugs are known to induce paradoxical reactions when their concentration is abnormally elevated respect to the age of the subject. In a 1 day-old newborn, admitted to the Cardiology Unit of the "Bambino Gesù" Children Hospital of Rome due to a prenatal diagnosis of tetralogy of Fallot, a paradoxical reaction with a spontaneous resolution was observed. The analysis of urines, performed by gas chromatography/mass spectrometry after a drug screening, showed the presence of the active metabolite 1-hydroxymidazolam at 9.7 μg/mL with no detectable trace of its precursor midazolam and of the alternative metabolite 4-hydroxymidazolam. On the basis of these evidences, we speculated that 1-hydroxymidazolam, produced by the mother's liver enzymes, passed to the newborn whose reduced volume of distribution (for the shunted circulation) favoured the onset of such a reaction. Hence, the toxicological approach should be carried out on both mother and child wherever the clinical picture may appear unexplainable or does not match with the other findings collected.

Keywords

Midazolam, 1-hydroxymidazolam, paradoxical reaction, tetralogy of Fallot, cardiac shunt, toxicological analysis.

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Introduction

Benzodiazepines (BDZ) with short half-life like midazolam (MDZ) are effectively used as sedative premedication to relieve intrapartum pain [1]. The BDZ have been proven to have no adverse effects over the newborn, so that they are considered safe drugs to use in caesarean section as well as in normal vaginal delivery [2, 3]. The data on the pharmacokinetics (PK) of MDZ and of its major active hydroxylated metabolite, 1-hydroxymidazolam (1-OHM), derive by the usage in the intensive care units [4]. However, little is known regarding the fate of this drugs if the MDZ is administered when the baby is affected by congenital cardiocirculatory defect. In the particular case of the tetralogy of Fallot (TOF), in which left and right circulations are shunted, the PK of MDZ can differ from that estimated for an healthy individual of the same age due to a difference in volume of distribution (VD) [5, 6]. Therefore unpredictable MDZ effects can take place. In such circumstances, the clinical signs can be helpful to guide the clinician, but only through the laboratory testing it becomes possible to make a clear diagnosis. Regarding this point, the immunometric tests for drugs screening represent a fundamental first-line tool for the clinician. However, for BDZ, which are highly structurally related compounds, it is necessary to perform a second-line analysis to achieve a higher specificity with a more sophisticated technique. Particularly, the gas chromatography coupled with the mass spectrometry (GC/MS) makes it possible to precisely identify the analytes, therewith providing a fine quantitation of them.

In order to address the value of drug-screening tests and mass-spectrometry confirmatory analysis in the neonatal care unit diagnostics, we present a case of a newborn with a congenital circulatory shunt who had signs of 1-OHM intoxication.

Case report

A 1 day-old newborn was admitted to the Department of Medical and Surgical Neonatology of the "Bambino Gesù" Children Hospital of Rome, due to a prenatal diagnosis of TOF. He was born to a 33 year-old mother following an uncomplicated pregnancy by normal spontaneous vaginal delivery at 39^{+6} weeks gestational age. Stain of meconium in the amniotic fluid was reported. Birth weight was 3,650 g, length was 53 cm, the Apgar score was 9 at 1 minute and 9 at 5 minutes. The SaO_2 was 95% (at 21% FiO_2), vital signs were in normal range and no cyanosis was observed. The echocardiographic assessment confirmed the wide ventricular septal defect, an overriding aorta with right-sided arc and moderate sub-pulmonary stenosis. The laboratory parameters were all in the reference range (RR), except for the total bilirubin at 3.77 mg/dL (RR: 0.25-1.00 mg/dL), the conjugated bilirubin at 0.3 mg/dL (RR: 0.08-0.25 mg/dL), the AST at 71 IU/L (RR: 5-40 IU/L), the ALT at 22 IU/L (RR: 5-40 IU/L), and creatinine phosphokinase at 1,058 IU/L (RR: 32-294 IU/L). Lastly, neither the neurological examination, nor the cranial ultrasound examination showed any positivity. The child was therefore considered to be in good clinical conditions.

Within few hours after his admission, the infant began to be restless and jittery, with difficult feeding and inconsolable cry regardless of the attempts made to treat or calm him down. At the maximum of its criticality, the clinical picture was characterized by a permanent state of agitation, a vigorous and continuous crying, the SaO_2 sometimes below 90% and a respiratory frequency largely above 65 breaths per minute. Subsequently, the clinical evaluation led to an attribution of a grade 5 on the Neonatal/Infant Pain Scale (grimace, vigorous crying, change in breathing pattern, fussy arousal). Due to the lack of evidences of physical discomfort (like hypoglycaemia, intubation, invasive procedures or abnormal intestinal meteorism), as well as for the difficulty to explain the clinical picture, a toxicological screening was ordered by the attending physician. The urine sample, which was collected about 24 hours after birth (diuresis was 2.19 mL/kg/h), showed a strong positivity for the class of BDZ, corresponding to a level > 1,000 ng/mL (cut-off value: 200 ng/mL).

In order to confirm the findings of the screening test, a GC/MS analysis in full scan mode was performed. Particularly, the same urine sample was

treated with beta-glucuronidase to release BDZ from the glucuronic acid, and successively derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Using this kind of analysis it was possible to show the presence of the BDZ metabolite 1-OHM, whereas its alternative form 4-OHM as well as the parental compound MDZ were undetectable, given a lower limit of detection of 1 ng/mL. Afterwards, the GC/MS analysis was repeated in single ion monitoring mode in order to achieve quantitation, using the deuterated 1-OHM (1-OHM-d4) as internal standard. Particularly, 1-OHM was shown to have a concentration as high as 9.7 µg/mL (corresponding to 9,700 ng/mL) (**Fig. 1**). The toxicological screening was scheduled to follow the evolution of the clinical picture. Surprisingly, it was possible to observe a progressive normalization of the clinical signs within the next few hours afterwards. Noticeably, with no need of any pharmacological intervention, the breastfeeding became more comfortable, with other signs of agitation and irritation disappearing within the same day. A urine specimen collected after 1 month showed BDZ < 30 ng/mL, as confirmed by the GC/MS analysis. Noteworthy, although the mother had received a single intravenous bolus of MDZ (2.1 mg), a toxicological analysis performed on her, and particularly on the breastmilk collected during the hospitalization, showed no trace of BDZ (in this case the GC/MS analysis was performed instead of the immunochemical testing due to the high fat content which required an adequate extraction of the sample) [7].

Discussion of the case

Newborns show a very limited spectrum of signs, and symptoms when either adverse effects or intoxication emerge from drugs administered to the mother during labor. So the clinical diagnosis may require the supporting evidences provided by instrumental and laboratory tests. When the clinical picture appears puzzling, the "toxicological hypothesis" taken into consideration is most often regarding a withdrawal syndrome [8]. As long as drug abuse in pregnancy can be clinically misrecognized [9], a confirmation through the analysis of body fluids (mostly urines) is mandatory.

With respect to the case we presented, there were no evidences concerning a BDZ abuse during pregnancy. The absence of any drug in the mother's breastmilk excluded body fat accumulation due to a prolonged exposure [10], as well as acute extra-uterine intoxication due to breastfeeding [10-

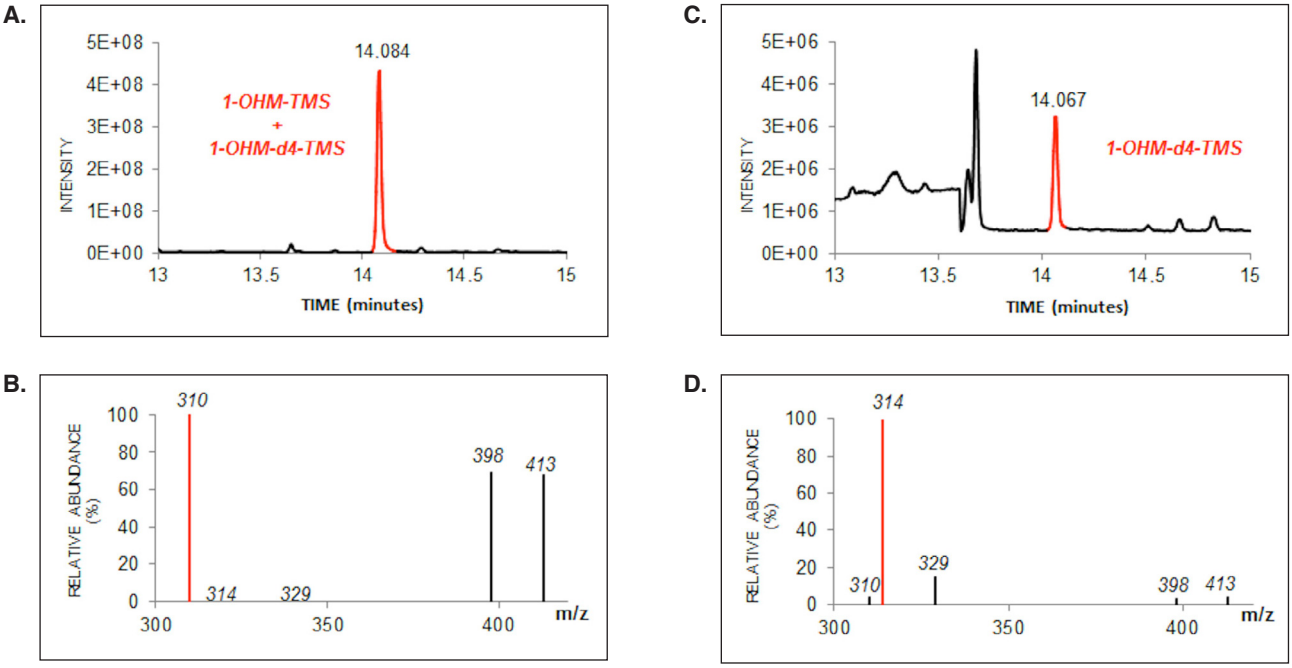

Figure 1. GC/MS analysis of urine samples in single ion monitoring mode; the peak in the chromatogram of the first day sample (**A**) is given by the 1-OHM and its deuterated internal standard; the mass spectrum (**B**) shows the abundance of the ion at m/z 310 which is specific of the 1-OHM-TMS molecule; the peak in the chromatogram of the urine sample collected at discharge (**C**) shows the sole internal standard, as confirmed by the intensity in mass spectrum (**D**) of the ion at m/z 314 which is specific of the 1-OHM-d4-TMS. The analytes are in the chemical form of trimethylsilyl-derivatives (TMS).

13]. The only clue was represented by the MDZ bolus she had received to relieve the intrapartum agitation, and respect to which the 1-OHM found in the newborn specimen was a consistent finding. However, this clinical history raised the concern regarding how such a safe drug could have caused the clinical pattern we observed in the newborn [3, 14, 15]. In this regard there were two elements to take into consideration: first, the clinical picture was neither an intoxication nor a withdrawal syndrome, and secondly the infant had a congenital circulatory shunt. Therefore, the only reliable hypothesis seemed to orient towards an acute intra-uterine exposure to BDZ which caused a paradoxical reaction (PR) in the neonate after the birth [16].

Discussion

The PR is defined as a condition in which a sedative agent produces excitatory rather than depressive effects after its administration. Such a phenomenon has been shown to depend on the maturation of the gamma-aminobutyric acid (GABA) signalling [17]. Through its action on the post-synaptic GABA receptor type A (GABA-A), the GABA signalling usually induces hyperpolarization of the cell. However, because of a lower expression of the potassium-chloride cotransporter type 2, the same stimulation tends to cause a depolarization due to a lack of potassium ions entrance. As a consequence, the modulatory effect on nociception turns into a potentiation of the nociceptive behaviour. In humans, the PR to MDZ has been shown to be either age and dose related [16]. When the MDZ dosage is halved, the rate of PR is reduced to about 50%, with age conferring the higher probability to develop a PR to MDZ (the odds ratio is more than 5). The PR to MDZ has a quick onset in children (within few minutes after administration), with the excitatory manifestations that break out after a period of relative calm or sedation [18].

To understand how the TOF might have favoured the onset of a PR, it must be taken into consideration the effect that a cardiac shunt can have on PK. As long as the systemic circulation contributes to the body volume in which the drug distributes, any change it undergoes in turn affects the VD (**Fig. 2**). It has been shown that for such drugs like fentanyl (a synthetic opioid), the severity of the cardiac defect correlates with the change in VD, with mild abnormalities leading to smaller VD reduction [5, 19]. In this regard, animal models have provided direct evidences on such a relationship. Particularly, in lambs with a surgically induced right-to-left cardiac shunt, it was observed a doubling of the lidocaine arterial peak, along with a halving of both VD and total body clearance [6]. Noticeably, a 36% reduction of the blood level at which lidocaine induced convulsions was also reported. It is noteworthy to mention that MDZ concentration can be affected disproportionately even by a moderate change in the VD, for it is very small especially in infants (about 1 L/kg) [4]. Hence, regardless of any collateral effect over clearance and half-life, the most relevant effect that the TOF shunt produced was trough the increase of MDZ blood level, which caused the drug to reach the threshold for the PR in the infant [20].

However, no trace of MDZ was found in the urine sample. In pregnancy, the MDZ is promptly delivered to the newborn because of its fast placental transfer (more than 60% of maternal dose is found in the umbilical cord blood), whereas 1-OHM shows a slower kinetics (about 30% less than its parental compound [21]). Notwithstanding that, in adults the MDZ tends to disappear quite promptly

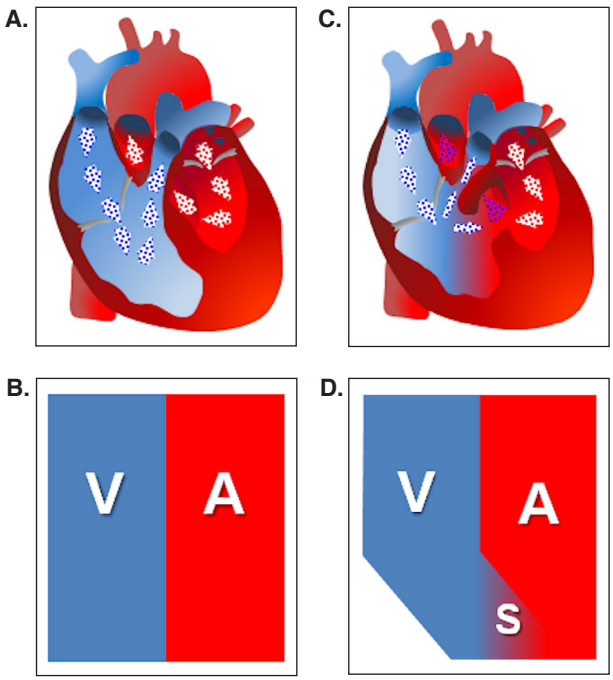

Figure 2. Systemic circulation and its relationship with the volume of distribution (VD); in the normal circulation (**A**) the venous and the arterial blood never merge, and the respective compartments are well separated (**B**); on the contrary, in the tetralogy of Fallot (**C**), the two circulatory systems are shunted, with part of their respective volume which is shared and therefore smaller (**D**).

from circulation, for it is efficiently converted into its hydroxylated metabolites within 2 or 3 hours. This is due to the intestinal and hepatic cytochrome CYP3A, which converts MDZ into its major metabolite 1-OHM or into the minor metabolite 4-OHM (**Fig. 3**). Conversely, in the foetus, the MDZ is metabolized quite slowly by the CYP3A7 enzyme isoform, which is about 100 times less active and has a higher rate of 4-OHM production (with the shift taking place shortly after the birth) [22-25]. According to the latter, if the newborn had received mostly MDZ from the mother, the first urine sample would have shown MDZ and 1-OHM (mainly produced by the mother's liver) with a minor amount of 4-OHM (mainly produced by the foetal liver). Conversely, nothing but the 1-OHM was found, and moreover at a level far distant from the capability of an immature liver. Hence, considering that the hydroxylated form is still pharmacologically active, it is reasonable to speculate that the PR was induced by the 1-OHM rather than by the MDZ [26].

We would remark that the glucuronide-conjugated metabolites of MDZ are also active, and thus they could have played a part in the development of the PR [27]. Noteworthy, by their analysis we could have further confirmed the maternal provenience of 1-OHM. In fact, the activity of the hepatic uridine 5'-diphospho-glucuronosyltransferase (UGT), which forms hydroxyl-midazolam-glucuronide (OHMG, in either *O*- and *N*-glucuronide forms), is very low in foetus and increases after the birth (**Fig. 3**) [28-30]. Hence, after a quick metabolism by the maternal liver, the OHMG would have crossed the placental barrier and reached the foetal circulation [31]. Unfortunately, the sample hydrolysis necessary before the GC/MS analysis did not make it possible to assess the level of glucuronide-conjugates in the urine specimen.

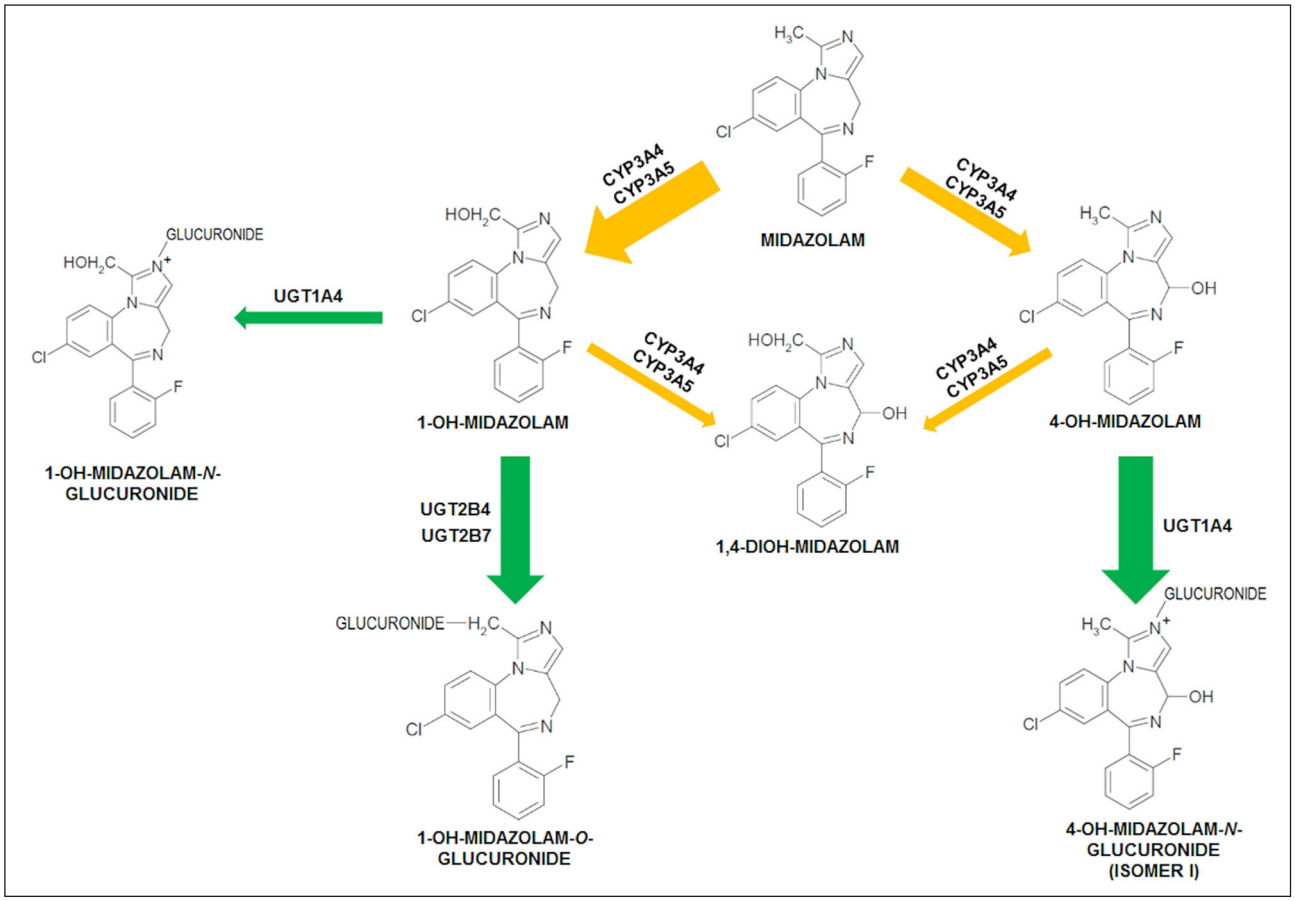

Figure 3. The metabolism of MDZ. The metabolism of MDZ involves two enzymatic steps, with the cytochrome P450 3A4/3A5 enzymes that hydroxylate the MDZ to form OHM, and the UGT 2B4/2B7 and 1A4 enzymes that conjugate OHM with the glucuronic acid to form OHMG; in the newborn, the CYP3A7 preferentially produces 4-OHM instead of the 1-OHM. The thickness of the rows represents the relevance of the metabolic pathway with respect to the formation of each specific metabolite.

Conclusions

The toxicological approach in the newborn seems to be mandatory if an unexplained clinical picture is observed after medications have been administered to a pregnant woman during labor. Such atypical clinical patterns could emerge in presence of congenital cardiocirculatory defects of the newborn, in which the hemodynamic abnormalities could affect the PK of drugs (especially if they have small VD), possibly producing unexpected signs with a difficult clinical explanation. The scarcity of data in the issued literature suggests that further investigations should be undertaken both to confirm the hypothesis that we speculated herein and to clarify the clinical impact of pathologic hemodynamics on the utilization of drugs thought to be otherwise safe in the management of labor and delivery.

Abbreviations

1-OHM: 1-hydroxymidazolam
4-OHM: 4-hydroxymidazolam
BDZ: benzodiazepines
CNS: central nervous system
GABA: gamma-aminobutyric acid
GABA-A: gamma-aminobutyric acid receptor type A
GC/MS: gas chromatography/mass spectrometry
MDZ: midazolam
OHM: hydroxy-midazolam
OHM-d4: hydroxy-midazolam-tetradeuterated
OHMG: hydroxy-midazolam-glucuronide
PK: pharmacokinetics
PR: paradoxical reaction
RR: reference range
TOF: tetralogy of Fallot
UGT: uridine 5'-diphospho-glucuronosyltransferase
VD: volume of distribution

Declaration of interest

The Authors declare that they have no conflict of interest.

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