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Nitrous oxide and vitamin B12 in sickle cell disease: Not a laughing situation

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ABSTRACT

Nitrous oxide (N_2O) is widely used as an anesthetic or an analgesic. N_2O prolonged and recurrent administration is known to affect vitamin B12 metabolism with subsequent clinical consequences. We report herein the case of a 13-year-old girl with sickle cell disease exhibiting severe neurological and biochemical signs of functional vitamin B12 deficiency due to prolonged and repeated exposure to N_2O. This was an incentive to prospectively investigate functional vitamin B12 deficiency in patients affected by sickle cell disease regularly exposed to N_2O. We measured plasma concentrations of vitamin B12, total homocysteine, methionine and methylmalonic acid in 39 patients with sickle cell disease between 2015 and 2016. No patients developed neurological symptoms related to N_2O administration but 19 patients (49%) had biochemical abnormalities suggesting mildly disturbed vitamin B12 metabolism e.g. decreased B12 vitamin, hypomethioninemia, or slightly increased methylmalonic acid or homocysteine. The clinical case highlight the potential severe deleterious effects of N_2O over exposure on B12 vitamin metabolism in particular in patients affected with sickle cell disease. Conversely, when used without excess even repeatedly, there seem to be no overt clinically relevant abnormalities in vitamin B12 metabolism as observed on the cohort of 39 sickle cell disease affected patients.

1. Introduction

N_2O, used for more than 150 years, acts supraspinally to induce analgesia by activation of opioidergic neurons in the periaqueductal gray matter and noradrenergic neurons in the *locus coeruleus*, A5 and A7 areas of the brainstem. N_2O was considered to be completely benign until 1956 [1] when several tetanus patients were reported with acute bone marrow aplasia after prolonged N_2O anesthesia [2]. Later, neurological symptoms such as numbness, sensitive deficiency, hyporeactive tendon reflexes were reported to be attributed to N_2O abuse [3]. Since then, several studies described neuropathy following prolonged exposure to nitrous oxide.

N_2O neurological toxicity is related to its inhibition of vitamin B12 metabolism. In the sixties, an interaction between N_2O and the cobalt atome of the cobalamin (vitamin B12) was demonstrated [4]. Precisely, N_2O oxidizes the cob[*I*]alamin (Co^+) into cob[*II*]alamin (Co^{2+}), which affects its binding to the vitamin B12-dependent enzymes namely methionine synthase (EC 2.1.1.13) and methylmalonyl-CoA mutase (EC 5.4.99.2) [5]. Consequently, homocysteine and methylmalonic acid (MMA) accumulate while methionine is depleted (Fig. 1). The functional deficit of vitamin B12 leads to myelinopathy such as subacute neurodegeneration of the spinal cord but also megaloblastic bone marrow failure and abnormalities in other rapidly regenerating tissues such as gastrointestinal tract and skin [6]. Homocysteine accumulation
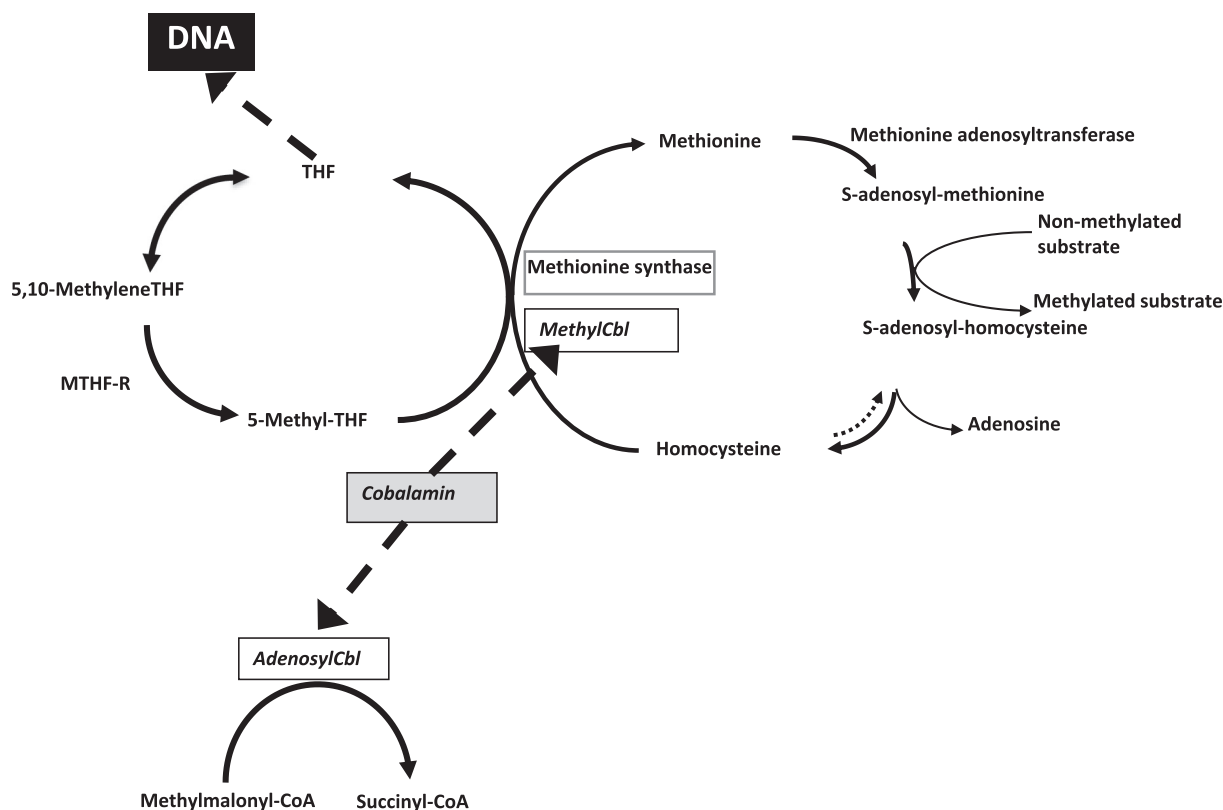
Fig. 1. Vitamin B12 metabolism schematic view.
MTHF-R: methyltetrahydrofolate reductase; THF: tetrahydrofolate.

itself can induce thrombotic events [7,8].

N$_2$O is used in the first line of treatment of vaso-occlusive crisis of children with sickle cell disease [9]. Thus, these children are highly exposed to N$_2$O, leading to a higher risk of potential side effects. Moreover, sickle cell disease is responsible for chronic hemolysis with subsequent risk of vitamin B12 overconsumption. Children with sickle cell disease are also at risk of thrombotic vasculopathy, which can be aggravated by hyperhomocysteinemia that promotes thrombo-embolic events [10]. Only few patients with sickle cell disease were reported with functional vitamin B12 deficiency revealed by neurological signs as a consequence of N2O over exposure [11–13].

We report herein a 13 year old girl with sickle cell disease with neurological and biochemical signs of severe functional vitamin B12 deficiency due to prolonged and recurrent exposure to N$_2$O and compare this case with other similar published cases. This single case of severe N$_2$O toxicity was an incentive to prospectively investigate functional vitamin B12 deficiency in patients affected by sickle cell disease. We measured plasma concentrations of vitamin B12, total homocysteine, methionine and MMA in 39 patients with sickle cell disease exposed to N$_2$O between 2015 and 2016.

2. Materials and methods

2.1. Study design

We conducted a monocentric observational prospective study in Robert-Debré University Hospital Department of Paediatrics between November 2015 and November 2016 (agreement N° 2015-02-08-SC/ ethical research committee "Ile-De-France II" / CNIL N°1,827,740).

We included all children between one month and 17 years of age with sickle cell disease (SS, SC or Sβ0), hospitalized for a vaso-occlusive crisis, with a need for a blood sample and a non-opposition from the legal guardians. We excluded all children whose haemoglobin level was

below 5 g/dl, who had been transfused in the last 3 months, who were in a transfusion program, who did not need a blood test or who already had 2 blood samples taken for the study. For each patient, duration of exposure to N$_2$O, the total number of vaso-occlusive crisis and the number of emergency room visit since diagnosis were collected. All data were recorded anonymously from the medical records.

2.2. Samples

Blood was drawn at the end of each hospitalization. Blood with heparin added as anticoagulant was used for analysis of plasma of total homocysteine (tHCy), methionine (Meth), MMA and vitamin B12. Blood without anticoagulant was used for serum folate measurement.

2.3. Laboratory measurements

Plasma MMA, tHCy) and Meth were analyzed by ESI-LC-MS/MS method adapted from Imbard et al. [14] (Acquity-I Class UPLC- XevoTQD, Waters).

Plasma Vitamin B12 and serum folates were measured by competitive immunoenzymatic methods (Automate Access Beckman Coulter).

2.4. Statistical analyses

Descriptive statistics were used to summarize baseline demographics and laboratory parameters. We reported the mean ± standard deviation and [min;max] of continuous data.

3. Case report

This girl was diagnosed with homozygous S/S sickle cell disease at 4 years of age in Gabo. At age 13, she exhibited an acute chest syndrome, complicated by a disseminated osteomyelitis. She was

Table 1
Laboratory parameters and various treatments used.

Time	day 0	day +32	day +68	day +80	day +148	day +183	day +214
Treatments:							
Nitrous oxide	Yes	Yes	None	None	None	None	None
Vitamin B12	None	1 mg/d PO (5 days)	None	1 mg/d IM (2 days) 1 mg/d PO (5 days)	None	1 mg/d PO (7 days)	None
tHcy (5–9 µM)	**155**	**66**	**23**	6	7	6	
Meth (15–27 µM)	**11**	**11**	**10**	15	18	19	**18**
Plasma MMA (< 0.4 µM)			2.9	0.5	< 0.4		
Urine MMA (< 20 µmol/mmol creatinine)	**900**		**41**	**38**	13	16	
Plasma vitamin B12 (140–780 pM)	226		146	685	153		332
Hb (11.3–14.4 g/dL)	12.6	**9.4**		**8.7**	**10.0**	**10.6**	**10.4**
Mean corpuscular volume (75–88 fL)	**90.6**	84.6		86.5	86.7	87.5	80.0

Treatment are presented with their dose in mg/days (mg/d) and their duration within brackets.
BMI: Body mass index; Hb: Haemoglobin; IM: intramuscular; MMA: methylmalonic acid; Meth: methionine; PO: Per os; tHcy: total homocysteine reference ranges are indicated between brackets. Data outside reference range are in bold.

transferred to France where she received important amounts of N_2O due to high pain (minimal of 1.5 h daily during 7 months). She further exhibited complete loss of any movements in the lower limbs with absence of deep tendon reflexes compatible with peripheral neuropathy. No electrophysiological studies were possible due to the severity of the clinical condition. However, due to the possibility of overt N_2O exposure, serum vitamin B12, plasma tHCy, Meth and MMA were measured (day 0). Plasma tHcCy and urinary MMA were highly elevated (> 10 fold) while plasma methionine was decreased (Table 1). Vitamin B12 and folate were in the normal range. These results suggested a functional vitamin B12 deficiency. N_2O was initially maintained due to the high pain intensity and oral vitamin B12 was added during 5 days. At day 32, N_2O was discontinued allowing rapid normalization of metabolic parameters (Table 1). Two months later, she received intramuscular vitamin B12 injection during 2 days followed by 5 days of oral vitamin B12 due to mild elevation of tHcy. This allowed normalization of homocysteine concentration while pain and signs of neurological toxicity disappeared after a few weeks (Table 1).

4. Results of the prospective study

4.1. Population description

Sixty-one patients met the inclusion criteria. All patients received N_2O at each hospitalization for vaso-occlusive crisis with at 6 to 12 L per minute with a maximum of 1 h but it was not possible to precisely quantify the duration of administration. We excluded 22 patients who had received red blood cell transfusion in the last 3 months. In total, 39 patients were analyzed (21 girls and 18 boys). The mean age of our population was 10.6 ± 5.3 years [1.4;17.8]. Thirty-two patients had homozygous sickle cell anemia (HbSS) while 7 had one HbS allele and one HbC allele (HbSC). Twenty-three patients were treated by hydroxycarbamide. Among the 39 patients, 7 had a splenectomy and 4 had a cerebral vasculopathy. Patients exhibited 3.1 ± 3.0 [0;12] vaso-occlusive crisis per year during the previous year; and 1.9 ± 1.2 [0;5] during the 3 months period prior to inclusion.

4.2. Laboratory parameters

Demographic data and laboratory parameters are detailed in Table 2.

Mean vitamin B12 concentration was in the normal range, 295 ± 183 pM. Five patients (13%) had a decreased vitamin B12 concentration.

Mean methionine concentration was in the normal range,

19.6 ± 5.3 µM. Six patients (15%) had a mild decreased methionine concentration.

Mean tHcy concentration was in the normal range (< 10 µM). Five patients (13%) had a slight increase of the tHcy between 10 and 19 µM.

Mean MMA plasma concentration was in the normal range (< 0.4 µM). Two patients had a slight increase of the plasma MMA concentration and one patient had an MMA increase at 2.1 fold of normal values.

5. Discussion

We described the case of a young girl with S/S sickle cell disease who presented a peripheral neuropathy due to a functional vitamin B12 deficiency (i.e. with normal serum vitamin B12) ascribable to high N_2O exposure. This led us to systematically investigate B12 vitamin metabolism in patients with sickle cell disease and hospitalized for a vaso-occlusive crisis receiving N_2O. Our study revealed that almost half of the patients exhibited biological hallmarks of B12 vitamin metabolism but without neurological, hematological, or gastrointestinal symptoms suggestive of functional vitamin B12 deficiency.

However, our study has several limitations. Precise quantification of N_2O dose was not possible due to the mode of administration (on demand). Moreover, the number of patients included was limited.

Overall, including our case report, seven patients affected with sickle cell disease and presenting with a functional vitamin B12 deficiency due to over exposure to N_2O are reported in the literature. Ogundipe et al. [13] described in 1999 three adult patients with peripheral neuropathy and decreased walking ability. In 2013, Chaugny et al. described a sensorimotor polyneuropathy in a 20 years old patient affected by sickle cell disease [11]. Of note, similar to our case, highly elevated tHcy levels were also reported in this patient who exhibited a pulmonary embolism. Recently, Neveu et al. described two patients, one with peripheral while the other was asymptomatic despite a dramatic increase of homocysteine. All patients improved after N_2O withdrawal and vitamin B12 supplementation [12].

All these reports led us to prospectively investigate functional vitamin B12 deficiency in children with sickle cell disease during vaso-occlusive crisis in our hospital. In contrast with our first observation, none of the 39 sickle cell other patients developed neurological symptoms in relation with N_2O administration. Regardless, 49% had mild biochemical abnormalities i.e. either low vitamin B12, elevation of plasma tHCy or MMA, or decrease of Meth. These values remained very moderately abnormal and were not associated with symptoms that could be directly associated with vitamin B12 deficiency. Our data show that reasonable use of N_2O in a hospital setting in sickle cell

Table 2
Laboratory results of the study.

Patient	Age at inclusion (years)	Sex	Basal Hb rate	BMI	Number of vaso-occlusive crises in 12 months	Number of vaso-occlusive crises in 3 months	Vitamin B12 (140–780 pM)	Methionine (15–27 µM)	tHcy (5–9 µM)	AMM (< 0.4 µM)	Serum folates (> 18 µg/L)	RBC folates (390–1900 µg/L)
1	7.8	Male	9		1	1	593	**14**	3	NC	98	2325
2	5.4	Female	7.5		0	0		**9**	6	< 0.4		
3	2.0	Male	8.5	16.48	2	2	581	**10**	3	0.1	97.7	3614
4	2.0	Male	8.5	16.48	2	2	811		5	0.1		
5	13.1	Male	7	16.23	5	3	235	23	6	0.14	47.5	1334
6	1.9	Female	9		0	0		22	9	< 0.4	95.1	1234
7	17.3	Male	9	20.55	4	2	**90**	19	**11**	< 0.4	434.7	1732
8	13.0	Male	8	**11.56**	3	1	348	18	4	< 0.4	357	2037
9	14.8	Female	10	21.28	1	1	**136**	20	7	< 0.4		
10	8.2	Female	8.5	14.01	2	4	303	16	6	< 0.4	918.6	**> 1974**
11	9.5	Female	8.5	18.66	6	1	243	**11**	4	< 0.4	197	2509
12	13.7	Female	7	20.12	3	3	188	20	7	**0.44**	464	2504
13	13.7	Female	7	20.12	3	4		17	5	< 0.4		
14	2.6	Male	9	15.07	1	1		22	4	< 0.4		
15	17.5	Male	7.5	**16.87**	12	2		15	8	**0.54**		
16	10.1	Male	8.5	16.84	3	1	147	22	8	< 0.4	1250	**> 754**
17	6.3	Female	9	15.20	0	1		27	6	< 0.4		
18	17.1	Female	9.5	22.43	4	1	171	**13**	**10**	< 0.4	21.1	2674
19	3.1	Female	9	16.91	1	2	419	19	4	< 0.4	164.3	**> 4110**
20	11.9	Male		16.23	2	1		22	8	< 0.4		
21	15.8	Male	8	**16.18**	12	2	267	23	**11**	< 0.4		
22	10.2	Male	8.5	**14.48**	3	2	**121**	22	6	< 0.4		
23	12.8	Female	7.5	15.16	1	1		23	9	< 0.4		
24	1.7	Female	8.5		1	1		29	4	< 0.4		
25	1.4	Female	8.5	16.02	1	1		29	3	< 0.4		
26	14.4	Female	8	25.10	8	2		18	5	< 0.4		
27	4.3	Male	10.6	14.32	3	2	331	16	3	0.3	53.4	3900
28	8.4	Female	8.5	14.01	3	5	511	21	6	< 0.4	48.2	2760
29	17.4	Male	9	20.55	5	3	**110**	25	7	< 0.4	1408.5	**> 440**
30	12.2	Female	8.5	17.27	1	1	222	16	5	< 0.4	42.1	
31	12.6	Female	7	16.12	1	3	270	22	9	< 0.4	**> 54.8**	
32	17.8	Male	7.5	**17.58**	3	1	140	16	5	< 0.4	50.8	**> 4541**
33	13.3	Female	7.5	18.55	2	4	364	20	6	< 0.4	1500	
34	12.8	Female		19.63	6	4		**14**	7	< 0.4		
35	11.7	Male	9	19.52	3	1	**24**	NC	NC	< 0.4		
36	16.6	Male	10	21.87	9	2		30	**19**	< 0.4		
37	12.8	Female	9	17.58	1	2	254	28	7	**0.85**		
38	15.6	Male	8	**16.22**	2	1	298	17	**13**	< 0.4	142.2	2315
39	16.5	Female	9.4	20.25	0	1	495					

BMI: Body mass index; Hb: Haemoglobin; MMA: methylmalonic acid; Meth: methionine; RBC: Red Blood Cell; tHcy: total homocysteine Reference ranges are indicated between brackets. Data outside reference range are in bold.

disease patients do not severely impact vitamin B12 metabolism. In line with our results, in 2016, the French medicine agency ANSM (Agence Nationale de Sécurité du Médicament) reminded to the Heath care professionals the duration of the administration of N_2O should not exceed one hour a day during 15 consecutive days [15].

Besides sickle cell disease patients and N_2O overexposure, a few case reports have described N_2O toxicity in the rare situations of acquired or inherited vitamin B12 metabolism defects, without overexposure to N_2O. Selzer et al. [16] described a 3 months old child with neurologic deterioration including severe hypotonia, absence of deep tendon reflexes, seizures and apnea leading to death. He had received 25 days prior to death twice N_2O (one for a biopsy of a mass of the left leg which revealed an infantile fibrosarcoma and the second for the ablation of the tumor). Further investigations disclosed normal serum vitamin B12 but decreased Meth and increased tHCy. Post mortem investigations revealed 5,10-Methylenetetrahydrofolate reductase deficiency (MTHFR) deficiency. Felmet et al. [17] described in 2000, an eight months old patient who presented progressive coma associated with profound hypotonia and athetoid movements, six days after an anesthesia with N_2O. He also presented a recent decline in growth velocity and mild developmental delay due to a profound vitamin B12 deprivation from maternal origin. He partially improves after vitamin B12 supplementation. Mc Neely et al. [18] described a six months old

child who presented a neurologic degradation 3 weeks after receiving N_2O anesthesia for a craniostenosis. Laboratory parameters revealed profound vitamin B12 deficiency partially due to maternal deficiency. The patient partially improved after intra muscular vitamin B12 administration. Those three cases emphasize that N_2O even without overexposure could be responsible for an acute neurological degradation when primary or secondary vitamin B12 metabolism dysfunction preexists.

Many studies have also described N_2O exposure in the context of recreational use [3,19–27]. The picture is often a sensorimotor neuropathy and/or a medullary injury sometimes up to a combined sclerosis of spinal cord. Laboratory parameters found a vitamin B12 concentration normal or variably decreased, associated with signs of functional B12 vitamin deficiency (high tHcy, low Meth and MMA accumulation) in the range of those observed in our study.

To conclude, our data highlight the potential deleterious impact of N_2O overexposure on vitamin B12 metabolism. However, regular but not excessive use of N_2O in sickle cell disease patients can induce minor abnormalities in vitamin B12 metabolism though without obvious clinical signs and therefore is to be regarded as safe. Nevertheless, N_2O administration should indeed be carefully monitored and quantified as any drug especially because unsuspected circumstances such as asymptomatic vitamin B12 deficiency can trigger severe neurological

degradation. Last, functional B12 deficiency should be investigated by measuring metabolic parameters (vitamin B12, tHcy, Meth and MMA) prior to N_2O administration in at-risk patients or in any patient with neurological symptoms or excessive nitrous oxide consumption.

Author contributions

CD, AI, JFB, MS designed the study; CD, BK, ML, JG, JS, SP, LH, AF, MB, MS, JFB and AI participate to the data collection and analysis. CD, AI, JFB, and MS participate to the writing and editing of the manuscript. All authors reviewed the manuscript.

Declaration of Competing Interest

The authors declare no competing interests.

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