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An autism cohort study of cobalt levels following vitamin B12 injections

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The effects of methylcobalamin (vitamin B12) injection ($75\,\mu g\,kg^{-1}$) on cobalt levels in autism spectrum disorders (ASDs) and potential toxic effects of cobalt on human neurons were evaluated. A cohort of ASDs ($n = 72$) presenting to the Genetic Centers of America were evaluated for the frequency of methylcobalamin injections and cobalt levels using Laboratory Corporation of America (LabCorp) testing. Potential toxic effects of cobalt (cobalt(II) nitrate hexahydrate) to human neurons grown *in vitro* were evaluated. Methylcobalamin injections significantly increased the mean levels of plasma cobalt (6.83-fold) and urinary cobalt (51-fold) in comparison to unexposed subjects, and significant positive correlations were found between the frequency of methylcobalamin injections and the levels of plasma and urinary cobalt (injections every second day induced cobalt levels in excess of the LabCorp cobalt occupational maximum exposure limits). The LC_{50} in human neurons following 24 h incubation with cobalt is 559 μM. Research must be conducted to determine optimal therapeutic methylcobalamin doses.

Keywords: autistic; cyanocobalamin; hydroxycobalamin; neurotoxicity; toxicity

Introduction

There are well-recognized neurological-psychiatric sequelae of the impaired intracellular synthesis of adenosylcobalamin and methylcobalamin (cobalamin C disease), which is an inborn error of metabolism (Roze et al. 2003). Vitamin B12 deficiency can arise for various reasons including a mother adhering to a vegan diet during pregnancy and while breastfeeding (Casella et al. 2005), or through strict adherence to a vegan diet (Cundiff and Harris 2006).

The methionine cycle involves the regeneration of methionine via the vitamin B12-dependent transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine in the methionine synthase reaction. Methionine may then be activated by methionine adenosyltransferase to form *S*-adenosylmethionine (SAM), the primary methyl donor for most of the cellular methytransferase reactions including the methylation of DNA, RNA, proteins, phospholipids, and neurotransmitters. The transfer of the methyl

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group from SAM to various enzyme-specific methyl acceptors results in the formation of *S*-adenosylhomocysteine (SAH). The reversible hydrolysis of SAH to homocysteine and adenosine by the SAH hydrolase (SAHH) reaction completes the methionine cycle. Adenosine is further metabolized by adenosine kinase for purine synthesis or catabolized by adenosine deaminase. Homocysteine can be either remethylated to methionine or irreversibly removed from the methionine cycle by cystathionine β-synthase (CBS). Two important consequences of a decrease in methionine cycle turnover are a decreased synthesis of SAM for normal methylation activity and a decreased synthesis of cysteine and glutathione for normal antioxidant activity (Finkelstein 1998).

Abnormalities of methylation have been described in subjects diagnosed with an autism spectrum disorder (ASD). Several investigators have reported on metabolic endophenotypes that may be associated with decreased transmethylation and transsulfuration of methionine (James et al. 2004; Geier and Geier 2006; James et al. 2006; Geier and Geier 2007; Geier et al. 2009a; Geier, Kern, and Geier 2009b). Further, it was demonstrated that there were significantly increased rates of allele frequency or gene–gene interactions associated with impaired transmethylation and transsulfuration in ASDs for the following genes: reduced folate carrier (RFC 80G > A); transcobalamin II (TCN2 776G > C); catechol-O-methyltransferase (COMT 472G > A); methylenetetrahydrofolate reductase (MTHFR 677C > T and 1298A > C); and glutathione-S-transferase (GST M1) (James et al. 2006).

As a result, it was suggested that methylcobalamin injections may play an important role in the treatment of subjects diagnosed with an ASD. Investigators using the injectible form of methylcobalamin ($75\,\mu g\,kg^{-1}$, twice a week) in subjects diagnosed with an ASD observed significant decreases in the concentrations of adenosine and oxidized glutathione, significant increases in the concentrations of methionine, cysteine, and total glutathione, as well as the ratios of SAM : SAH and total glutathione : oxidized glutathione (James et al. 2004, 2009).

An examination of the molecular structure of methylcobalamin reveals that it contains 4.38% of cobalt by weight. As a result, the dosing schedule described by previous investigators for subjects diagnosed with an ASD (James et al. 2004, 2009) would be expected to result in an instantaneous dose of cobalt $= 3.285\,\mu g\,kg^{-1}$. It was previously reported that the entire body content of cobalt is less than 1 mg, primarily stored in the muscles, bone, liver, and kidneys (Reavley 1999). Overall, this means that one injection of $75\,\mu g$ methylcobalamin kg^{-1} could result in an instantaneous exposure to cobalt equal to about 25% of the entire body-burden of cobalt (total body cobalt = 1 mg/75 kg average adult bodyweight = $13.3\,\mu g$ cobalt kg^{-1} bodyweight), and this would be repeated every several days for an indefinite period of time.

The aim of the present study was to evaluate the potential effects of methylcobalamin injections on cobalt levels in a cohort of subjects diagnosed with an ASD. The first phase of the study attempted to evaluate the distribution patterns of cobalt following a methylcobalamin injection. The second phase of the study attempted to evaluate the potential toxic effect of cobalt on human tissue culture cells.

Materials and methods

The Institutional Review Board (IRB) for the Institute of Chronic Illnesses approved this retrospective experimental study.

Cohort study – phase I

Subjects

In the present study, a retrospective chart review was conducted to identify a cohort of subjects that was presented to the Genetic Centers of America for outpatient genetic/developmental evaluations from 2005 to 2008. Charts were reviewed to identify the subjects that were diagnosed with ASDs, based upon the criteria in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV), had documented information regarding their present use of methylcobalamin supplementation at the time of initial clinical presentation, and had subsequent lab testing at the Laboratory Corporation of America (LabCorp), Inc., for urine and blood cobalt levels. The subjects meeting the above criteria were screened to identify the subjects who had been administered methylcobalamin injections at least once every three days (exposed group) or were receiving no methylcobalamin injections (unexposed group) for inclusion in the present study. A total of 72 subjects were identified for inclusion in the present study. Table 1 summarizes the overall demographics of the exposed and unexposed groups examined in the present study.

Evaluation

Subjects included in the present study had been tested for urine and plasma levels of cobalt. These tests were performed on morning blood and first morning urine samples collected following an overnight fast. By definition, anyone in the present study with urine or plasma levels below the LabCorp's level of detection ($1.0\,\mu g\,L^{-1}$) was assumed to have zero cobalt.

Controls

In addition to the cohort comparison conducted in the present study based upon exposure, subjects in the exposed group were evaluated for their blood and urine levels of cobalt in comparison to the occupational exposure reference ranges proved by LabCorp. LabCorp cobalt testing has set its maximal occupational exposure limit for plasma cobalt at $1.0\,\mu g\,L^{-1}$ and occupational exposure limit for urinary cobalt at $15\,\mu g\,L^{-1}$.

Table 1. Demographic information for the subjects diagnosed with an ASD.

Descriptive information	Exposed ASD[a] ($n=24$)	Unexposed ASD[b] ($n=48$)
Gender/age		
Male/female (ratio)	21/3 (7 : 1)	40/8 (5 : 1)
Mean age in years ± SD (range)	9.3 ± 3.5 (4–18)	8.9 ± 3.7 (4–18)
Race (*n*)		
Caucasian	87.5% (21)	83.3% (40)
Minorities[c]	12.5% (3)	16.7% (8)

Notes: SD = standard deviation. All subjects examined in the present study were diagnosed with ASDs and had presented for outpatient developmental/genetic evaluations at the Genetic Centers of America from 2005 to 2008.

[a]Exposed ASD is defined as any subject receiving methylcobalamin injections at least once every three days.

[b]Unexposed ASD is defined as any subject not receiving methylcobalamin injections.

[c]Includes participants of Hispanic, Black, Asian, or mixed ancestry.

Statistical analyses

The statistical package contained in StatsDirect (Version 2.4.2) was utilized. Overall blood and urinary cobalt levels were compared in the exposed and unexposed groups using the non-parametric Mann–Whitney *U*-test statistic. The null hypothesis was that, between exposed and unexposed subjects, there should be no difference between the overall distribution of blood or urinary cobalt levels. In addition, among those in the exposed group, the non-parametric linear regression (continuity corrected) test statistic was utilized to evaluate the relationship between the urinary and blood levels of cobalt and the frequency of methylcobalamin injections. The null hypothesis was that the slope of the line would be equal to zero for the relationship between the urinary and blood levels of cobalt and the frequency of methylcobalamin injections. For all the statistical tests in the present study, a two-tailed p-value < 0.05 was considered statistically significant.

Human tissue culture – phase II

Human cell cultures

Cultures of SH-SY-5Y human neuroblastoma isolated by the European Collection of Cell Cultures were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The methods employed in the present study were previously described (Geier, King, and Geier 2009c). For neuroblastoma cells, the culture medium consisted of Dulbecco's Modified Eagle's Medium/Ham's F12, 50/50 1× with L-glutamine (MEM-F12) (Mediatech, Inc., Manassas, VA, USA), 15% fetal bovine serum (FBS) sterile filtered (Equitech-Bio, Inc., Kerrville, TX, USA), and 1% MEM non-essential amino acid (MEM NEAA) solution 100 × (Sigma-Aldrich). In all cases, the cells were grown following a standardized procedure at 37°C, 95% humidity, and 5% CO_2 in 40-mL tissue culture (Nunclon^{TM} delta surface) flasks (NUNC^{TM}, Rochester, NY, USA).

Cells were gown in flasks until confluent, and then the cell cultures were trypsinized (Trypsin, INTERGEN® Company, Purchase, NY, USA). The disaggregated cells were seeded evenly into COSTAR® (Corning International, Corning, NY, USA) 96-well (100 µL per well), cell-culture-cluster, flat-bottom, tissue-culture, treated plates with lid. The seeded cells were grown following a standardized procedure for at least one day at 37°C, 95% humidity, and 5% CO_2 in the 96-well cell culture plates with appropriate cell media and FBS concentration prior to treatment with the studied cobalt compound.

Compound

Cobalt(II) nitrate hexahydrate ($Co(NO_3)_2 \cdot 6H_2O$, CAS No. 10026-22-9) was purchased from Sigma-Aldrich (St. Louis, MO, USA). A 100 mM cobalt(II) nitrate hexahydrate stock solution was prepared for cobalt(II) nitrate hexahydrate by dissolving it into MEM-α culture medium, and the resultant solution was sterilized by filtration through 0.20 µm NALGENE® Filter Unit (Nalge Nunc International, Rochester, NY, USA).

Cobalt-induced cell cytotoxicity in human neuroblastoma cells

Cell cytotoxicity was assessed in human neuroblastoma cells using the resazurin-based cell survival assay kit (TOX-8, Sigma-Aldrich). The resazurin assay measures cellular oxidative/reduction potential. The original media was removed from each well of the 96-well cell culture plates, and was replaced with 200 μL per well dilutions of cobalt(II) nitrate hexahydrate (1μM–10 mM) in appropriate cell media. In the control wells, the same procedure was followed except that the media added contained no added cobalt. Cells were incubated following a standardized procedure for 24 h at 37°C, 95% humidity, 5% CO_2, and gentle shaking (100 rpm) in the 96-well cell culture plates. Subsequently, 50 μL of resazurin-based solution (20% concentration, dissolved in appropriate cell media) was added to each well. The 96-well cell culture plates were gently shaken, and incubated for 12 h at 37°C, 95% humidity, and 5% CO_2.

The 96-well cell culture plates were transferred to a $VERSA_{max}$ (maintained at 37°C) for assaying. The contents of the study wells in the 96-well culture plates were then assayed for absorption at 600 and 690 nm. Each test dilutions and the controls were repeated in eight wells. The 690 nm absorbance value was subtracted from the 600 nm absorbance value to determine a measure of the level of cell viability in each well evaluated. The net values determined for each cobalt dilution were normalized to controls set at 100%. From the 24 h incubation time, approximate LC_{50} values were determined for each cell type, and the results (mean and SEM) were expressed as a percent of the control mean ($[mean_{Test} \pm SEM_{Test}]/mean_{Control} \times 100\%$).

Visual cytotoxicity induced in human neuroblastoma cells

Cytotoxicity in human neuroblastoma cells was assayed using microscope inspection with microscope digital image capture. The original media was removed from each well in the 96-well cell culture plates. The media was replaced with 200 μL per well dilutions of cobalt that were added at about the LC_{50} derived from the resazurin-based assay (100 μM – 1 mM) in appropriate cell media. In control wells (containing no added test compound), the same procedure was followed except that only the cell media was added. Cells were incubated following a standardized procedure for 24 h at 37°C, 95% humidity, 5% CO_2, and gentle shaking (100 rpm) in the 96-well cell culture plates. The wells were then examined using an inverted UNITRON® microscope (Bohemia, NY, USA) with the 5× objective. A Big Catch™ digital eye-piece camera (EM-035M) and Scope Photo Image software (Torrance, CA, USA) were used to capture digital images of the cells.

Statistical analyses

The statistical package contained in StatsDirect (Version 2.4.2) was utilized. The parametric *t*-test statistic was used to compare cells exposed to increase the exposure of cobalt(II) nitrate hexahydrate in comparison to the unexposed controls. A two-tailed p-value < 0.05 was considered statistically significant. In addition, LC_{50} values were determined for the oxidative/reduction activity assay. The linear regression test statistic was utilized to examine the linear portion of the curve derived from the assay

response curve developed for the cellular oxidative/reduction activity to determine the LC_{50}.

Results

Cohort study – phase I

Table 2 compares the relative mean levels of plasma and urine cobalt observed among the subjects diagnosed with an ASD exposed to methylcobalamin injections at least every three days in comparison to unexposed subjects. It was found, on the average, that exposed subjects receiving methylcobalamin injections had significantly increased the mean levels of plasma cobalt (6.83-fold) and urinary cobalt (51-fold) in comparison to unexposed subjects.

Figure 1 evaluates the relationship between the frequency of methylcobalamin injections with blood and urinary cobalt levels in exposed subjects. It was observed that significant positive correlations were observed between the frequency of methylcobalamin injections and the levels of plasma cobalt ($\tau_b = 0.37$) and urinary cobalt ($\tau_b = 0.37$). Overall, it was found that methylcobalamin injections given to study subjects every second day resulted in average plasma and urinary cobalt levels in excess of the LabCorp cobalt maximal occupational exposure limits. In addition, there was a significant positive correlation ($\tau_b = 0.50$) between blood and urine cobalt levels for the subjects in the exposed ASD group.

Human tissue culture – phase II

The results of the dose-dependent assessment of cobalt(II) nitrate hexahydrate inducing the cell cytoxicity in human neuroblastoma cells following 24 h incubation is summarized in Figure 2. Cobalt(II) nitrate hexahydrate concentrations ≥ 1 mM induced significant cell cytotoxicity that was measured using the resazurin-based cell survival assay kit. The calculated LC_{50} in human neuroblastoma cells following 24 h

Table 2. Plasma and urinary cobalt levels observed in subjects diagnosed with an ASD.

Lab testing	Exposed ASD[a] ($n = 24$)	Unexposed ASD[b] ($n = 48$)
Plasma cobalt ($\mu g\,L^{-1}$)		
Mean ± SE (range)	0.82 ± 0.19 (0.0–3.2)[c]	0.12 ± 0.10 (0.0–4.6)
Median (IQR)	0.50 (0.0–1.5)	0.0 (0.0–0.0)
Urine cobalt ($\mu g\,L^{-1}$)		
Mean ± SE (range)	10.7 ± 6.5 (0–156.7)[c]	0.21 ± 0.13 (0–5.6)
Median (IQR)	2.15 (0–5.2)	0.0 (0.0–0.0)

Notes: SE = standard error of the mean. IQR = inter-quartile range. All subjects examined in the present study were diagnosed with ASDs and had presented for outpatient developmental/genetic evaluations at the Genetic Centers of America from 2005 to 2008. All lab testing was done by LabCorp.

[a]Exposed ASD is defined as any subject receiving methylcobalamin injections at least once every three days.

[b]Unexposed ASD is defined as any subject not receiving methylcobalamin injections.

[c]The non-parametric Mann–Whitney *U*-test statistic revealed a significant ($p < 0.001$) difference between the exposed and unexposed ASD diagnosed groups.

incubation with cobalt(II) nitrate hexahydrate was 559 µM. Figure 3 visually illustrates that 1 mM concentrations of cobalt(II) nitrate hexahydrate incubation with human neuroblastoma cells for 24 h induced significant cellular degeneration in comparison to the unexposed controls.

Discussion

The present study is the first of its kind to evaluate the potential distribution patterns of cobalt in the human body following methylcobalamin injections, as well as the potential toxic consequences of cobalt to human tissue culture cells. It was observed that using methylcobalamin injection (75 µg/kg) at least once in every three days to the subjects diagnosed with an ASD induced significant, many-fold increases in cobalt levels in the urine and plasma in comparison to the cobalt levels in unexposed

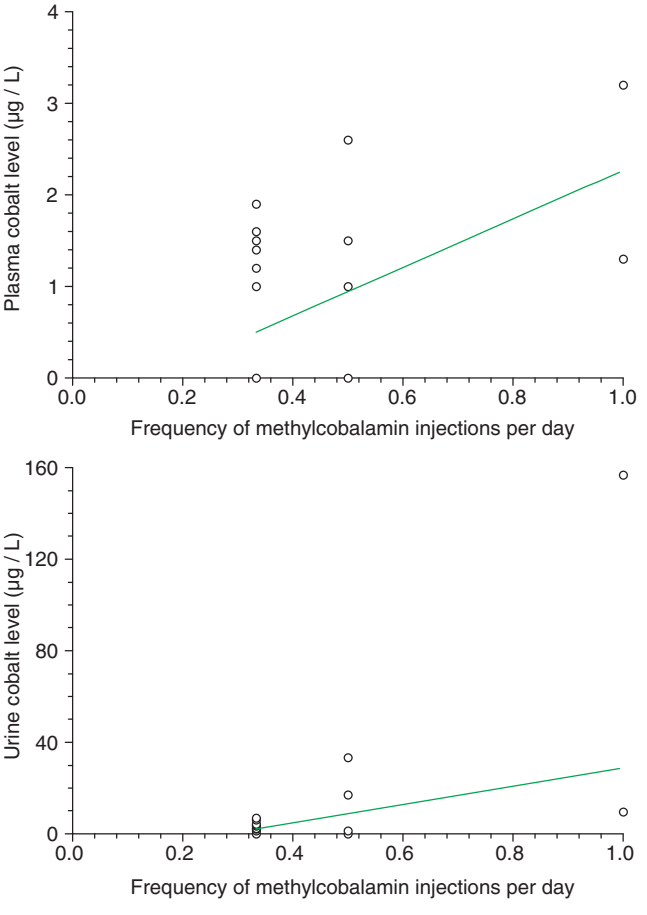

Figure 1. Frequency of methylcobalamin injections and cobalt levels in the plasma and urine observed in subjects diagnosed with an ASD.
Notes: Plasma cobalt $\tau_b = 0.37$ ($p < 0.05$); Urine cobalt $\tau_b = 0.37$ ($p < 0.05$). Frequency of methylcobalamin injections per day: 1 = every day; 0.5 = every second day; and 0.3 = every third day.

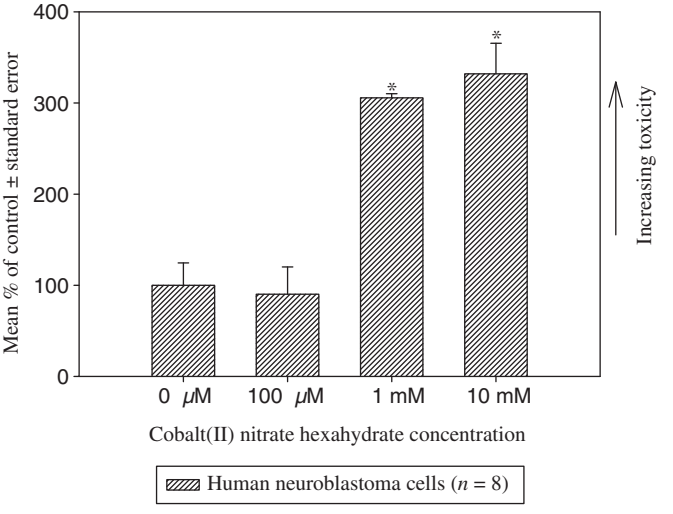

Figure 2. Dose-dependent assessment of cobalt(II) nitrate hexahydrate-induced cell cytotoxicity in human neuroblastoma cells following 24 h incubation.
Notes: Cobalt(II) nitrate hexahydrate LC_{50} in human neuroblastoma cells = 559 μM following 24 h incubation. Cell viability was measured using the Resazurin Dye Cell Viability Assay.
*$p < 0.01$ (cobalt exposure concentration in comparison with the 0 μM control).

subjects diagnosed with an ASD. In addition, there were significant positive correlations between the increasing frequency of methylcobalamin injections to the subjects diagnosed with ASD and cobalt levels in the plasma and urine. Further, using methylcobalamin injections every second day was able to increase average urinary and plasma cobalt levels in excess of the LabCorp occupational cobalt exposure limits. Finally, it was determined that cobalt incubated with human neuroblastoma cells for 24 h was able to induce significant cytotoxicity.

Historically, there is limited information on the distribution pattern and toxic effects of vitamin B12 in animal model systems. Following vitamin B12 (hydroxocobalamin) intraperitoneal administration (70 mg kg^{-1} day^{-1}) for a 21-day period to rats, investigators observed significant increases in the examined tissue concentrations of cobalt in the diaphragm and myocardium (other organs were not examined) (Pery-Man et al. 1996), and no significant adverse histological effects of vitamin B12 administration was observed in the tissues examined. In contrast, investigators observed that intravenous administration of a single several-hundred milligram-per-kilogram dose of vitamin B12 (hydroxocobalamin) to rabbits produced significant acute histological changes to organs, including the kidney, liver, and heart (Hobel et al. 1980). Importantly, since neither of these two studies attempted to study the potential adverse histological effects of chronic long-term low doses of vitamin B12 in organs such as the brain, one cannot make any concrete extrapolation from the results reported in these animal studies to the injection of methylcobalamin into humans.

There are limited human data on the distribution of radioactive vitamin B12 (the isotope used was ^{60}Co) administered to children (Cooperman 1972). It was observed upon autopsy that the highest levels were present in the pituitary gland and the liver,

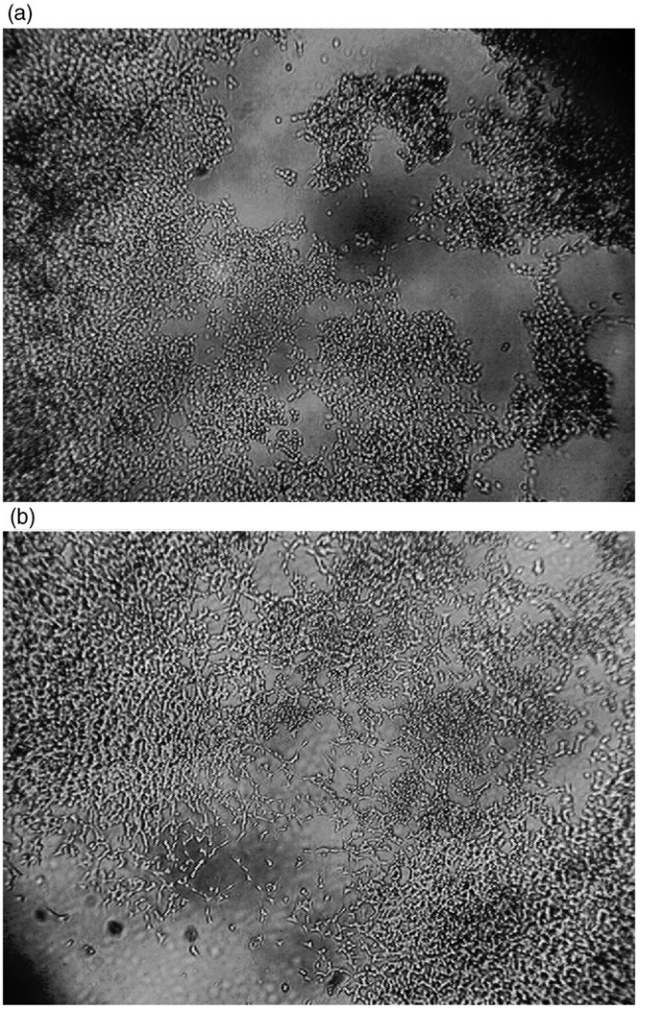

Figure 3. A visual examination of cobalt(II) nitrate hexahydrate-induced cell cytotoxicity in human neuroblastoma cells following 24 h incubation. (a) 1 mM cobalt(II) nitrate hexahydrate. (b) 0 μM cobalt(II) nitrate hexahydrate.
Notes: Visual captures using an inverted UNITRON® microscope with the 5× objective. A Big Catch™ digital eye-piece camera (EM-035M) and Scope Photo Image software were used to capture digital images of the cells.

followed by the kidneys, gastrointestinal tract, lungs, heart, pancreas, spleen, and adrenals.

An apparent concern for potential toxicity and increased tissue concentrations of cobalt following vitamin B12 administration is that several investigators have evaluated the cytotoxic effects of cobalt on human neuroblastoma cells (Olivieri et al. 2001, 2002). These previous studies revealed dose-dependent cytotoxic effects in comparison to those observed in the present tissue culture experiments. Further, investigators reported that cobalt also significantly adversely affected intracellular reduced glutathione levels and significantly increased the secretion of β-amyloid. One of the investigators commented, based upon their results, that their "findings are significant as cobalt is an essential nutritional requirement, usually

bound to cobalamin (vitamin B12), for all animals which in the unbound form could lead to neurotoxicity'' (Olivieri et al. 2001). In addition, these investigators reported that elevated brain levels of cobalt in subjects diagnosed with Alzheimer's disease were previously observed (Olivieri et al. 2001). The Sigma-Aldrich material safety data sheet for cobalt(II) nitrate hexahydrate describes that exposure may induce adverse effects in behavior, allergic reactions, gastrointestinal dysfunction, and gross metabolic changes.

While the present study was not designed to evaluate the long-term chronic distribution patterns of cobalt following methylcobalamin injections, a case was identified in the retrospective chart review of a 9-year-old Caucasian male, diagnosed with an ASD, who received methylcobalamin injections ($75\,\mu g\,kg^{-1}$). As shown in Figure 4, this case is unique in which heavy metal tests (that included urinary cobalt levels) were conducted at LabCorp on this subject about every 2 weeks, for a 6-month period. The subject's primary care doctor during the period slowly increased the methylcobalamin dosing regiment from every third day to every day, as shown in Figure 4. The result was a significant increase in the subject's urinary cobalt levels. Moreover, the increases observed in urinary cobalt levels appear to be consistent with those predicted from the acute exposure data examined in the present study.

Strengths/limitations

The strength of the present study is that a single moderate-sized study subject cohort, all with the same condition, were presented to the same clinic and evaluated using the same type of laboratory testing. Further, within the cohort there were significant variations in the frequency of exposure to methylcobalamin injections. Overall, exposure ranged from a subject group that had never been exposed to

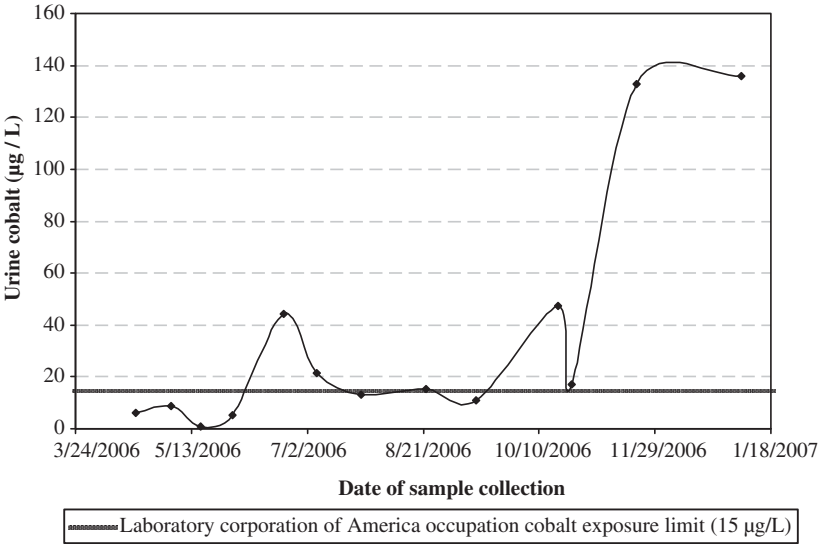

Figure 4. A case example of urinary cobalt levels following increasing the frequency of methylcobalamin injections (increased gradually from every third day to every day).
Note: The subject was a 9-year-old Caucasian male who was undergoing routine monitoring for urine toxic metal levels about every 2 weeks.

methylcobalamin injections to those exposed to a uniform methylcobalamin dose ($75\,\mu g\,kg^{-1}$) either on an every day, every other day, or every third day basis. Another strength of the present study was that accurate measurements were made regarding the actual cytotoxicity to human cells in tissue culture. It was possible to calculate the dose-dependent cytotoxicity induced by cobalt exposure in human neuroblastoma cells following a specified incubation period and assay of cell viability.

One of the weaknesses of the present study is that it was not possible to determine the exact form of the cobalt being measured, namely, whether the cobalt measured in the urine and plasma in the methylcobalamin form, a breakdown product, or ultimately as a free form of cobalt. This might have significant implications to the actual toxicity of the cobalt to human tissue. Similarly, while the present study was able to demonstrate a direct relationship between the frequency of methylcobalamin injections and levels of cobalt in the urine and blood, it was unable to examine the various tissue concentrations of cobalt or the forms of cobalt present in the tissue. Once again, such data would help to significantly determine the potential toxic consequences of methylcobalamin injections.

Another weakness of the present study is that it evaluated only the acute distribution of cobalt following methylcobalamin injections. It does not provide insights into what the distribution pattern of cobalt may look like in the human body following long-term administration. It is clear that the case reports presented show that there may be much more information to be gleaned from such studies.

In our own clinical practice of subjects diagnosed with an ASD, we routinely use methylcobalamin as a treatment to assist those with diagnosed disturbances within the methionine-cycle and the transsulfuration pathway. In light of the elevations observed in the present study, we have been careful to administer significantly lower doses than the injection of $75\,\mu g$ methylcobalamin kg^{-1} every third day. We presently use sublingual preparations of $50\,\mu g$ methylcobalamin administered on a daily basis, and have found that most of the children following this treatment regimen have urinary/plasma cobalt levels that are below the limits of detection by LabCorp or below the LabCorp occupational maximal exposure limits to cobalt, but have apparently adequate blood levels of vitamin B12.

Conclusions

The present study is the first of its kind to attempt to evaluate and describe some of the distribution patterns of cobalt in humans following the administration of methylcobalamin injections. Methylcobalamin injections were able to induce plasma and urinary concentrations of cobalt in excess of LabCorp occupational maximal exposure limits, but the exact form of cobalt, the tissue levels, and ultimately potential tissue toxicity remain unclear. Further, cobalt was observed to induce significant cellular cytoxicity in human neuroblasma cells, including cellular degeneration.

It is clear that there may be significant potential benefits to the administration of methylcobalamin to subjects diagnosed with an ASD based upon the significant abnormalities identified within the methionine cycle-transsulfuration pathways and genetics SNPs within these pathways, but much further research needs to be conducted regarding the optimal therapeutic doses. This will ensure that the doses

used remain as safe as possible, while providing the best possible benefits to the subjects receiving them. Until such time, it is important that physicians prescribing methylcobalamin injections for subjects should routinely monitor blood and urine cobalt levels, and, to be safe, ensure that their dosing regimens do not result in blood and/or urine cobalt levels in excess of permissible maximal exposure safety limits. These concerns and caveats are all important in light of the rapid expansion of the use of methylcobalamin injections in the treatment of a number of different conditions.

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