CME

Unintentional Manganese Delivery in Neonatal Parenteral Nutrition

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ABSTRACT

Iatrogenic manganese (Mn) neurotoxicity is a safety concern in neonates receiving parenteral nutrition (PN). Prior studies suggest Mn contamination of PN ingredients represents an unintended source of Mn delivery. In order to determine the relative contribution of unsourced Mn to total Mn exposure in neonatal PN, this study measured Mn concentrations in neonatal PN solutions using inductively coupled plasma mass spectrometry. Solutions prepared using a standard fixed dose neonatal multiple trace element product were compared with test solutions prepared using individual trace element ingredients not including Mn. The standard solutions (n = 6) contained a mean (SD) Mn concentration of 56.63 μg/L (0.94), compared with 6.04 μg/L (0.39) in the test solutions without added Mn (n = 6). This study suggests that neonatal PN contains significant quantities of Mn not intentionally added during PN preparation. Further studies are needed to identify individual ingredient sources of unintentional Mn, and the feasibility of Mn omission strategies.

Key Words: manganese, neonate, parenteral nutrition, preterm, safety

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What Is Known

- Neonatal manganese exposure from routine daily parenteral nutrition is physiologically excessive and potentially neurotoxic.
- A portion of the total daily manganese exposure in parenteral nutrition is from unseen and unintended manganese delivery, possibly because of ingredient contamination.

What Is New

- Omitting manganese when preparing neonatal parenteral nutrition still achieves clinically appropriate daily manganese delivery because of the unseen manganese.
- Exploiting the unintentionally existing manganese in parenteral nutrition represents a research tool for designing parenteral nutrition capable of reducing manganese exposure in neonates.

In the United States, the trace element manganese (Mn) is routinely added to neonatal parenteral nutrition (PN) as part of an Food and Drug Administration (FDA)-approved, neonatal-specific, fixed-dose, multiple trace element (MTE) combination product along with chromium, copper, and zinc, named Multitrace-4. The 5 μg/kg daily Mn dose recommended in the product labeling originates from a nutritional guideline developed for adults and children without any neonatal data (1). At this dose, and taking into account the 100% bioavailability from the intravenous route of administration, the daily Mn load from PN is approximately 100 times greater than from breastmilk (2). Considering that neonates have underdeveloped hepatobiliary Mn excretory pathways, Mn overexposure and neurotoxicity from routine PN is a safety concern in this population, especially for those born premature (2). Reformulating the approved product to contain the recommended 1 μg/kg daily Mn dose, as is present in the European Medical Agency (EMA)-approved product (eg, Peditrace) could alleviate this concern (3,4). Clinically significant amounts of Mn is unintentionally present, however, in many PN ingredients, which confounds the ability to deliver a reliable daily Mn dose regardless of MTE Mn content (5–8). The source of this ''extra'' Mn may be because of natural contamination during the parenteral products manufacturing process. Simply not using any Mn-containing MTE products when admixing PN, and instead using chromium, copper, and zinc individually, has been suggested by some experts (9,10). Such an approach relies on the unintended Mn quantity to deliver a daily obligatory Mn supply. Although creative, such a workaround presents some safety risks of its own by requiring a more complicated PN compounding process and delivering an unpredictable daily Mn exposure. Furthermore, most studies of unintentional Mn content in neonatal PN are over 25 years old. Newer data with contemporary ingredients and PN-compounding procedures are needed before risk reduction strategies that account for unsourced Mn that can be tested and deployed.

We designed the following study to quantify the amount of Mn unintentionally present in neonatal PN.

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METHODS

Sample Preparation and Composition

PN solutions designed to simulate those routinely seen in clinical neonatal care were aseptically prepared at the study site institution's hospital pharmacy in a Class 5 ISO laminar-airflow hood using non-DEHP ethylene vinyl acetate bags and an automated compounding device (Exacta-Mix 2400, Baxa Corporation, Englewood, CO), operated by an experienced hospital pharmacy technician authorized to perform neonatal PN preparation and under the direct supervision of the investigator (J.S.). The test solutions were prepared without Mn whereas the standard solution contained Mn at the 5 $\mu g \cdot kg^{-1} \cdot day^{-1}$ dosage from the standard trace element mixture (Table 1 and Supplemental Digital Content, *http://links.lww.com/MPG/B812*). We considered it necessary to analyze standard PN solutions as previous investigators have found MTE preparations to be key sources of Mn contamination (6). Each test and standard solution was prepared in triplicate to yield 3 100 mL samples each. The process was repeated after a 7-day span to account for inter-process variability because of ingredient lot and equipment operator differences. The final total number of prepared solutions was, therefore, 12; 6 test solutions and 6 standard. Immediately after preparation, each solution was transferred to a precleaned high-density polyethylene (HDPE) container using a new single-use all plastic infusion set (Alaris 2200–0500, CareFusion, San Diego, CA). The container was then stored under refrigeration (2–8 °C) until shipment to the off-site laboratory (Physis Environmental Laboratories, Inc. Anaheim, CA).

Sample Analysis

Determination of total Mn in study samples was accomplished by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) using US Environmental Protection Agency Method 200.8 (11). This method utilizes stringent quality control and sample handling procedures necessary to avoid contamination and ensure the validity of analytical results during sampling and analysis for metals.

Each sample was digested via a wet oxidation procedure using Optima grade concentrated nitric acid suitable for ultratrace elemental analysis. Samples were acidified to pH <2 in their original sample container (250 mL HDPE), mixed, and held for a minimum of 24 hours, and rechecked for pH. This process was repeated until sample pH remained at <2 after 24 hours. The resulting preserved acidified and digested sample can be held at room temperature up to 6 months before analysis. A 50 mL aliquot of the acidified digestate was then analyzed and quantified using an Agilent 7700x Inductively Coupled Plasma Mass Spectrometer. The system operating conditions were first checked daily before analyzing samples by tuning according to instrument parameters. ICP-MS tuning solution containing Cobalt, Lithium, Yttrium, and Thallium at 1 ppb was used. Once instrument tune limits were satisfied, a calibration curve was established by measuring the ion counts of a minimum of 5 dilution concentrations of commercially available calibration stock standard solutions. After the initial calibration and before analyzing any samples, an initial calibration verification using a second source standard was performed and a continuing calibration verification performed every 10 analyses and at the end of every sample batch run. Sample material in solution was introduced into the ICP-MS by pneumatic nebulization into a high purity grade argon plasma where energy transfer processes cause desolvation, atomization, ionization, detection, and identification with a mass spectrometer on the basis of the mass-to-charge ratio (m/z) for Mn (55/1). The concentration of Mn was determined by comparing the ion counts measured in the unknown sample to the calibration curve. The lower Mn detection limit is 0.005 μg/l.

TABLE 1. Test and standard parenteral nutrition content

	Test PN solution	Standard PN solution
Dextrose, %	10	10
Amino acids*, %	3	3
Na†, mEq/L	60	60
K‡, mEq/L	20	20
Ca, mEq/L	30	30
Mg, mEq/L	3	3
Multivitamins, mL/L	20	20
Heparin, U/mL	1	1
Cu, μg/L	200	200
Cr, μg/L	2	2
Zn, mg/L	4	4
Mn, μg/L	0	50
Se, μg/L	20	20

*Includes supplemental cysteine 40 mg per g amino acids.
†As NaCl 20 mEq/L + NaAcetate 20 mEq/L + NaPhosphate 20 mEq/L.
‡As KCl.

Data Analysis

Mean Mn concentrations between test and standard solutions were expected to be different by design and no statistical assessment of difference was conducted. To explore variability in Mn concentration between samples prepared on different days, mean Mn concentrations between the same test or standard solutions prepared 1 week apart were compared using the paired student's *t*-test. A *P* value <0.05 was considered statistically significant.

RESULTS

Measured Mn concentrations (μg/L) of all 12 samples are shown in Table 2. The mean (SD) Mn concentration of all 6 test solutions was 6.04 (0.39) compared with 56.63 (0.94) in the 6 standard solutions. The mean intra-group values were statistically significantly different on the 2 different sample preparation days for the test ($P = 0.016$) but not for the standard solutions ($P = 0.195$).

Assuming a 100 $mL \cdot kg^{-1} \cdot day^{-1}$ PN intake, test solutions would provide 0.56 to 0.65 $\mu g \cdot kg^{-1} \cdot day^{-1}$ Mn based on the lowest and highest measured Mn concentration values. The standard solutions would provide 5.5 to 5.8 $\mu g \cdot kg^{-1} \cdot day^{-1}$.

DISCUSSION

This study directly compared neonatal PN solutions prepared with and without Mn in order to determine the quantity of unsourced Mn in neonatal PN. Our results are consistent with those of similar studies conducted over the past 30 years (range reported: 5.1–7.6 μg/L) (5,7,8,12). The key difference in the present study

TABLE 2. Measured manganese concentration in test and standard parenteral nutrition solutions

	Test PN solution (μg/L Mn)				Standard PN solution (μg/L Mn)			
	1	2	3	Mean	1	2	3	Mean
Week 1	6.516	6.085	6.467	6.357	55.355	56.917	56.173	56.148
Week 2	5.734	5.596	5.814	5.715	57.090	58.085	56.188	57.121

PN = parenteral nutrition.

compared with others is that we tested PN solutions containing individual trace elements minus Mn, rather than not including any TE at all (5), as the latter approach may be less desirable in clinical practice. We also used the current standard ICP-MS method (11) of Mn measurement instead of the atomic absorption spectrophotometry (AAS) graphite furnace method used in older studies (7,8,12). The former method has greater accuracy, less potential for interference, and lower detection limits. At the concentrations being studied, the AAS detection limit of 0.2–0.4 μg/L is, however, not necessarily inferior and may explain the similar results between older studies and ours.

The calculated daily Mn delivery from our no-added Mn test solutions approaches the 1 μg/kg daily supplemental Mn dose currently recommended for neonates and infants (3,4,9). The potential clinical significance of this finding is that neonatal PN prepared without adding any Mn may still be capable of delivering an appropriate daily Mn supply. Our data are insufficient to recommend such a practice and clinical studies are needed to test this approach. A potential caveat to adopting this approach in clinical practice is the complicated nature of individually adding TE to PN during the compounding process. We did find an intragroup difference in Mn concentration among test PN solutions based on preparation day. This may have been because of variability associated with human handling of multiple individual TE ingredients in the test PN group. It may also have been because of differences in unsourced Mn in the 7 of 17 ingredients that came from different lot numbers (see Supplemental Digital Content, *http://links.lww.com/MPG/B812*). The reported differences, however, were not clinically significant.

Notably, the 6 μg/L Mn concentration in our no-Mn-added test solutions was equal to the difference between the intended 50 μg/L and measured 56 μg/L Mn concentrations in the standard solutions prepared with the Mn-containing MTE product. This suggests that the individual TE ingredients used in the test solution are not a major source of Mn contamination, or that the fixed dose MTE product has a similar amount of Mn contamination as the individual TE ingredients. This needs to be confirmed through direct measurement of these products. A weakness of our study is that we did not identify, which individual ingredients were responsible for the observed contamination. Other groups have determined that Mn is present as a contaminant in many of the ingredients used in compounding PN, including the MTE product itself (6,12). There is a need for following up these older studies using contemporary products to determine the current state of unintentional Mn exposure in routine neonatal care.

Quantifying unintended Mn in neonatal PN has clinical importance when considering the supraphysiologic and potentially toxic exposure neonates experience when their providers use routine approved parenteral multiple trace elements products. In adults and children beyond the neonatal period, PN-related Mn toxicity is associated with excessive Mn dosage, lengthy PN duration, and hepatobiliary comorbidities that impair Mn excretion. Whenever excess exposure occurs, Mn accumulates in the globus pallidus and striatum regions of the brain; subcortical neurons that regulate voluntary movement. Symptoms of PN-related Mn neurotoxicity include tremor, gait disturbance, dystonic movements, hypokinesia, altered mental status, and seizures (13,14). Neonates are a unique group at risk of Mn overload and neurotoxicity from intravenously administered Mn because of their increased neuronal barrier permeability and developmentally lower basal biliary Mn excretion rate (15,16). The combination of these underlying physiological risks with the iatrogenic risk from routine but unnecessarily high Mn exposure in PN represents a very real health and safety concern. A recent prospective observational study in 58 neonates receiving routine PN containing a normal daily Mn dose found a positive correlation between brain deposition of Mn and PN Mn exposure during hospitalization (5). We and others have demonstrated that despite active omission of Mn from PN during compounding, Mn is still unintentionally present at a concentration capable of delivering a sufficient daily dose. Omitting Mn during PN compounding may, therefore, be a sensible approach in the clinical setting to reduce the potential health risks. Such an approach requires testing in a prospective clinical study with appropriate safety endpoints before it can be encouraged and adopted in clinical practice. Potential regulatory approaches to reduce risk include changing the formulation of the approved neonatal MTE product by eliminating the Mn content, and requiring manufacturers of PN ingredients to label their products with the Mn contamination concentration as they already are required in the United States to do for aluminum contamination (17).

In conclusion, neonatal parenteral nutrition contains a clinically significant supply of unintended manganese. Given the potential neurodevelopmental harms from excess manganese exposure during early life, further studies are needed to identify these Mn sources and the possible value of manganese omission strategies during PN compounding.

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