Brief communication

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Elemental impurities in injectable iron products for swine

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Summary

Elevated levels of arsenic, chromium and lead were detected in multiple injectable iron products following concurrent analysis by two laboratories. Only one product possessed concentrations of all three elements of concern that were undetectable or below the parenteral daily exposure limit for humans for each heavy metal, respectively.

Keywords: swine, elemental impurities, iron deficiency anemia

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Resumen – Impurezas elementales en los productos de hierro inyectable en cerdos

Se detectaron elevados niveles de arsénico, cromo y plomo en múltiples productos de hierro inyectables después análisis concurrentes en dos laboratorios. Solo un producto contenía concentraciones de los tres productos valorados que no fueron detectables o bajo los límites de exposición parenteral para humanos, para cada uno de los metales pesados, respectivamente.

Résumé – Éléments d'impuretés dans des produits de fer injectable pour les porcs

Des quantités élevées d'arsenic, de chrome et de plomb ont été détectées dans de multiples produits de fer injectable à la suite d'analyses simultanées effectuées dans deux laboratoires. Un seul produit possédait des concentrations de ces trois produits dans des quantités non-détectables ou sous la limite d'exposition quotidienne parentérale pour les humains pour chaque métal lourd, respectivement.

The use of injectable iron for the prevention of iron deficiency anemia is nearly an industry standard in swine production throughout the world. Since initial reports in the mid-twentieth century detailed a piglet's need for supplemental iron, 200-mg doses of injectable iron have routinely been given to every pig as per product label directions.[1-3] More recently, it has been shown that genetic improvements leading to larger litter sizes and rapid growth rates are resulting in piglets outgrowing their available iron stores prior to weaning, even when given an iron injection at birth.[4] Therefore, an additional 200-mg dose of iron prior to weaning has been shown to provide improved post-weaning growth performance.[5-7]

With an ever-increasing desire to produce a safe food supply, it is important to ensure that the products used in all phases of swine production are safe for use in food-producing animals. Though each of the iron products reported in this publication are approved for use in swine by the regulatory authorities in their respective countries, no inclusion limits for elemental impurities have been established for parenteral veterinary products. Guidelines are available, however, for human pharmaceutical products through guidance documents USP <232>[8] and ICH Q3D[9] which are being adopted as required standards for human drugs by many authorities including the United States and the European Union.[9]

The nature of the manufacturing process of pharmaceutical-grade iron dextran and gleptoferron requires one or more sources of raw materials, including elemental iron. Without appropriate quality control, it is reasonable that other elemental impurities may accompany iron in the raw material used during formulation. The purity of the final product will then depend on the steps employed to remove any such impurities. Additional quality and consistency challenges are presented by the fact that parenteral veterinary iron products are classified as non-biological complex drugs. Non-biological complex drugs are unique in that their structures cannot be fully characterized by physiochemical analysis and replication of the final active pharmaceutical product relies on specific and highly controlled manufacturing processes.[10] Altogether, this information indicates that adherence to high standards of manufacturing is paramount to creating a parenteral veterinary iron product that is safe, efficacious, and consistent. Therefore, the aim of the present project was to evaluate parenteral veterinary iron products for the presence of impurities that would be undesirable for intramuscular injection in food-producing animals.

Materials and methods

Sample submission

In total, 16 iron products from eight countries, each approved for the treatment of iron deficiency anemia in swine, were evaluated by the Toxicology and Nutrition Laboratory at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL). Fifteen of the 16 samples were also evaluated for the same analytes at an independent laboratory. Samples were submitted to each laboratory in their original unopened containers with the exception of the two products from China, which had been inspected by US customs during the shipping process, and the bottle stopper had been punctured. Prior to submission for testing, a random number was assigned to each vial using a random

SLR, SME: Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa.

CWO: Pharmacosmos Inc, Watchung, New Jersey.

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number sequence generator (**www.random.org**). The original product label was removed and a label with the assigned random identification number was adhered to each vial. All product-specific information was withheld from the laboratories until testing was complete.

Analysis of samples

Samples were analyzed for arsenic, cadmium, chromium, cobalt, lead and mercury using inductively coupled plasma mass spectrometry (ICP/MS; Analytik Jena Inc, Woburn, Massachusetts) at the ISU VDL. Analysis was performed with the ICP/MS in collisional reaction interface mode with hydrogen as the skimmer gas and the autosampler rinse solutions consisting of 1% nitric acid, 2% hydrochloric acid, and 4 ppm gold. Standards for elemental analyses were obtained from Inorganic Ventures (Christiansburg, Virginia) while digestion tubes, syringe filters, trace mineral grade nitric acid, and trace mineral grade hydrochloric acid were obtained from Fisher Scientific (Pittsburgh, Pennsylvania). Each sample was processed and analyzed following the established standard operating procedure for the fluid heavy metal panel.

To begin the analysis, samples were first digested in 70% nitric acid at 60°C for ≥ 1 hour. To do so, a 0.25-mL portion of each sample was transferred to a 15-mL centrifuge tube, and 0.25 mL of 70% nitric acid was added. All samples were digested for a minimum of 1 hour at 60°C. After digestion, all samples were diluted to 5 mL using 18MΩ water and vortexed to mix. Sample digests were then centrifuged for 5 minutes at 1900*g* and forced through 0.45-µm filter discs. Filtered samples were then diluted 1:500, 1:50, and 1:10 to accommodate the varying concentrations of the elements. To avoid carryover, the dilutions and the original digest were analyzed by ICP/MS from highest dilution to no dilution. Additionally, a blank sample of 1% nitric acid was analyzed between dilutions and 1% and 10% nitric acid was analyzed after each injectable solution sample. For quality control, certified reference materials were analyzed with each batch, additionally bismuth, indium, lithium, scandium, terbium and yttrium were used as internal standards for the ICP/MS.

Utilizing ICP/MS, a secondary analysis was performed by an independent laboratory following analysis at the ISU VDL. The limit of detection for arsenic, chromium and lead for both laboratories was 0.1 ppm.

Results

Results from the present study showed that a 200-mg injection of many of the iron products tested contained a concentration of one or more elemental impurities that exceed the permitted daily exposure (PDE) limit established for humans via parenteral exposure (Table 1). Only one injectable product, Uniferon (Pharmacosmos Inc, Watchung, New Jersey), was found to have non-detectable levels of both arsenic and lead, and was also the only product with chromium levels that would not exceed human PDE limits. The remaining elements included in the testing (cadmium, cobalt, and mercury), were either not detected, or were detected at levels well below PDE limits for humans and are therefore not reported. Briefly, the presence of chromium was detected in all the injectable iron products tested with eight (ISU VDL) and 11 (independent laboratory) products containing concentrations exceeding the human PDE by greater than 25%. Of the products with arsenic concentrations greater than 0.1 ppm, nine (ISU VDL) and eight (independent laboratory) samples exhibited concentrations exceeding the human PDE by greater than 25%. Likewise, of the products found to have detectable levels of lead, 10 (ISU VDL) and eight (independent laboratory) exhibited concentrations exceeding the human PDE by greater than 25%. Both laboratories agreed analytically on one iron product that possessed concentrations of arsenic, chromium and lead greater than the allowable PDE established for humans. In contrast, only one iron product was found to have concentrations of all three elements of concern that were undetectable or below the human PDE for each heavy metal, respectively.

Discussion

While injecting animals with products containing elemental impurities is potentially contrary to a practitioner's responsibility to "first, do no harm," there is currently no published data supporting the level of risk associated with injection of such impurities in swine. As a result, acceptable concentrations of elemental impurities such as arsenic, chromium and lead in animal drugs have not been established by the US Food and Drug Administration Center for Veterinary Medicine (CVM). It is therefore necessary for sponsors of veterinary drug products to apply risk-based control strategies for these impurities and to establish appropriate acceptance criteria. Thus, it is recommended that the USP <232>[8] and ICH Q3D[9] limits for elemental impurities, which are for humans, be used as a starting point for establishing a suitable limit for animal drug impurities. Adjustment of these limits may be justified following consideration of species and dosage; however, it remains the responsibility of the sponsor of a veterinary drug product to ensure that elemental impurities in the final drug are controlled within safe limits (AskCVM, oral communication; May 9, 2017).

For these reasons, the PDEs referenced and used as basis for comparison in this article are based on the ICH Q3D guidelines for maximum allowable levels of metal impurities when administering a drug to treat disease in humans.[9] Although these guidelines specify that exposures higher than the PDE may be acceptable in certain cases, such as intermittent treatment, the burden is on the manufacturer to demonstrate that it is acceptable in a given case. In this context, arsenic and lead merit particular scrutiny as they belong to the highest risk group as specified in the guidelines (Class 1 – highest degree of toxicity combined with reasonable risk of being found in pharmaceuticals).[9] Furthermore, the guidelines deal primarily with exposure in adult humans, whereas the use of injectable iron in piglets would correspond to use in infants. For these reasons, the human PDEs appear a reasonable starting point for evaluating whether certain levels of heavy metal impurities may be problematic for piglet health.

Generally speaking, the degrees of toxicity of arsenic and chromium are dependent on their respective valences. Arsenic(III) is more toxic than either As+5 or organic arsenic.[11] However, potential toxicological effects of arsenic can result in ataxia, paresis, and blindness following demyelination of nerves. The lethal oral dose of sodium arsenite, an inorganic arsenic, is approximately 200 mg/kg.[12] The more toxic and orally absorbable form of chromium is Cr+6, while Cr+3 is poorly absorbed orally and is considered less toxic. The maximum tolerable oral dose for Cr+3 in mammals, since Cr+6 is rarely ingested, is 100 mg/kg of more soluble forms of Cr+3.[13] Sperm motility of boars may potentially decrease with excess Cr+6 resulting in inhibited fertility.[14] Speciation of either arsenic or chromium contaminants within the injectable products to determine their potential toxicity could not be determined at the time of analysis. Swine are relatively resistant to lead toxicosis, but affected animals may exhibit tremors, seizures, and inappetance.[15]

Table 1: Detected content of arsenic, chromium and lead in parenteral iron products for swine, tested at two laboratories*

Brand name	Manufacturer†	Country	Molecule	Concentration (mg/mL)	Arsenic‡			Chromium			Lead‡		
					ISU	Lab 2	PDE¶	ISU	Lab 2	PDE¶	ISU	Lab 2	PDE¶
					µg/200mg dose§		µg/kg	µg/200mg dose§		µg/kg	µg/200mg dose§		µg/kg
Aspen Anem-X 100	Sparhawk	United States (USA)	ID	100	3.4	2.0		30.2	27.0		<0.1	<0.1	
Durvet Iron-100	Sparhawk	USA	ID	100	4.0	1.9		36.2	32.9		<0.1	<0.1	
Ecotin 200	Iven Laboratories	Spain	ID	200	0.2	0.4		36.0	49.5		4.9	5.8	
FerroForte	Bimeda	Canada	ID	200	1.7	1.6		12.0	35.0		0.3	<0.1	
Ferrohipra 200	Hipra	Belgium	Glep	200	<0.1	<0.1		25.0	24.1		2.0	1.1	
GleptoForte	Ceva	USA	Glep	200	0.9	<0.1		32.4	29.6		0.6	0.5	
Gleptosil	Ceva	Germany	Glep	200	2.2	1.2		21.0	28.9		<0.1	<0.1	
Gleptosil	Sogeval	United Kingdom	Glep	200	1.4	0.5		18.0	27.0		<0.1	<0.1	
Prolongal	Bayer	Belgium	Glep	200	<0.1	<0.1	0.3	33.2	28.9	22.0	2.6	0.6	0.1
Uniferon 200	Pharmacosmos	USA	ID	200	<0.1	<0.1		0.4	0.7		<0.1	<0.1	
Ursoferran	Serumwerk	Germany	Glep	200	<0.1	<0.1		25.0	36.0		0.4	0.4	
Ursoferran	Serumwerk	Russia	Glep	200	0.1	<0.1		19.0	21.9		1.1	1.2	
VetOne	Sparhawk	USA	ID	100	2.0	2.2		19.4	36.7		1.2	1.5	
Viloferron	iron4u	Denmark	Glep	200	<0.1	<0.1		29.0	29.0		3.1	0.3	
Xue Duo Bang	Guangxi Research Institute of Chemical Industry	China	ID	100	1.4	1.8		65.4	39.8		0.6	<0.1	
Xue Wei Bao**	Guangdong Wens Dahunong Biotechnology	China	ID	100	1.8	NA		28.6	NA		<0.1	NA	

* All values are rounded to the nearest one significant figure. Yellow highlighted cells indicate the element was present at ≤ 25% higher than the daily limits established for humans. Blue highlighted cells exceed the human daily exposure limit by > 25%.

† Marketing Authorization holder/NADA owner.

‡ Values reported as < 0.1 µg/200 mg dose were below the limit of detection for the assay.

§ For all 200 mg/mL products the reported elemental concentrations in µg/200 mg dose are equivalent to parts per million. For 100 mg/mL products, detected concentrations in parts per million were doubled to represent a typical 200 mg dose.

¶ Permitted daily exposure is the published daily exposure limit for an adult human. Values were converted to µg/kg assuming 50 kg as a conservative adult human body weight and using inclusion limits reported in USP < 232 >[8] and ICH Q3D[9] for human pharmaceutical products.

** Sample was not available for testing at both laboratories.

ISU = Iowa State University Veterinary Diagnostic Laboratory; Lab 2 = independent laboratory; PDE = permitted daily exposure; ID = Iron Dextran; Glep = Gleptoferron; NA = not applicable.

Although swine may be more resistant to arsenic and lead toxicity relative to other species, and diagnosed toxicities are uncommon, food safety must be taken into account and was not considered in establishing human PDE limits for these elements. Though we do not fully understand the potential risk(s) in swine associated with parenteral exposure to these impurities, the United States Department of Agriculture's Food Safety and Inspection Service currently does not allow for a detectable level of lead in any meat, while arsenic must be below 0.5 ppm in uncooked skeletal muscle tissue from swine.[12,16] Chromium, however, has a high volume of distribution with low accumulation in tissue, and therefore is not likely a toxicological concern for humans.[13] The combination of limited information and potential risk warrants that further research be done to determine the pharmacokinetics and tissue levels of heavy metal impurities subsequent to parenteral injection, or to expect that drug sponsors take steps to reduce or eliminate the level of impurities in parenteral products used for food-producing animals.

The present data shows that arsenic, chromium and lead can inadvertently be administered with iron injections to pigs depending on the product used. Because there is little information on the subject; in the absence of further investigation, practitioners and producers should consider taking steps to minimize the risk of any potential food safety, toxicological, or clinical impact(s) of parenteral administration of unintended heavy metals prior to the use of products containing such impurities.

Implications

- Under the conditions of this study, most of the 16 injectable iron products tested contain levels of arsenic, chromium or lead exceeding the human PDE for each respective impurity.
- Uniferon was the only product tested with undetectable levels of arsenic and lead while having a level of chromium lower than the human PDE.
- Given the limited knowledge of the properties of arsenic, chromium and lead when injected parenterally in swine, further research is warranted to fully characterize the consequences of exposure.
- Any potential risk associated with parenteral exposure of arsenic, chromium or lead to piglets can be avoided by using an injectable iron product with levels of these impurities below known human PDE limits.
- Manufacturers of injectable iron products for swine should take all steps necessary to ensure their product is void of any potentially harmful impurities, including heavy metals.

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Conflict of interest

Funding for analytical testing of iron injectable products was provided by Pharmacosmos. Pharmacosmos is the manufacturer and sponsor of Uniferon and co-author Chris Olsen is employed by Pharmacosmos.

Disclaimer

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