Personal View – Final

Cobalt chloride doping in racehorses: concerns over a potentially lethal practice

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Abstract

Recent reports from Australia suggest that cobalt chloride is being used in racehorses competing in New South Wales and Victoria. Although these are the first such reports, it is suspected that cobalt chloride may have been used illegally for some time. Cobalt chloride is a well-established hypoxia mimetic that is used experimentally to stabilise hypoxia-inducible factor 1 α (HIF-1 α) at the cellular level. It can induce hypoxia-like responses, increase blood erythropoietin (EPO) levels, promote erythropoiesis and enhance the oxygen carrying capacity of blood. However, prolonged administering large quantities of cobalt salts can lead to serious health problems such as cardiomyopathy. Supraphysiological doses of cobalt chloride may result in systemic cytotoxicity, myocardial dysfunction and acute inflammatory responses in the myocardium leading to cardiac arrest and death. In this paper we review the current evidence for the illicit use of cobalt chloride in racehorses. There are genuine concerns that non-veterinarians are sourcing misleading information from the internet as there are websites claiming that cobalt chloride can improve equine health and performance. It is the duty of veterinary surgeons working in the racing industry to ensure that owners, trainers and race officials are aware of the dangers of ‘amateur’ use of a potentially fatal compound.

(202 words in abstract)

Keywords: Cobalt chloride; Doping; Racehorse; Hypoxia mimetic; Erythropoietin

Introduction

Blood doping is an illegal and unacceptable way of enhancing athletic performance by increasing the oxygen carrying capacity of blood (Lippi et al., 2005). Currently used blood doping methods usually involve stimulation of erythropoiesis by using erythropoietin (EPO) or its recombinant form (Debeljak and Sytkowski, 2012). EPO is the hormone responsible for controlling erythropoiesis in bone marrow therefore erythropoiesis-stimulating agents (ESAs) and metal salts that can substitute for and simulate the erythropoietic actions of EPO have been used as potential performance-enhancing agents (Lippi et al., 2006; Duh et al., 2008). Although these agents may possibly have some physiological effects, there are significant risks associated with the illicit use of these substances in athletes (Franz, 2009).

The popular press frequently publishes revelations about the possible use of anabolic steroids and other banned substances in racehorses. The most recent report comes from Australia and involves the detection of cobalt chloride in racehorses competing in New South Wales and Victoria ¹. Although these convictions are the first in the country, it is suspected that cobalt chloride doping may have been practiced for some time.

Cobalt chloride is a well-established hypoxia mimetic and inducer of hypoxia-like responses, which can cause gene modulation at the hypoxia inducible factor pathway to stimulate EPO transcription and increase its levels in blood (Ho et al., 2014). Cobalt (symbol Co, atomic number 27) is a transition metal in the periodic table. In biological systems cobalt is at the active centre of coenzymes such as cobalamins, the most common example of which is vitamin B₁₂. Therefore, cobalt is an essential trace micronutrient that is important for the formation of the vitamin B₁₂ complex. As an activator of enzymes it is involved in the

¹ <http://www.theage.com.au/sport/horseracing/stewards-find-first-cobalt-chloride-irregularity-in-victoria-20141204-120dwm.html> (accessed 8 March 2015)

oxygen-carrying function of red blood cells and can replace the co-factor zinc in some enzymes. There are no published reports of cobalt dietary deficiency. A variety of foods including nuts, green leafy vegetables, fish and cereals contain cobalt and it unlikely for humans to develop dietary deficiencies.

Currently there is no evidence to suggest that cobalt chloride can enhance human or equine performance. The rationale for its use in racehorses is likely to be based on preclinical research done in cell lines and some anecdotal evidence from in vivo studies in laboratory animals, suggesting that cobalt chloride *may* have the same effect as EPO on erythropoiesis in bone marrow. Cobalt chloride is not a prescription medication and various cobalt salts are available for purchase from a variety of commercial sources. The salts are inexpensive, easily accessible, not subject to medicines regulation and orally active. Therefore, ill-informed and unscrupulous trainers can easily obtain cobalt chloride and administer it to horses. However, regulatory bodies have recently implemented a urinary threshold of 2000 ng/mL and a plasma threshold of 10 ng/mL for the control of cobalt abuse in non-race day or out-of-competition samples (200ug/l plasma in Australia and 100ug/l in Hong Kong) (Ho et al., 2014).

Aside from the lack of evidence for enhanced athletic performance in horses, one of the key concerns is the paucity of information about the long-term safety of cobalt chloride administration and toxicity, especially in vital organs. In the US there have been reports of unexplained deaths in horses that were found to have elevated blood levels of cobalt chloride. Although cobalt salts have medical applications for the treatment of anaemia (Bowie and Hurley, 1975; Duckham and Lee, 1976), cobalt can be highly toxic. Cobalt exerts well-known and well-documented neurotoxic effects (Catalani et al., 2012) in addition to its toxic actions on the thyroid, the heart and the haematopoietic system. High doses of cobalt in

patients exposed to abnormal levels from damaged hip prostheses induce optic and auditory neuropathy (Apostoli et al., 2013). Furthermore, there are reports that cobalt exposure may lead to fatal cardiomyopathy and ischemic heart disease in cobalt-exposed workers (Barborik and Dusek, 1972; Jarvis et al., 1992; Centeno et al., 1996) and in regular beer drinkers who have consumed beer from breweries with cobalt contamination (Alexander, 1972). It is also worth commenting that cobalt-drug interactions are unknown. Racehorses commonly receive NSAIDs and, in non-UK racing jurisdictions, can race on furosemide medication.

However, the lay public does not have access to detailed information about the potential risks and many trainers do not have the scientific knowledge to assess the risk: benefit ratio for the use of cobalt salts. Unfortunately, the Internet is a source of inaccurate, conflicting and misleading information about cobalt and its salts. This is the introductory text that describes uses of cobalt chloride in horses on the [eHow website](http://www.ehow.com/info_8740774_use-cobalt-chloride-equines.html)²:

'Cobalt chloride, also nicknamed blue salt by the horse and cattle community, is often associated with the dietary needs of cows. Cobalt chloride isn't only for cattle, however. Horses can also benefit from supplements of this essential electrolyte, as nontraditional as their consumption of it may be. Horse owners should use caution in dispensing cobalt chloride to avoid overdoses and unnecessary iodine intake, but there are usually few risks involved.'

² http://www.ehow.com/info_8740774_use-cobalt-chloride-equines.html (accessed 8 March 2015)

The author of this non-refereed article is Kirsty Ambrose, a regular contributor to www.ehow.com. She holds a Bachelor of Arts in English literature from the University of Victoria and enjoys writing about pet care. Her article is a top hit on Google (5th item in a Google search (article accessed 8 March 2015) using the keywords ‘cobalt’, ‘chloride’ and ‘equine’). This style of writing clearly gives readers the impression that providing cobalt chloride to horses can improve their overall health. The paper has not gone any kind of peer-review and the author does not cite any scientific or clinical papers to back up the claim that ‘Horses can also benefit from supplements of this essential electrolyte’. Clearly cobalt is not a conventional electrolyte. It is a micronutrient and research suggests that micronutrients can be toxic in high concentrations.

Medical uses of cobalt and cobalt chloride

It is important to highlight some of the medical uses of cobalt and cobalt chloride. Cobalt-60 (⁶⁰Co) is a radioactive form of cobalt used in radiotherapy for targeting inoperable tumours ³. The concept of ⁶⁰Co radiotherapy was developed in the 1950’s by scientists at the University of Saskatchewan in Canada (Johns et al., 1952; Morrison et al., 1952). Although cobalt therapy has partly been replaced by linear accelerator radiation therapy (the electron beam), which can generate higher energy radiation, cobalt treatment still has a useful role in radiotherapy. ⁶⁰Co is also one of the most commonly used radio-isotopes for food irradiation ⁴ (Deitch, 1982).

Cobalt salts have proved to be effective therapies for stimulating erythropoiesis in both non-renal and renal anaemia. Cobalt chloride has been effective for the management of uraemic patients with refractory anaemia, especially in patients undergoing long-term

³ <http://www.epa.gov/radiation/radionuclides/cobalt.html> (accessed 8 March 2015)

⁴ http://www.epa.gov/radiation/sources/food_irrad.html (accessed 8 March 2015)

haemodialysis (Bowie and Hurley, 1975; Duckham and Lee, 1976). Cobalt chloride stabilizes the transcriptional activator hypoxia-inducible factor 1 α (HIF-1 α) and thus mimics hypoxia thereby stimulating EPO production (Fig. 1). However, as with any type of drug, there are also serious medical adverse effects associated with long-term use, especially in high concentrations. Safe and effective use in the human medical field has been dependent upon accurate prescribing and diligent monitoring by clinicians for adverse reactions. The same mechanisms involved in HIF-1 α activation may potentially have genotoxic (De Boeck et al., 2003) and carcinogenic (Simonsen et al., 2012) effects, through cobalt mediated inhibition of DNA repair (Lison et al., 2001). Oral intake of inorganic cobalt salts can cause severe organ damage, especially by inducing toxicity in the gastrointestinal tract, the thyroid, the heart and the sensory systems (Ebert and Jelkmann, 2014). These undesirable side effects should deter professional equine trainers (and human athletes) from using cobalt chloride and other cobalt salts as ‘chemistry set chemicals’ for stimulating erythropoiesis.

Cobalt activation of matrix metalloproteinases

Cobalt intake can bear risks to health and the potential effectiveness of cobalt salts and other ESAs continue to be an open and important question for sport and athletic regulatory bodies. More research is needed on cobalt physiology and pathophysiology and more effective strategies are needed to unmask the potentially deleterious effects of cobalt salts in horses. *In vitro* studies have demonstrated that cobalt chloride can up-regulate matrix metalloproteinases 2 and 9 in equine laminar keratinocytes (Medina-Torres et al., 2011). Cobalt chloride also induces cytotoxicity and upregulates MMP-2 in ligament cells (Song et al., 2012; Wang et al., 2012). Horses are at risk of developing laminitis and it is possible that in certain conditions sustained hypoxia within the hoof and up-regulation of MMPs may cause irreparable damage to the lamellar basement membrane, increasing the risk of laminitis

(Medina-Torres et al., 2011). Damage to other load-bearing connective tissues is also potentially possible. These equine-specific risks are currently unquantified.

Conclusions

Paracelsus the ‘father’ of toxicology wrote: ‘*All things are poison and nothing (is) without poison; only the dose makes that a thing is no poison.*’ Clearly, it is the **dose** that makes a substance poisonous. To our knowledge studies to determine therapeutic vs. toxic dose of cobalt chloride in horses have not been published. The schematic in Fig. 2 summarises our concept of therapeutic and supraphysiological doses of hypoxia mimetics.

Cobalt chloride has pro-apoptotic and anti-apoptotic biphasic effects but these largely depend on the cell type studied and the dose used. Although low quantities of cobalt chloride may potentially stimulate erythropoiesis without any lethal effects, we do not have enough information about the long-term effects of exposing horses to cobalt salts. Cobalt poisoning can occur following exposure to large amounts of cobalt (Goldfrank, 2011). There are also genuine concerns about the purity of chemical grade salts that are currently available and their interactions with other drugs. The cobalt salts currently available are not pharmaceutical grade substances. High doses of impure cobalt chloride may be associated with significant toxicity. It is irresponsible and unethical to administer them to horses.

Therefore, it is important that we continue the development of technologies and assays to detect and control the misuse of cobalt in horses (Ho et al., 2014) and remain prepared to review and refine the urinary and plasma threshold concentrations based on emerging new evidence. If this disturbing trend continues and more unexplained deaths occur, we must focus new research on the effects of physiologically relevant concentrations

of cobalt chloride on global patterns of gene expression, protein function and cytotoxicity in primary equine cells and tissues in vitro, make predictions about its bioavailability and pathophysiological effects in vivo and develop sensitive biomarkers to examine its effects on the myocardium.

The development of new ESAs and the use of cobalt-based chemical agents capable of acting as hypoxia mimetics and EPO-stimulating agents highlight the need for developing new and sensitive analytical mass-spectrometry methods for detecting the abuse of these substances in human sport and horseracing (Reichel, 2011).

A recent study examined the pharmacokinetics and pharmacodynamics of cobalt following a single intravenous administration to 18 horses (Knych et al., 2014). The authors showed that a single intravenous dose of cobalt chloride or cobalt gluconate had no effect on EPO concentrations, red blood cell parameters or heart rate in any of the horses studied. However, this study did not examine the effects of multiple intravenous doses. We suspect that this may actually be happening in horseracing.

We are concerned that some trainers will continue to use Google as the source of reliable information. It is the duty of veterinary surgeons working in the racing industry to ensure that trainers are aware of the dangers of ‘amateur’ use of a potentially fatal compound.

Conflict of interest statement:

The authors do not have any conflicts of interest to declare. They do not have any financial, personal or other relationships with other people or organizations that could have influenced their work.

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218 **References**

219

220 Alexander, C.S., 1972, Cobalt-beer cardiomyopathy. A clinical and pathologic study of
 221 twenty-eight cases. The American Journal of Medicine 53, 395-417.

222

223 Apostoli, P., Catalani, S., Zaghini, A., Mariotti, A., Poliani, P.L., Vielmi, V., Semeraro, F.,
 224 Duse, S., Porzionato, A., Macchi, V., et al., 2013, High doses of cobalt induce optic
 225 and auditory neuropathy. Experimental and Toxicologic Pathology 65, 719-727.

226

227 Barborik, M., Dusek, J., 1972, Cardiomyopathy accompanying industrial cobalt exposure.
 228 British Heart Journal 34, 113-116.

229

230 Bowie, E.A., Hurley, P.J., 1975, Cobalt chloride in the treatment of refractory anaemia in
 231 patients undergoing long-term haemodialysis. Australian and New Zealand Journal of
 232 Medicine 5, 306-314.

233

234 Catalani, S., Rizzetti, M.C., Padovani, A., Apostoli, P., 2012, Neurotoxicity of cobalt. Human
 235 and Experimental Toxicology 31, 421-437.

236

237 Centeno, J.A., Pestaner, J.P., Mullick, F.G., Virmani, R., 1996, An analytical comparison of
 238 cobalt cardiomyopathy and idiopathic dilated cardiomyopathy. Biological Trace
 239 Element Research 55, 21-30.

240

241 De Boeck, M., Kirsch-Volders, M., Lison, D., 2003, Cobalt and antimony: Genotoxicity and
 242 carcinogenicity. Mutation Research 533, 135-152.

243

244 Debeljak, N., Sytkowski, A.J., 2012, Erythropoietin and erythropoiesis stimulating agents.
 245 Drug Testing and Analysis 4, 805-812.

246

- Deitch, J., 1982, Economics of food irradiation. *Critical Reviews in Food Science and Nutrition* 17, 307-334.
- Duckham, J.M., Lee, H.A., 1976, The treatment of refractory anaemia of chronic renal failure with cobalt chloride. *The Quarterly Journal of Medicine* 45, 277-294.
- Duh, M.S., Weiner, J.R., White, L.A., Lefebvre, P., Greenberg, P.E., 2008, Management of anaemia: A critical and systematic review of the cost effectiveness of erythropoiesis-stimulating agents. *Pharmacoeconomics* 26, 99-120.
- Ebert, B., Jelkmann, W., 2014, Intolerability of cobalt salt as erythropoietic agent. *Drug Testing and Analysis* 6, 185-189.
- Franz, S.E., 2009, Erythropoiesis-stimulating agents: Development, detection and dangers. *Drug Testing and Analysis* 1, 245-249.
- Goldfrank, L.R., 2011. *Toxicologic emergencies*, Ninth Ed. McGraw Hill. New York, NY, USA.
- Ho, E.N., Chan, G.H., Wan, T.S., Curl, P., Riggs, C.M., Hurley, M.J., Sykes, D., 2015, Controlling the misuse of cobalt in horses. *Drug Testing and Analysis* 7, 21-30.
- Jarvis, J.Q., Hammond, E., Meier, R., Robinson, C., 1992, Cobalt cardiomyopathy. A report of two cases from mineral assay laboratories and a review of the literature. *Journal of Occupational Medicine* 34, 620-626.
- Johns, H.E., Bates, L.M., Watson, T.A., 1952, 1000 Curie cobalt units for radiation therapy. I. The Saskatchewan cobalt 60 unit. *British Journal of Radiology* 25, 296-302.
- Knych, H.K., Arthur, R.M., Mitchell, M.M., Holser, I., Poppenga, R., Smith, L.L., Helm, M.N., Sams, R.A., Gaskill, C.L., 2014, Pharmacokinetics and selected pharmacodynamics of cobalt following a single intravenous administration to horses. *Drug Testing and Analysis*. doi: 10.1002/dta.1737.
- Lippi, G., Franchini, M., Guidi, G.C., 2005, Cobalt chloride administration in athletes: A new perspective in blood doping? *British Journal of Sports Medicine* 39, 872-873.
- Lippi, G., Franchini, M., Guidi, G.C., 2006, Blood doping by cobalt. Should we measure cobalt in athletes? *Journal of Occupational Medicine and Toxicology* 1, 18.
- Lison, D., De Boeck, M., Verougstraete, V., Kirsch-Volders, M., 2001, Update on the genotoxicity and carcinogenicity of cobalt compounds. *Occupational and Environmental Medicine* 58, 619-625.
- Medina-Torres, C.E., Mason, S.L., Floyd, R.V., Harris, P.A., Mobasher, A., 2011, Hypoxia and a hypoxia mimetic up-regulate matrix metalloproteinase 2 and 9 in equine laminar keratinocytes. *The Veterinary Journal* 190, e54-59.

- Morrison, A., Dixon, W.R., Garrett, C., Johns, H.E., Bates, L.M., Epp, E.R., Cormack, D.V., Fedoruk, S.O., 1952, Multicurie Cobalt 60 Units for Radiation Therapy. *Science* 115, 310-312.
- Reichel, C., 2011, Recent developments in doping testing for erythropoietin. *Analytical and Bioanalytical Chemistry* 401, 463-481.
- Semenza, G.L., 1999, Regulation of mammalian O₂ homeostasis by hypoxia-inducible factor 1. *Annual Review of Cell and Developmental Biology* 15, 551-578.
- Semenza, G.L., 2000, Expression of hypoxia-inducible factor 1: Mechanisms and consequences. *Biochemical Pharmacology* 59, 47-53.
- Sethi, G., Shanmugam, M.K., Ramachandran, L., Kumar, A.P., Tergaonkar, V., 2012, Multifaceted link between cancer and inflammation. *Bioscience Reports* 32, 1-15.
- Simonsen, L.O., Harbak, H., Bennekou, P., 2012, Cobalt metabolism and toxicology--a brief update. *The Science of the Total Environment* 432, 210-215.
- Song, Z.C., Zhou, W., Shu, R., Ni, J., 2012, Hypoxia induces apoptosis and autophagic cell death in human periodontal ligament cells through HIF-1 α pathway. *Cell Proliferation* 45, 239-248.
- Wang, Y., Tang, Z., Xue, R., Singh, G.K., Liu, W., Lv, Y., Yang, L., 2012, Differential response to CoCl₂-stimulated hypoxia on HIF-1 α , VEGF, and MMP-2 expression in ligament cells. *Molecular and Cellular Biochemistry* 360, 235-242.

Figure Legends

Fig. 1: Physiological and pathophysiological regulation of hypoxia-inducible factor 1 α (HIF-1 α) by hypoxia and cobalt chloride. HIF-1 α is a basic-helix-loop-helix transcription factor that activates expression of genes encoding erythropoietin (EPO), glucose transporters (GLUTs), glycolytic enzymes, vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs) and other genes whose protein products increase oxygen delivery, facilitate metabolic adaptation to hypoxia (Semenza, 1999, 2000; Sethi et al., 2012). Cobalt chloride acts as a hypoxia mimetic by activating the expression of genes that contain a hypoxia response element. Proteasomal pathways degrade HIF-1 α during normoxia but this transcription factor is stabilized under hypoxic conditions and in the presence of hypoxia mimetics such as cobalt chloride.

Fig 2: Schematic illustrating the potential effects of high and low doses of hypoxia mimetics such as cobalt chloride. (A) Low (therapeutic) doses of cobalt chloride may stimulate erythropoiesis and result in enhanced oxygen carrying capacity. (B) Supraphysiological doses of cobalt chloride may result in systemic cytotoxicity, myocardial dysfunction and acute inflammatory responses in the myocardium leading to cardiac arrest and death.