Toxicology Letters 255 (2016) 43–46

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Skeletal muscle lactate overproduction during metformin intoxication: An animal study with reverse microdialysis

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HIGHLIGHTS

- Lactic acidosis is a life-threatening complication of metformin use.
- It may be due to excessive inhibition of lactate clearance through liver gluconeogenesis.
- We infused high dose of metformin in gluteus muscle of pigs with reverse microdialysis.
- Dialysate (interstitial) lactate level and lactate-to-pyruvate ratio rose over time.
- Lactate overproduction can contribute to metformin-induced lactic acidosis.

ARTICLE INFO

Article history:
Received 7 March 2016
Received in revised form 2 May 2016
Accepted 9 May 2016
Available online 10 May 2016

Keywords:
Metformin
Diabetes
Lactic acidosis
Mitochondria
Microdialysis

ABSTRACT

Lactic acidosis during metformin intoxication is classically mainly attributed to diminished lactate *clearance* through liver gluconeogenesis. Here we studied 6 healthy, sedated and mechanically ventilated pigs to clarify whether high dose of metformin also increases skeletal muscle lactate *production*. Each animal had two microdialysis catheters inserted in gluteus muscles, one per side. One catheter was infused with saline (control) while the other one was infused with metformin diluted in saline (1 M), both at a rate of 0.3 μl/min. Dialysate lactate concentration and lactate-to-pyruvate ratio, a marker of the balance between anaerobic glycolysis and aerobic (mitochondrial) metabolism, were measured every 3 h, for 12 h. Continuous infusion of metformin caused a progressive rise in dialysate lactate level ($p = 0.007$) and lactate-to-pyruvate ratio ($p < 0.001$) compared to that of saline, as for mitochondrial "poisoning". These findings suggest that skeletal muscle lactate overproduction contributes to the development of metformin-induced lactic acidosis.

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1. Introduction

Metformin is the 7th most frequently prescribed generic drug in the USA (>76 million prescriptions in 2014) (Health, 2015). It is a safe compound, when correctly used (Salpeter et al., 2010). Nonetheless, metformin can cause lactic acidosis, especially when renal failure leads to its accidental accumulation (Vecchio et al., 2014). The pathogenesis of metformin-induced lactic acidosis remains controversial (Lalau and Race, 2001). Therapy consists of vital organ support and (extracorporeal) drug removal. Mortality approaches 25–50% (Kajbaf and Lalau, 2014).

Lactic acidosis occurs whenever lactate *production* exceeds lactate *clearance*. Normally, skeletal muscle is a major source of lactate production (though anaerobic glycolysis) whereas liver is largely responsible for lactate clearance (through gluconeogenesis)

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http://dx.doi.org/10.1016/j.toxlet.2016.05.010
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(Consoli et al., 1990). One common cause of lactic acidosis is tissue hypoxia with low aerobic (mitochondrial) energy production. Anaerobic glycolysis then accelerates to preserve cellular energy charge and avoid death. As a result of increased lactate production, lactic acidosis develops (Kraut and Madias, 2014).

At therapeutic dose, metformin diminishes liver gluconeogenesis (Madiraju et al., 2014) without affecting skeletal muscle lactate release (Bailey and Puah, 1986). This is the reason why lactic acidosis during metformin accumulation is classically mainly attributed to exaggerated inhibition of lactate clearance (Wang et al., 2003). However, at toxic dose, metformin can limit mitochondrial function in virtually every tissue, similarly to hypoxia (Protti et al., 2010, 2012a,b). Lactate overproduction in skeletal muscle and other organs may then contribute to the pathogenesis of the syndrome.

Reverse microdialysis can be used for running toxicological studies in vivo, with no systemic side effects (Höcht et al., 2007). A thin catheter, made up of an inlet tube, a semi-permeable (dialysis) membrane and an outlet tube, is inserted in a region of interest. Drugs are infused in the inlet tube, reach the dialysis membrane and diffuse into the surrounding extracellular space. At the same time, endogenous molecules move into the catheter according to their interstitial levels. Dialysate is retrieved through the outlet tube, collected in vials and then analysed. Its composition reflects that of the interstitium in close contact with the catheter, especially if the catheter is perfused very slowly (Hutchinson et al., 2015).

Aim of this study was to verify whether metformin intoxication increases skeletal muscle lactate production in vivo. To do so, we used reverse microdialysis in healthy pigs to infuse very high dose of metformin in skeletal muscle while monitoring dialysate (interstitial) lactate level.

2. Methods

This work was approved by the Italian Ministry of Health and complied with international recommendations (Institute of Laboratory Animal Resources, 1996).

We studied 6 healthy pigs, weighting approximately 20 kg, equipped with arterial, central venous and pulmonary artery catheters. Animals were sedated with propofol (80–100 mg/h, iv) and medetomidine (50 μg/h, iv), paralyzed with pancuronium bromide (8–10 mg/h, iv) and infused with saline (50 ml/h, iv). They were mechanically ventilated and externally warmed. Heart rate, mean arterial pressure, cardiac output (thermodilution), arterial and mixed or central venous blood gas analysis were recorded every 3–6 h.

Each pig had two microdialysis catheters inserted in gluteus muscles, one per side (CMA 60, M Dialysis AB; Stockholm, Sweden). The dialysis membrane at the tip of these catheters has a permeability cut-off of 20,000 Da. After 6 h of stabilization, one catheter was infused with saline and the other one with metformin hydrochloride (Sigma-Aldrich; St. Louis, MO, USA) diluted in saline (1 M), both at a rate of 0.3 μl/min (CMA 106). Of note, molecular weight of metformin is around 166 Da. Vials connected to the outlet tube were replaced, and dialysate lactate and pyruvate levels were measured (ISCUS^{flex}), every 3 h, for 12 h. Dialysate lactate-to-pyruvate ratio was calculated as marker of the balance between anaerobic glycolysis and aerobic (mitochondrial) metabolism (Hutchinson et al., 2015).

2.1. Histological analysis

Three animals were finally sacrificed (KCl 40 mEq, iv bolus, under deep sedation). Gluteus muscles infused with high dose of metformin were fixed in formalin and embedded in paraffin after removing microdialysis catheters. Serial 3-μm thick longitudinal sections were stained with haematoxylin and eosin. A pathologist not aware of the aim of the study examined them. The other three animals were assigned to other research protocols.

2.2. Statistical analysis

Data are reported as mean and standard deviation (SD). Normality of distribution was verified with Shapiro-Wilk test. Interaction between time and drug (saline *vs.* metformin) in affecting dialysate composition was assessed with two-way repeated measure (RM) analysis of variance (ANOVA). Temporal changes in physiological and laboratory variables were analysed with one-way RM ANOVA. P values <0.05 were considered statistically significant (SigmaPlot version 11.0, Jandel Scientific Software; San Jose, CA, USA).

3. Results

Cardiorespiratory variables were stable (no significant changes over time) and normal over the entire duration of experiments. On average, heart rate was 97 ± 25 beats per minute, mean arterial pressure was 92 ± 13 mmHg, cardiac output (measured in 5 animals) was 2.3 ± 0.5 l/min, arterial haemoglobin concentration was 9.0 ± 1.0 g/dl, arterial oxygen saturation was $99 \pm 1\%$ and mixed (5 animals) or central (1 animal) venous oxygen saturation was $71 \pm 8\%$. Arterial glucose concentration remained constant (4.2 ± 0.7 mM at the beginning and 4.2 ± 1.0 mM at the end of experiments) ($p = 0.630$) while arterial lactate level slightly diminished, rather than augmented, over time (from 0.6 ± 0.3 mM to 0.5 ± 0.2 mM) ($p = 0.038$).

On average, continuous infusion of metformin progressively increased dialysate lactate concentration ($p = 0.007$) and lactate-to-pyruvate ratio ($p < 0.001$) compared to that of saline (Figs. 1 and 2). Dialysate pyruvate level did not differ between infusions over time ($p = 0.641$).

Six healthy, sedated and paralyzed pigs had two microdialysis catheters inserted in gluteus muscles, one per side, and infused with saline (black symbols) or metformin diluted in saline (1 M) (white symbols). Vials connected to the outlet tube of the two catheters were simultaneously collected every 3 h, for 12 h, so that "saline" acted as control for "metformin". Lactate level at time "6 h" of one animal was not available. It was estimated as mean of values recorded at time "3 h" and "9 h" from that same animal (see also

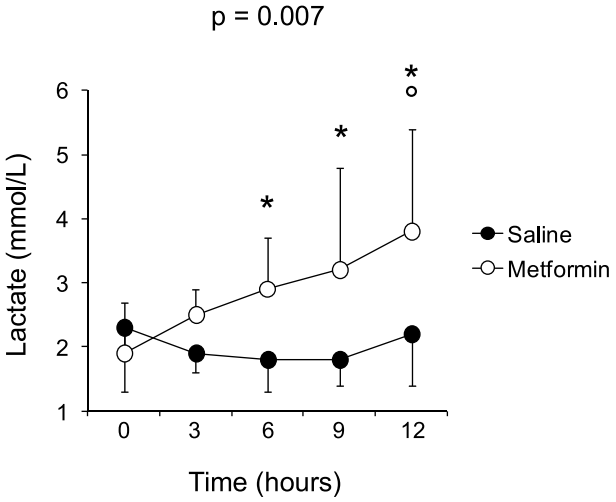

Fig. 1. Dialysate (skeletal muscle interstitial) lactate levels during continuous infusion of saline or metformin with reverse microdialysis.

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