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Short communication

Dichloroacetate Prevents Hypoxic Lactic Acidosis in Rats

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ABSTRACT

Lactic acidosis has different causes, including hypoxia. It is notably treated with dichloroacetate, which stimulates the activity of the pyruvate dehydrogenase enzyme complex in the mitochondrial inner membrane. However, there has been controversy over its efficiency in hypoxic lactic acidosis, particularly in the cerebral tissue and the cerebrospinal fluid. Assess the efficiency of dichloroacetate in the prevention of hypoxia-induced lactic acidosis. We used adult rats, 3 months old, with a weight of 250-300 grams. Anesthesia was achieved by intraperitoneal injection of pentobarbital (Nembutal®), at the dose of 50 mg/kg. For the induction of hypoxia, the rats were given to inspire a gas mixture containing 11% O₂ during 45 minutes. There were 20 rats in the dichloroacetate group and 20 in the control group. The dichloroacetate group rats were given dichloroacetate 300 mg/kg in slow IV injection before the induction of hypoxia. We measured lactate concentration in the blood and in the cerebrospinal fluid before the induction of hypoxia and at the end of 45 minutes of hypoxia, by spectrophotometry based on enzymatic principle. In normoxia, the lactate concentration was 1.84 ± 0.11 mmol/L in the blood and 2.00 ± 0.15 mmol/L in the cerebrospinal fluid. After 45 minutes of hypoxia, lactate concentration was 2.15 ± 0.36 mmol/L in the blood and 2.87 ± 0.16 mmol/L in the cerebrospinal fluid for the dichloroacetate group versus respectively 5.28 ± 0.91 mmol/L and 5.33 ± 0.58 mmol/L for the control group, $p < 0.01$. The study shows that Dichloroacetate is efficient for the prevention of hypoxic lactic acidosis.

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Key Words: hypoxia, lactic acidosis, prevention, dichloroacetate.

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INTRODUCTION

Lactic acid is a strong acid which is dissociated and the increase of its concentration provokes an increase in body fluids H^+ concentration and a decrease of pH, resulting in metabolic acidosis. Different conditions can lead to lactic acidosis, be it acute or chronic. A common clinical cause is congenital lactic acidosis; there is also acquired lactic acidosis. Due to metabolic acidosis, these conditions can become life-threatening and necessitate intensive treatment.

The lactate-lowering properties of dichloroacetate have been studied under experimental and clinical conditions (Stacpoole *et al*, 2003, Marangos *et al*, 1999). Dichloroacetate is used to prevent the rise in lactate level in different experimental designs in order to study two series of experiments, one with hyperlactatemia and another with normolactatemia. Dichloroacetate has notably been used to study cerebral metabolism, particularly the compartmentalization of glucose and lactate metabolism between astrocytes and neurons (Itoh *et al*, 2003). Such compartmentalization studies have also been carried out on the heart, using dichloroacetate (Lloyd *et al*, 2003). Dichloroacetate significantly decreases blood, cerebrospinal fluid and intracellular lactate concentrations. Clinical efficacy of dichloroacetate has been observed in congenital and acquired lactic acidosis (Stacpoole *et al*, 2003). It is also efficient in preventing exercise-induced hyperlactatemia in patients with mitochondrial defects (Duncan *et al*, 2004). However, due to its mode of action by increasing aerobic oxidation of glucose and lactate, there has been controversy over its efficiency in hypoxic lactic acidosis, particularly in the cerebral tissue and the cerebrospinal fluid, because in these conditions oxygen is lacking, so that enhancement of aerobic oxidation of lactate would not be effective. There have been controversial reports on this issue, better results being obtained in young animals (Miller *et al*, 1990, Corbett *et al*, 1998). In this context, we carried out a study on the efficiency of dichloroacetate in the prevention of hypoxic lactic acidosis.

MATERIALS AND METHODS

Adult WISTAR rats, three months old, weighing between 250 and 300 grams were used. Anesthesia was carried out with pentobarbital (Nembutal[®]), in intraperitoneal injection, at the dose of 50 mg/kg. A plastic cannula was introduced in the trachea and connected to a BioScience UK respiratory apparatus. The tidal volume was set to 3 mL and the respiratory rate to 50 per minute. There were 20 rats in the dichloroacetate group and 20 rats in the control group. The dichloroacetate group rats received 300 mg/kg of dichloroacetate in slow IV injection in the femoral vein during 10 minutes with a Harvard Apparatus infusion/withdrawal pump before induction of hypoxia.

The control group did not receive any pre-treatment. In both groups, hypoxia was induced with an inspired gas mixture containing 11% O_2 during 45 minutes. Samples of blood and cerebrospinal fluid, approximately 20 μ L, were taken before the induction of hypoxia and at the end of 45 minutes of hypoxia. Blood was sampled from the femoral artery, while cerebrospinal fluid was sampled from the cisterna magna (cisterna cerebellomedullaris).

Assays of lactate concentration in the blood and in the cerebrospinal fluid were carried out before the induction of hypoxia and at the end of 45 minutes of hypoxia in both rat groups. The lactate assay was based on enzymatic principle. L-lactate reacts with NAD^+ in presence of lactic dehydrogenase (LDH) at pH 9-9.6 to form pyruvate and $NADH + H^+$. The reaction is displaced to the right thanks to an excess of NAD^+ and the withdrawal of pyruvate due to its reaction with hydrazine. Lactate concentration was determined by spectrophotometric measurement of the $NADH$ formed, at the wave length of 340 nm, with a Zeiss spectrophotometer. Comparison of the mean increase in blood and cerebrospinal fluid lactate concentration in the dichloroacetate and the control group was done on the computer using the Student *t* test.

These experiments were carried out in the laboratory of normal and pathologic physiology, Ghent University, Belgium

Table 1.

Blood and cerebrospinal fluid lactate concentration in rats in normoxia and in hypoxia, dichloroacetate and control groups (mean \pm SD, in mmol/L).

	Normoxia	Hypoxia (dichloroacetate pretreatment)	Hypoxia (control group)	P
Blood	1.84 \pm 0.11	2.15 \pm 0.36	5.28 \pm 0.91	< 0.01
Cerebrospinal fluid	2.00 \pm 0.15	2.87 \pm 0.16	5.33 \pm 0.58	< 0.01

RESULTS

Results of blood and cerebrospinal lactate concentration in normoxemia and after 45 minutes of hypoxemia in dichloroacetate pre-treated rats and in control rats are presented in table 1.

DISCUSSION

Pretreatment with dichloroacetate in slow IV injection at the dose of 300 mg/kg is efficient in the prevention of hypoxic lactic acidosis. The efficiency of dichloroacetate makes it an efficient drug for laboratory purposes. It permits to block the rise in plasma lactate concentration during hypoxia, permitting to achieve different experimental conditions, when studying hypoxic conditions, with or without high plasma lactate concentration.

It is also a promising drug for clinical use, particularly for the treatment of acquired lactic acidosis that it rapidly and efficiently reduces. There are reports of efficacy of dichloroacetate in the treatment of lactic acidosis as a severe and lethal complication of different regimens of highly active antiretroviral therapy of HIV and AIDS (Shaer and Rastegar, 2000; Davies *et al*, 2001). Clinical trials are progressively proving efficacy of dichloroacetate in the treatment of congenital lactic acidosis in children (Barshop *et al*, 2004; Berendzen *et al*, 2006; Stacpoole *et al*, 2006). Dichloroacetate has the advantage of being a well-tolerated drug, even in these cases of chronic treatment. Lactic acidosis is also a complication of severe malaria; dichloroacetate has proven efficient in reducing lactic acidosis not only in experimental malaria (Rae *et al*, 2000), but also in African children (Maitland *et al*, 2005). Therefore, when lactic acidosis is recognized as a

complication of severe malaria, dichloroacetate can be used as an adjuvant therapy to the primary antimalarial drugs, which theoretically would reduce mortality (Krishna, 1997), but this effect still remains to be proven (Maitland *et al*, 2005).

In conclusion, dichloroacetate is efficient in preventing hypoxic lactic acidosis in experimental models, which makes it useful in many experimental designs when studying lactate metabolism and properties. This effect should be beneficial in clinical cases of lactic acidosis as a complication of highly active antiretroviral therapy or of severe malaria. Clinical trials are needed to determine guidelines for its use in these cases.

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