Investigations of D-lactate metabolism and the clinical signs of D-lactataemia in calves

I. LORENZ, A. GENTILE, W. KLEE

Five clinically healthy calves received an intravenous injection of 25 g sodium D-lactate (223 mmol) in 100 ml sterile water and five control calves were given the same volume of 0·9 per cent sodium chloride. Two clinical examiners who were blinded to the status (test or control) of the calves observed that between eight and 40 minutes after the injections the calves that had received sodium-D-lactate could be distinguished with certainty from the control calves on the basis of their clinical signs, for example, an impaired palpebral reflex, somnolence and a staggering gait. One-compartment and two-compartment analyses of the changes in the plasma concentration of D-lactate, and its renal clearance, indicated that the calves metabolised considerable amounts of D-lactate.

D-LACTIC acidosis has been described as a disease only in human beings and ruminants. In cattle it was first reported as a result of acute ruminal acidosis due to grain overload, a condition which is easily reproducible and has been the subject of many scientific studies. The results showed that although mammals have no specific D-lactate dehydrogenase ruminants are able to metabolise D-lactate more efficiently than had been assumed (Stangassinger 1977). More recently, ruminal acidosis of milk-fed calves has been investigated; a low pH in the reticulorumen of young calves can be induced by forced feeding (by tubing or drenching), a dysfunction of the reticular groove reflex, and by the reflux of abomasal contents. The fermentation of carbohydrates in the liquids in the rumen by bacteria produces the isomers of lactic acid, among other compounds (Dirr 1988). It has been shown that a marked D-lactic acidosis can be induced in calves with ruminal acidosis by the instillation of whole milk into the reticulorumen (Gentile and others 2002).

In calves with neonatal diarrhoea the intestinal losses of bicarbonate and the formation of L-lactate by anaerobic glycolysis as a result of tissue hypoperfusion have long been considered to be the main causes of metabolic acidosis. However, since Grude and others (1999) first reported high serum concentrations of D-lactate in calves with neonatal diarrhoea that did not have abnormal ruminal contents, further evidence has been obtained that hyper-D-lactataemia frequently occurs in diarrhoeic calves (Omole and others 2001, Lorenz 2002). In human beings, substantial amounts of D-lactic acid are produced after the resection of large portions of the small intestines, when undigested carbohydrates are transported into the large intestine. The similarity of the clinical symptoms of the so-called short-bowel syndrome (incoordination, ataxia, loss of memory, disorientation, headaches, slurred speech and disturbance of consciousness up to coma) described by Uribarri and others (1998) to the clinical signs observed in diarrhoeic calves with metabolic acidosis led to the assumption that these signs are influenced more by the concentration of D-lactate than by the degree of acidosis. In a study of calves with naturally acquired diarrhoea, Lorenz (2004) has shown that changes in behaviour, and particularly in posture, can be better explained by an increase in serum D-lactate concentration than by a decrease in base excess. The disturbance of the palpebral reflex appears to be due almost completely to high levels of D-lactate. In the present study all the calves had base excess values less than –10 mmol/litre, and it was the aim of the study to investigate whether clinical signs could be induced by hyper-D-lactataemia in the absence of acidosis. Previous studies of the metabolism of D-lactate in ruminants have used adult animals (Stangassinger 1977), and an additional objective was to investigate the ability of young calves to eliminate D-lactate from the blood.

MATERIALS AND METHODS

Ten clinically healthy, male Holstein-Friesian calves up to 14 days old were obtained from a local dealer; their farm of origin and history of colostrum intake were not considered. Their mean (sd) bodyweight was 42·7 (4·5) kg.

On each of five consecutive days, a pair of the calves were randomly assigned to be treated either with D-lactate or with normal saline. Once a calf had received D-lactate it was excluded from further trials. After the morning feeding of 1·5 litres of whole milk, the calves were weighed, and an intravenous catheter (FEP radio-opaque, non-pyrogenic, G 14 [2·0 x 70 mm]; Delta Med Medical Services) was inserted into a jugular vein, and a urine collecting bag (Urodress; Convatec, Bristol-Meyers Squibb) was fixed around the prepuce for the continuous collection of urine. The two calves were sampled simultaneously throughout the 24 hours of the study. One calf was given 25 g sodium-D-lactate (223·1 mmol) in 100 ml sterile water, and the control calf was given the same volume of 0·9 per cent sodium chloride; both solutions were warmed and injected intravenously within one minute. Blood samples were taken before the injection, and at two minute intervals for 10 minutes, at 10 minute intervals for 90 minutes, and two, four and 24 hours after the injection into vials containing a glycostatic anticoagulant containing EDTA and potassium fluoride. The intravenous catheter was rinsed by the aspiration and re-injection of blood continuously throughout the first 10 minutes, and then before each sampling. The blood samples were centrifuged immediately and the plasma was stored at –20°C. Blood gases were analysed before the injection, to check that the values were within the normal ranges, and 30 minutes after the injection, to assess any changes in acid-base status that might have been responsible for any clinical signs. Urine was collected after two, four and 24 hours. Before the injection, and at the end of sampling periods, urination was provoked by massaging the prepuce. The volume of urine collected was measured, it was cooled, well mixed and a sample was taken and stored at –20°C.

The calves were examined before the injection by two examiners to check that they were in good health, and at short intervals up to four hours. Special attention was paid to changes in posture, gait and behaviour, and to the palpebral and sucking reflexes. The examiners did not know whether the calves had received D-lactate or saline.

The concentrations of D- and L-lactate in plasma and urine were determined by enzymatic methods on an automatic analyser (Hitachi 705) three days after the last sampling. L-lactate was oxidised to pyruvate by the specific enzyme lactate oxidase (Diagnostic kit: Lactate, 1822837; Roche Diagnostics) and D-lactate was determined by using D-lactate dehydrogenase as described by Lorenz and others (2003).

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I. Lorenz, DrMedVet,
W. Klee, DrMedVet,
Clinic for Ruminants, University of Munich, Sonnenstraße 16, D-85764 Oberschleißheim, Germany
A. Gentile, Dr, Dipartimento Clinico Veterinario, University of Bologna, Bologna, Italy

TABLE 1: Mean (sd) plasma concentrations of D-lactate in five calves up to 24 hours after injection

Time of sampling (minutes)	D-lactate concentration (mmol/l)	Time of sampling (minutes)	D-lactate concentration (mmol/l)
Before injection	0·1 (0·07)	50	8·1 (0·9)
2	16·4 (2·0)	60	7·7 (1·0)
4	13·5 (1·9)	70	7·5 (1·1)
6	12·7 (1·6)	80	7·3 (1·1)
8	11·9 (1·5)	90	7·1 (0·8)
10	11·1 (1·7)	120	6·8 (0·6)
20	9·6 (1·0)	240	5·5 (0·6)
30	8·9 (1·0)	1440	0·7 (0·5)
40	8·5 (0·9)		

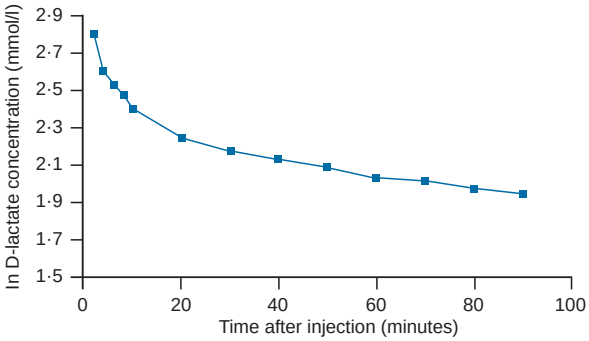

FIG 1: Natural logarithms of the mean plasma D-lactate concentrations in five calves after the injection of 223 mmol sodium D-lactate

The mean concentrations of D-lactate were subjected to one-compartment (Dost 1968) and two-compartment analysis (Greenblatt and Koch-Weser 1975). The renal clearance of D-lactate was calculated for the period from 120 to 240 minutes after the injection from the formula:

$$\text{Clearance} = \frac{U \times V}{P}$$

in which U is the concentration of D-lactate in the urine, V is the minute volume of urine, and P is the mean of the plasma concentrations of D-lactate at 120 minutes and 240 minutes.

The study was carried out under the supervision of the local 'Centralised Veterinary Service' for the Welfare of Experimental Animals at the University of Bologna and with the approval of the Ethical Review Committee of Animal Experimentation of the University of Bologna.

RESULTS

Table 1 gives the mean (sd) plasma concentrations of D-lactate at the different sampling times. There was no difference between the base excess values of the samples taken before the injection of D-lactate and 30 minutes later. There were no changes in the plasma concentrations of L-lactate throughout the investigation period, with values ranging from 0·15 to 1·19 mmol/l.

Fig 1 shows a semilogarithmic plot of the changes in the mean plasma concentrations of D-lactate. From 30 minutes after the injection the rate of decline was considered to be linear (r=0·99). The parameters derived from the one-compartment and two-compartment analyses are listed in Table 2. The renal clearance between 120 and 240 minutes after the injection was 27·5 ml/minute, much lower than the clearances derived from the one-compartment and two-compartment models.

Between eight and 40 minutes after the start of the injection, the calves that had received sodium-D-lactate could be distinguished with certainty from the control calves on the basis of their clinical signs. They had an impaired palpebral reflex; in all of them the eyes closed after a short delay and as if in slow motion, in one the eyes closed incompletely and in two there was a delay before their eyes reopened. Three of the calves were somnolent, and the other two appeared quiet and withdrawn. Four of the calves had a markedly staggering, 'drunken' gait, but the gait of the other was less severely affected. In four of the calves, long periods were recorded during which they stood motionless or slightly wavering or tottering with their heads lowered and their ears drooping. Four of the calves lay down more often and/or for longer periods than the control calves and had to be helped to rise, and four assumed unphysiological postures either while standing, for example, a sawhorse stance, or while lying down, for example, with one foreleg extended backwards parallel to the body, for long periods.

No impairment of the sucking reflex was observed in any of the calves. After four hours all the calves appeared clinically normal, and they drank their midday feed of 1·5 litres of whole milk readily.

TABLE 2: Parameters derived from the one-compartment and two-compartment analyses of the plasma D-lactate concentration curve

One-compartment		Two-compartment	
Slope	–0·00379	V1 (central compartment) (l)	12·3
Intercept with ordinate (mmol/l)	2·29	V2 (peripheral compartment) (l)	9·9
Theoretical volume of distribution (l)	22·6	Vd (l)	22·2
Half-life (minutes)	183	Clearance (ml/min/kg)	84·0
Total clearance (ml/min/kg)	85·6		

DISCUSSION

Although D-lactic acidosis has been known as a disease of ruminants (Dunlop and Hammond 1965) and human beings (Oh and others 1979) for decades, the mechanisms underlying the accompanying neurological signs are not understood. It has not even been determined whether D-lactic acid itself, or other substances produced under the same conditions, are toxic to the brain (Uribarri and others 1998). Under natural conditions the accumulation of D-lactate is usually accompanied by metabolic acidosis, and this may also contribute to the clinical signs.

Various attempts have been made to estimate the degree of metabolic acidosis in calves with neonatal diarrhoea on the basis of their clinical signs (Kasari and Naylor 1984, 1985, 1986, Naylor 1989, Geishauser and Thünker 1997, Wendel and others 2001), including changes in posture and/or behaviour. Recent research by Lorenz (2004) has provided strong evidence that even in calves with neonatal diarrhoea the neurological signs are triggered by the same mechanisms as in ruminal acidosis in cattle, or short-bowel syndrome in human beings. The results of the present study show that with the exception of any impairment of the sucking reflex, all the signs that have been attributed to metabolic acidosis can be reproduced by inducing hyper-D-lactataemia without acidosis. The plasma levels produced in this study were within the range of those observed in diarrhoeic calves (Lorenz 2004). The mechanism by which the signs may be produced is unknown, and the question is beyond the scope of this study. Cross and Callaway (1984) suggested that the effects of D-lactate may be caused by its interference with pyruvate metabolism.

In human beings it has been observed that the plasma concentration of D-lactate at which clinical signs occur is variable with a minimal reported value of 3·1 mmol/l (Uribarri and others 1998), and in the present study the intensity of the signs shown by the calves varied. Underlying deficiencies, for example, caused by malnutrition, have been suggested to explain this phenomenon (Hudson and others 1990). The calves were apparently adequately fed, but as the feeding regimen on their farm of origin was not known, minor deficiencies, for example, of vitamins, cannot be excluded.

When the first case of D-lactic acidosis was described in human beings (Oh and others 1979), the slow metabolism of D-lactate in mammals was considered to be the main factor responsible for its accumulation in the blood. At this time it had already been demonstrated that ruminants could metabolise D-lactate efficiently, although more slowly than L-lactate (Stangassinger 1977). It is now known that this is also true for human beings (Oh and others 1985). In ruminants and in human beings the rate of elimination of D-lactate from the blood decreases as its blood concentration increases (Stangassinger 1977). The concentrations of D-lactate induced in the calves in this experiment were higher than in any previous report of experimentally induced D-lactataemia, and it is therefore not surprising that the plasma half-life of D-lactate (183 minutes) was longer than that reported before (Stangassinger 1977). Nevertheless, the calves were able to eliminate considerable amounts of D-lactate from the blood. The mean value for its renal clearance was much lower than the glomerular filtration rate (Klee 1985), providing strong evidence of the tubular reabsorption of D-lactate. The fact that the total clearance and the clearance predicted by the two-compartment model were much greater than the renal clearance indicates that D-lactate was being metabolised by the calves. It is therefore uncertain whether the accumulation of D-lactate frequently observed in neonatal calves with diarrhoea (which in the study of Lorenz [2004] ranged up to 18·4 mmol/l) may be due only to its overproduction in the gastrointestinal tract, or may also be triggered by other factors. Ruminants lack D-lactate dehydrogenase, and the non-specific enzyme D-2-hydroxy acid dehydrogenase is considered to be responsible for D-lactate metabolism (Tubbs 1965, Cammack 1969). In vitro, the activity of this enzyme is diminished by a low pH (Tubbs 1965); the tendency of calves with diarrhoea to develop metabolic acidosis due to the loss of bicarbonate may therefore be one reason for the occurrence of hyper-D-lactataemia in these animals.

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