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The effect of administration of 2-iminobiotin at birth on growth rates, morbidity and mortality in piglets under farm conditions

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Abstract

It has since long been demonstrated that perinatal asphyxia in pigs can result in perinatal mortality, decreased viability/vitality at birth and reduced survival rates until 10 days of age. In human perinatology much interest is presently focussed on strategies to prevent adverse outcome arising from birth asphyxia. Given the rather high perinatal and postnatal losses in the pig industry, it is very interesting to explore rescue strategies to reduce adverse effects of postischemic organ damage caused by perinatal asphyxia also in newborn piglets.

The aim of this study was to determine the effects of postnatal treatment of piglets with 2-IminoBiotin (2-IB), a selective inhibitor of neuronal and inducible NitricOxideSynthase (nNOS and iNOS), under farm conditions on postnatal growth rates, morbidity and mortality.

In total 81 piglets from 15 litters were used. Immediately after birth, blood was collected from the umbilical artery and piglets were alternately assigned to either a control (saline) or drug (2-IB; 0.18 mg/kg bodyweight every 4 h during 24 h) group. Administration of control or drug started with an intravenous injection in the umbilical vein immediately after birth, followed by 6 intraperitoneal injections at 4 h intervals, starting 4 h after the intravenous injection. Piglets were checked for growth, morbidity and mortality until the experiment was finished (average age: 44 days).

Postnatal treatment with 2-IB resulted in significantly increased growth rates at 10 days of age ($P=0.02$) (165 g/day compared to 140 g/day in controls), independently of health status and birth weight. At weaning 2-IB treated piglets tended to show higher growth rates ($P=0.06$). Growth rates at the end of the experiment, morbidity and mortality were not affected by treatment.

It is hypothesized that 2-IB reduces the rather limited, nearly 'physiological' harmful effects occurring in the gastrointestinal tract, resulting from a short period of hypoxia-ischemia experienced during birth. This might explain the significantly increased growth rates at 10 days of life in 2-IB treated piglets. Selective inhibition of nNOS and iNOS might also result in increased availability of L-arginine for protein synthesis in newborns resulting in higher postnatal growth rates. However these issues need further investigations.

In conclusion, this study showed a positive effect of immediate postpartum administration of 2-IB during the first day after birth on growth rates up to 10 days of age. Furthermore, no negative effects of 2-IB treatment on piglet health and survival were found.

Keywords: 2-iminobiotin; Inducible Nitric Oxide Synthase; Neuronal Nitric Oxide Synthase; Perinatal asphyxia; Hypoxia-ischemia; Piglet; Growth rates; Morbidity; Mortality

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1. Introduction

Perinatal hypoxia-ischemia (birth asphyxia) can lead to actual loss of neonates but can also give rise to neurological deficits due to brain damage in surviving newborns (Vannucci and Perlman, 1997). Thus it is obvious that in human perinatology much research is focussed on strategies to prevent the severe, adverse outcome arising from perinatal hypoxia-ischemia. A similar approach might be of value in domestic animals.

The neuronal rescue strategies mentioned in literature are diverse and can grossly be divided into nonpharmacological (e.g. hypothermia, hyperglycaemia) and pharmacological intervention methods (Vannucci and Perlman, 1997; Volpe, 2001). Regarding the latter, in recent years the compound 2-IminoBiotin (2-IB) has been studied for its neuroprotective properties in perinatal hypoxia-ischemia (Nijboer et al., 2007; Peeters-Scholte et al., 2002a,b,c; van den Tweel et al., 2005). 2-IB is an inhibitor of both neuronal (constitutive) Nitric Oxide Synthase (nNOS) and inducible Nitric Oxide Synthase (iNOS), but not of endothelial Nitric Oxide Synthase (eNOS) (Sup et al., 1994). These enzymes catalyse the conversion of L-arginine to L-citrulline with concomitant formation of the free radical Nitric Oxide (NO) (Sup et al., 1994). Excessive production of NO by nNOS and iNOS after hypoxic–ischemic insults is considered to play a major role in neuronal injury (review by Samdani et al. (1997)).

Neuroprotective effects of 2-IB on both short- and long-term outcome have been demonstrated in studies performed in artificially asphyxiated newborn piglets and rat pups respectively (Peeters-Scholte et al., 2002b; van den Tweel et al., 2005). Besides using piglets as experimental model for human neonates, the pig itself can also be considered as a target animal as similar problems due to hypoxia-ischemia are encountered during the birth process. It has since long been demonstrated that perinatal asphyxia in newborn piglets, experienced in utero or during delivery under normal farm conditions, is a predominant cause of perinatal mortality (Randall, 1978). Additionally, perinatal asphyxia can result in birth of less viable piglets (Randall, 1971), reduced early postnatal vitality and decreased growth and survival rates until the age of 10 days (Herpin et al., 1996). To our knowledge, the currently applied on-farm pharmacotherapeutic intervention methods to reduce neonatal mortality are induction of farrowing with (a synthetic analogue of) prostaglandin F 2α and/or administration of oxytocin during farrowing. However, induction of farrowing will only reduce neonatal mortality when it is accompanied by intensive supervision (Holyoake et al., 1995). Furthermore, doses of oxytocin ranging from 0.111 to 0.167 IU/kg can also have adverse effects in that they induce higher mortality rates in newborn piglets compared to control animals (Mota-Rojas et al., 2002, 2005).

Regarding the above mentioned and the fact that in modern pig farming rather high perinatal mortality rates are still encountered (Leenhouwers et al., 1999; O'Reilly et al., 2006) it is relevant, both from an economic and animal welfare point of view, to study new compounds such as 2-IB in order to reduce neonatal mortality and possibly improve neonatal viability and vitality. Considering the fact that the limited information on short term, beneficial effects of 2-IB in the pig is available only from experimental, laboratory conditions, the aim of this study was to determine the effects of a postnatal treatment of piglets with 2-IB under farm conditions on postnatal growth rates, morbidity and mortality.

2. Materials and methods

Experiments were approved by the Ethical Committee of the Veterinary Faculty of Utrecht University (The Netherlands).

2.1. Animals and housing

A batch of 15 sows and gilts (all Dutch Landrace × Large White crossbred), accommodated at the experimental pig farm The Tolakker at the Faculty of Veterinary Medicine in Utrecht, was used in this study. Approximately 3 to 6 days before the expected date of farrowing, animals were transferred from the pregnant sow unit to individual farrowing crates in the farrowing unit. The average ambient temperature was maintained at about 20 °C. Pregnant sows were fed 1.15 to 1.75 kg of a commercial sow diet twice daily (07.30 and 16.00 h). Piglets were provided with a separate bedding consisting of wood-shavings, heated by a 150-Watt infrared lamp. As part of normal farm routine, sows were removed from the individual farrowing crates at an average litter age of 30 days (range 26–36 days) and the weaned litters remained in the pen.

2.2. Experimental procedures at birth

Animals were monitored for signs of an impending farrowing. All farrowings occurred spontaneously (not-induced). When the birth interval between two subsequent piglets exceeded a period of 2 h, vaginal exploration was performed and (if possible) a piglet

was manually extracted. Based on a clinical diagnosis of uterine inertia, on average 8 I.U. of oxytocin were administered intramuscularly to the sow (Oxytocine-s®, 10 I.U. oxytocin/ml, Intervet). In case of aggressive behaviour of the sow towards the piglets, 1 to 2 mg/kg azaperone (Stressnil®, 40 mg azaperone/ml, Janssen Pharmaceuticals) was administered intramuscularly. These intervention methods had to be applied during farrowing in three different sows, resulting in three manually extracted piglets, one piglet born after vaginal exploration only, four piglets born after vaginal exploration and oxytocin administration and three piglets born after azaperone administration. All these piglets were included in this study.

Immediately after birth of a liveborn piglet, a blood sample (0.3 to 0.8 ml) from the umbilical artery was collected in a heparinised syringe, regardless of the condition of the umbilical cord (intact or ruptured). Samples were preferably obtained in the period of apnea prior to the onset of regular respiratory movements. Due to practical limitations, 3 to 7 liveborn piglets per litter were used for the collection of data (i.e. not all piglets in a litter were used). Within 2 min after collection, blood samples were analysed with the iStat® Portable Clinical Analyser (i-Stat Europe, Birmingham, United Kingdom) for pH, pCO_2, HCO_3^- and BE_{ecf} (Base Excess of extra-cellular fluid) at a standard temperature of 37 °C (no corrections for body temperature were made). We previously demonstrated that the iStat® is a reliable analyser for acid-base balance values in newborn piglets (van Dijk et al., 2006).

Immediately after blood sampling, piglets were alternately assigned to either a control (saline) (0.9% NaCl) or a drug treated group. 2-IminoBiotin (2-IB) was dissolved in a mixture of saline (0.9% NaCl) and Phosphate Buffered Saline (Sigma Aldrich Chemicals, Zwijndrecht, The Netherlands) and administered in a dose of 0.18 mg per kg bodyweight every 4 h during 24 h. The dose of 2-IB was derived from experiments conducted in artificially asphyxiated piglets (Peeters-Scholte et al., 2002b).

Regardless of the treatment (control or drug), the first injection was given intravenously (IV) in the umbilical vein, and this was followed by 6 intraperitoneal (IP) injections at time intervals of 4 h, starting at 4 h after the intravenous injection.

Intact umbilical cords were not ruptured for and during blood sampling while restraining the piglet and injecting the control or drug dose into the umbilical vein.

IP injections were performed in the ventral wall, placing the needle perpendicular to the abdomen just between the second teat (counted from caudal) and the linea alba while the piglet was suspended on its hind legs. Piglets treated with either control or drug were ear notched to enable individual follow-up observations.

As every other experimental piglet was assigned to the drug group, treatment of the last experimental piglet from a sow determined the treatment (control or drug) of the first experimental piglet in the following sow. At least one control and one drug treated piglet were present per sow (maximal four control or drug treated piglets per sow).

Furthermore, the following data were collected at birth for each control and drug treated piglet: preceding birth interval (time elapsed since birth of the previous (live- or stillborn) piglet), position in the birth order (and the related variable rank, defined as (position in birth order − 1)/(total number born − 1)), presentation at birth (anterior or posterior), condition of the umbilical cord directly after expulsion (intact or ruptured), sex and birth weight. Mummies were not included in litter size or calculation of birth intervals. When a piglet was born inside the fetal membranes, these were ruptured immediately in order to obtain a blood sample from the umbilical artery. Weak piglets were not assisted in establishing respiration and attempts to suckle.

For each sow, breed, parity (parity one: gilts giving birth to their first litter), litter size and when available total duration of the expulsive stage of farrowing (time in minutes between birth of the first and birth of the last piglet in a litter) were recorded. Litter size included both live- and stillborn piglets (mummified piglets were excluded). Piglets showing no heartbeats at birth (determined by palpation of the ictus cordis) or only showing irregular slow heartbeats, which ceased within a few minutes after birth, were classified as stillborn.

2.3. Follow-up

Ear notched piglets were never cross-fostered to another sow. According to farm routine, the male piglets were castrated at day three after birth and all piglets were given 1 cc Duphafral Ferrodextran® B12 (100 mg ferrodextrane and 30 μg cyaonocobolamine per ml, Fort Dodge) and were tail docked.

Piglets were monitored daily for health status (diarrhoea, lameness and/or clinical signs indicating an infection of the respiratory tract) and mortality rates were established until the end of the experiment, at an average age of 44 days (range: 41 to 46 days). Sows were also checked daily for health status, including measurement of rectal temperature (twice daily until the third day after farrowing) and feed and water intake until weaning.

Table 1
Parity, litter size, duration of farrowing, piglet birth weights per litter, individual birth intervals and rank for litters and piglets involved in the study

Variable	*N*	Mean (S.D.)	Range (min–max)
Parity [a]	15	5.2 (3.5)	1–14
Tnb [b]	15	13.3 (3.1)	8–19
Nba [c]	15	11.0 (1.9)	8–14
Nbd [d]	15	2.3 (2.7)	0–8
Duration of farrowing (min) [e]	10	209 (118)	100–449
Piglet birth weight per litter (g)	15	1492 (228)	1095–2030
Individual birth interval (min) [f]	77	21 (23)	0–101
Rank [g]	81	0.48 (0.28)	0.06–1.00

[a] Parity = parity of the sow.
[b] Tnb = total number born per litter.
[c] Nba = number born alive per litter.
[d] Nbd = number born death per litter.
[e] Duration of farrowing = duration of the expulsive stage of farrowing (time interval between birth of first and last piglet in a litter). In five litters time of birth of the first piglet(s) was not available, therefore these litters were not used for the calculation of this parameter.
[f] Individual birth interval = time between birth of two subsequent (live- or stillborn) piglets in minutes.
[g] Rank = relative position in the birth order defined as (position in birth order − 1)/(total number born − 1).

Individual weights of the control and drug treated piglets and mean piglet weights per litter were determined at birth, at the age of 10 days and at weaning. Control and drug treated piglets were also weighed at the end of the experiment.

All control and drug treated piglets that died during this experiment were sent to the Department of Pathobiology (Faculty of Veterinary Medicine, Utrecht) for general pathological and histological examination. Furthermore, selected piglets from the control and drug group were euthanised with T61® (200 mg embutramide, 50 mg mebezoniumjodide, and 5 mg tetracaïnehydrochloride per ml, Intervet) at the end of the experiment for general pathological examination and histology. These latter piglets were pair-wise selected according to the following criteria: one drug and one control piglet, both piglets originating from the same litter with nearest similar acid-base values (pH, pCO_2, HCO_3^- and BE_{ecf}) at birth, same sex and nearest similar body weights (g) at birth.

2.4. Statistical analysis

All data have been analysed with SAS (SAS/STAT, 1990). The mixed procedure was used to analyse the variables affecting variability in growth at the age of ten days ((piglet weight at 10 days − birth weight)/10) (GRT), growth at weaning ((piglet weight at weaning − birth weight)/(age at weaning in days)) (GRW), and growth at the end of the experiment ((piglet weight at end of experiment − birth weight)/(age at end of experiment in days)) (GRE).

The following model was used to analyse the different growth rates: $y_{ijk} = \mu + \text{group}_i + \text{health}_j + \text{birth weight}_k + e_{ijk}$.

Table 2
Results of the model analysing factors affecting growth rates of piglets at 10 days (GRT), at weaning (GRW) and at the end of the experiment (GRE)

Class variable	GRT (*n*=74)		GRW (*n*=70)		GRE (*n*=70)	
	P-value	LSmeans	*P*-value	LSmeans	*P*-value	LSmeans
Group [a]	0.0224	–	0.0647	–	0.1013	–
C	–	140.43	–	189.65	–	212.39
D	–	165.05	–	209.30	–	228.47
Health [b]	0.1189	–	0.0496	–	0.1620	–
0	–	216.43	–	257.47	–	270.13
1	–	146.70	–	190.43	–	197.74
2	–	192.46	–	185.50	–	225.62
3	–	112.03	–	–	–	–
4	–	96.08	–	164.51	–	188.22
Continuous variable	**GRT (*n*=74)**		**GRW (*n*=70)**		**GRE (*n*=70)**	
	P-value	Estimate [c]	*P*-value	Estimate	*P*-value	Estimate
Birth weight (g)	<0.0001	0.08697	0.0002	0.08378	<0.0001	0.08626

[a] Group = treatment group (C = control; D = 2-IminoBiotin).
[b] Health = health status during experiment (0 = healthy; 1 = diarrhoea; 2 = lameness; 3 = clinical signs indicating a respiratory infection; 4 = diarrhoea and lameness).
[c] Estimate = regression coefficient.

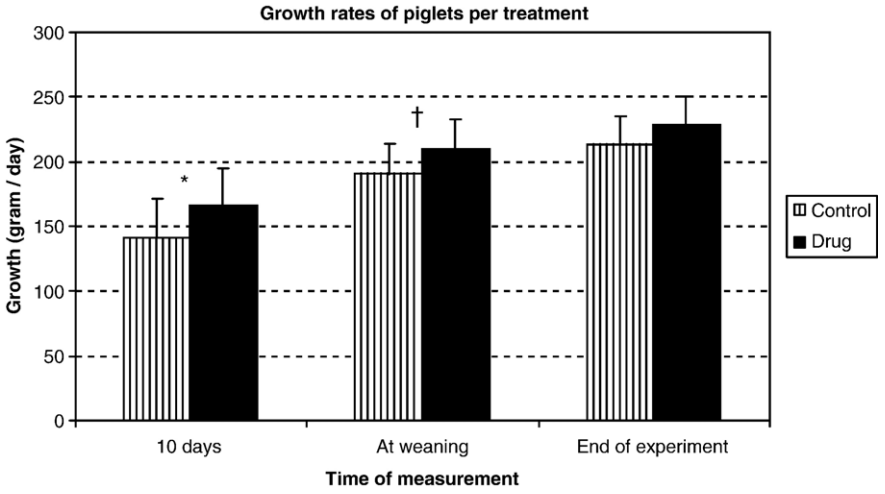

Fig. 1. LSmeans (SEM) for growth rates at 10 days, at weaning and at the end of the experiment for control and drug treated piglets are shown. *Significant differences (P-value ≤ 0.05); † tendency for significant differences ($0.05 < P$-value < 0.1).

Corrections for the class variables sow and parity and the continuous variable litter size were included as random effects. Parity was divided into two classes (class 1 = parity one and two; class 2 = parity 3 and higher).

The following class variables were included as fixed effects: group and health. Group refers to either saline (control; C) or 2-IB (drug; D) administration. Health refers to the health status of the piglets involved in the experiment: healthy throughout the experiment (0), diarrhoea (1), lameness (2), clinical signs indicating respiratory infection (3) and class 4 represents the combination of class 1 and 2. The continuous variable birth weight (in g) was included as a fixed effect.

To analyse the variables affecting variability in survival of piglets until the end of the experiment, the age of the piglets was analysed with the same mixed model as used for the analysis of growth. For piglets surviving throughout the experiments, age was set at 47 days (i.e. when all observations were stopped).

P-values equal to or less than 0.05 were considered to be significant. When a class variable was significant, multiple comparisons were performed with adjustments according to Tukey–Kramer (pdiff in the mixed procedure of the SAS system).

3. Results

3.1. Descriptive results

In total 81 piglets originating from 15 litters were used. Mean parity, litter size, duration of the expulsive stage of farrowing, piglet birth weights per litter, individual birth intervals and rank are shown in Table 1. Daily monitoring of sows revealed no abnormalities with the exception of one sow that showed a high rectal temperature (>40 °C) for only one day within 48 h after parturition. However, feed intake of this animal was not affected.

Mean values for acid-base balance parameters at birth (pH, pCO_2, HCO_3^- and BE_{ecf}), individual birth intervals, rank and birth weight of control and drug treated piglets did not differ significantly between both groups. 55% of the control and 49% of the drug treated piglets were born in anterior presentation. Furthermore, 6 piglets (15%) of the control group and 3 piglets (7%) of the drug group were born with a ruptured umbilical cord.

No side effects of the intravenous and/or intraperitoneal injections were observed in the newborn piglets throughout the experiment.

Table 3
Health status of the control and drug treated piglets

Class variables	Control	Drug
Health [a]		
0	35	33
1	4	2
2	1	4
3	–	1
4	–	1
Total	40	41

Variables did not differ significantly between control and drug treated piglets.

[a] Health = health status during experiment (0 = healthy; 1 = diarrhoea; 2 = lameness; 3 = clinical signs indicating a respiratory infection; 4 = diarrhoea and lameness).

3.2. Postnatal growth, health and survival

Table 2 and Fig. 1 demonstrate that drug treated piglets showed significantly higher growth rates at 10 days of age in comparison to the control piglets, independently of health status and birth weight ($P=0.02$). Furthermore, at weaning a tendency was found for treatment to affect growth rates, with drug treated piglets still showing higher growth rates than control treated piglets ($P=0.06$). However, at the end of the experiment, no significant differences between growth rates of either control or drug treated piglets were observed.

Besides, increasing birth weights resulted in a significant increase of all three growth rates (see Table 2; $P<0.001$). Health status only affected growth rates significantly at weaning (see Table 2). No interaction between group (treatment) and health status on growth rates was observed (data not shown).

Table 3 shows the health status of control and drug treated piglets. Overall preweaning mortality in the experimental litters included in this study averaged 1.7 piglets (15%) per litter (S.D. 1.345; range 0–5), with 73% of deaths occurring within the first three days of life. Regarding effects of group (treatment) on survival rates of the experimental piglets, no significant effect was demonstrated throughout the experimental period. In 7 litters, one or two experimental piglets died, resulting in loss of 7 (17.5%) control and 4 (9.8%) drug treated piglets. Age, weight and cause of death (as diagnosed by postmortem autopsy) of these piglets are presented in Table 4. In the control group, piglet losses were predominantly observed after three days of life whereas in the drug group three out of four piglets died within the first three days of life (see also Table 4).

General pathological and histological examination of the pair-wise selected piglets that were euthanised at the end of the experiment, revealed no abnormalities for both control and drug treated piglets.

Table 4
Overview of control and drug treated piglets that died during the experiment

Cause of death [a]	Control piglets		Drug treated piglets	
	Age [b]	Weight [c]	Age	Weight
Dehydration	21	1620	1	1700
	–	–	14	2790
Septicemia	13	2895	–	–
	27	4750	–	–
Trauma	1	1480	1	735
	2	905	1	1305
Unknown [d]	4	790	–	–
	8	1835	–	–
Total number of piglets:	7		4	

[a] Cause of death as diagnosed by the Department of Pathobiology after general pathological examination and histology.
[b] Age (in days) of the piglets.
[c] Weight of the dead piglets (g).
[d] These two piglets were not sent to pathology.

4. Discussion

The study presented here is the first to demonstrate a significant increase in growth rates at 10 days of age after administration of 2-IminoBiotin (2-IB) at birth in piglets housed under normal, commercial farm conditions.

The stillbirth rate (17% of the total number of piglets born) observed in our study is high compared to figures recently reported by others, which ranged from 7.6 to 12.6% (Leenhouwers et al., 2003; Lucia et al., 2002). Two sows gave birth to 7, respectively 8 stillborn piglets as a result of a prolonged duration of the expulsive stage of farrowing and this might well explain this increased stillbirth rate. When these sows were excluded, stillbirth rates averaged 12%.

Peeters-Scholte et al. (2002b) studied the possible neuroprotective effects of 2-IB administration in 1 to 3 day old piglets after 60 min of experimentally induced cerebral hypoxia-ischemia (induced by occluding both common carotid arteries and reducing the fraction of inspired oxygen). At 24 h after the hypoxic–ischemic insult, 2-IB treated piglets showed a significantly improved cerebral energy state, strong reductions in vasogenic edema and a significant reduction in tyrosine nitration in the cerebral cortex in comparison to control piglets (Peeters-Scholte et al., 2002b). Long-term neuroprotective effects of 2-IB up to 6 weeks after hypoxia-ischemia were demonstrated by van den Tweel et al. (2005) in a neonatal rat pup model of hypoxia-ischemia.

It should be emphasised that data in the present study were in general obtained from normoxic piglets. The acid-base balance values at birth show that only one piglet may have experienced a (severe) mixed respiratory-metabolic acidosis (pH 7.095; pCO_2 9.48 kPa; BE_{ecf} −8 mmol/l) at birth according to the definition of Herpin et al. (1996), while all other piglets did not suffer from a (more or less) severe degree of asphyxia at birth. We previously demonstrated that an increasing position in the birth order is accompanied by a more pronounced degree of mixed respiratory-metabolic acidosis in liveborn piglets at birth (van Dijk et al., 2006). Because the majority of the piglets (75%) involved in the present study was born in the first and second one-third part of the litter, it is not surprising that only few, really acidotic newborn piglets were present.

Nevertheless, all newborn piglets experience a transient period of decreased pH and increased pCO_2 values immediately following birth and this is likely to arise from a short period of fetal hypoxia during birth (Randall, 1972). This implies that already before the occurrence of a (more or less) severe degree of hypoxia-ischemia, piglets suffer from a mild degree of hypoxia, associated with redistribution of circulation to vital organs as brain and heart (Randall, 1992) during birth. Silver et al. (1988) demonstrated significant differences in oxygen content in fetal carotid and femoral arteries during late pregnancy, indicating preferential oxygen supply to the anterior body parts. Similar results were found during hypoxia (Silver et al., 1988) and altogether this might render the neonatal gastrointestinal tract more susceptible to mild hypoxia.

Moreover, the target enzymes for 2-IB have been shown to be present in type VI myenteric neurons of the small intestines of newborn piglets (nNOS) (Brehmer et al., 2004) and in both the muscularis and mucosa from duodenum up to colon in 3 to 7 days old piglets (nNOS and iNOS) (Di Lorenzo and Krantis, 2001; Gookin et al., 2006). Therefore, 2-IB might ameliorate the (rather limited, almost physiological) adverse effects occurring at the level of the gastrointestinal tract resulting from a short period of hypoxia and ischemia experienced during birth, thereby improving neonatal growth rates (independently of birth weight and health status).

Considering the effects of 2-IB treatment on growth rates, it is also important to mention that selective inhibition of nNOS and iNOS might increase the availability of the essential amino acid L-arginine for protein synthesis in newborn piglets, thus enabling higher neonatal growth rates (Flynn et al., 2000; Wu et al., 2004). However, considering the complex role of L-arginine, L-citrulline and NOS in metabolism (Wu and Morris, 1998), this issue clearly needs further investigation.

Apparently the control treated piglets were able to compensate for their lower, early neonatal growth rates at a later age, as at weaning only a tendency was found for 2-IB treated piglets to show higher growth rates. At approximately 6 weeks of age no differences in growth rates were present anymore. The relative short half-life of 2-IB, resulting in short-term effects of 2-IB, might also have been responsible for this latter observation. Unfortunately, no kinetic parameters of 2-IB are presently known, therefore it remains unclear for how long pharmacologically active levels of 2-IB are present in piglets after a 24 h period of administration as conducted in this study. As both control and drug treated piglets received the same amount of fluid per kg bodyweight, a possible positive effect of fluid administration itself on growth rates can be precluded.

The absence of significant differences in growth rates at weaning and at the end of the experiment might also be attributed to the relative limited number of piglets involved.

Recently, Nijboer et al. (2007) questioned the suggested mode of action of 2-IB. Even with high doses of 2-IB they could not demonstrate a significant inhibition of Nitric Oxide production in vitro, using SK–N–SH neuroblastoma cells and peritoneal cells (mainly macrophages) derived from female rats. However, administration of a nonselective inhibitor of NOS (L-NMMA) and a selective inhibitor of iNOS (aminoguanidine) significantly reduced NO production by respectively nNOS in SK–N–SH neuroblastoma cells and iNOS in the rat peritoneal cells. Furthermore they reported a gender specific effect of 2-IB after hypoxia-ischemia in that only female rat pups experienced neuroprotection, possibly by preventing an increase in cytosolic cytochrome *c* and decreased levels of cleaved caspase 3 (Nijboer et al., 2007). The previously mentioned neuroprotective effects obtained in newborn piglets by Peeters-Scholte et al. (2002b) were also reassessed and an identical gender specific effect was present (Nijboer et al., 2007). Our data, however, revealed no interaction between treatment (control or drug) and sex (data not shown).

Health status of the piglets (see Table 3) was not significantly affected by 2-IB treatment, but a slightly lower number of piglets with diarrhoea and a slightly higher number of lame piglets were observed in the 2-IB group compared to the control group. However, in both treatment groups only one diseased piglet died.

Although no significant effect of 2-IB treatment on survival was demonstrated in our study, it is remarkable that preweaning mortality averaged 9.8% in the 2-IB group and 17.5% in the control group respectively. Another striking contrast was the finding that 75% of preweaning mortality in the 2-IB treated group occurred in the first three days of life, compared to 29% in the control group. No clear explanation for this phenomenon is available yet and future research should include larger numbers of animals to elucidate possible effects of 2-IB on postnatal morbidity and mortality more precisely.

In conclusion, this study showed a positive effect of administration of 2-IB during the first day after birth on neonatal growth rates up to the age of 10 days. Furthermore, no negative effect of 2-IB treatment on health and survival of piglets was demonstrated and this was also confirmed by the lack of pathological and histological abnormalities in healthy 2-IB treated piglets at the end of the observation period. Future research should increase the numbers of piglets involved to verify the effects of 2-IB treatment on growth rates at

weaning and at the end of the experimental period. Furthermore, asphyxiated piglets should be included in the treatment trial to evaluate possible beneficial effects of 2-IB treatment on postnatal growth and survival in asphyxiated piglets under farm conditions.

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