EFFECTS OF DEXAMETHASONE ON PHYSICAL PROPERTIES AND MINERAL DENSITY OF LONG BONES IN PIGLETS

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Abstract

The study was conducted to test the effect of dexamethasone introduced intramuscularly on volumetric mineral density and physical properties of the femur and humerus in piglets during 14 d of their neonatal life. The effective duration of dexamethasone action lasted together 38 d because sows were treated with dexamethasone during the last 24 d before farrowing. Control piglets were treated with physiological saline administered in the same way and amount. On the 14th d of life the piglets were euthanised and humeri and femora were isolated and frozen at –25°C until further analyses. Using quantitative computed tomography (QCT) method, volumetric bone density of the cortical and trabecular bone were estimated. Moreover, selected geometric and mechanical properties of the femur and humerus were determined. The obtained results indicate that the administration of dexamethasone in both prenatal and neonatal life of the piglets decreased volumetric bone density and mechanical and geometric properties of their bones.

Key words: piglets, dexamethasone, perinatal period, bone properties.

The process of neonatal bone formation is mediated by different factors and cytokines which play a fundamental role in the differentiation of bone marrow osteoprogenitor cells and ossification. The administration of dexamethasone as a potential glucocorticoid has direct and indirect effect on these processes (1, 6, 9). It inhibits osteoblasts function and finally decreases synthesis of collagen and extracellular matrix (1). Glucocorticoids play an important role in general growth and maintenance of bone mass in the skeleton. Steroid therapy induces bone loss mediated by the influence on the transcription of some regulatory factors which determine the ratio of bone turnover. Glucocorticoids increase bone resorption and decrease bone formation leading to diminishing bone mass and bone mineral density (1, 9, 10). The action of steroids on bone loss by acceleration of bone resorption and their role in the induction of osteoporosis occurs in Cushing’s syndrome in animals and humans. However, there are still lacking information about the influence of both maternal and neonatal administration of glucocorticoids on the mechanical and geometric properties and volumetric bone density in infants. Glucocorticoids by the modification of bone quality increase the risk of fractures of ribs and limb bones. Glucocorticoids are very often used as anti-inflammatory and immunosuppressive drugs in serious problems concerning children with rheumatoid arthritis and other systemic diseases. Glucocorticoid therapy is needed for children and youths with asthma as well (1). The drugs are administered during pregnancy in woman in order to improve the lung morphology in premature foetus. For these reasons the effect of commonly used glucocorticoid - dexamethasone on bone mineralization should be established.

The aim of this study was to define the effect of dexamethasone administration on the development and processes of mineralisation of bones in prenatal and neonatal life of piglets and to propose a new experimental model for future investigations of the effects of some drugs, hormones or biological factors acting during prenatal and neonatal life, since the piglets seem to be the closest animal species comparable to infants and growing children (5, 14).

Material and Methods

Experimental design and sampling procedure. The experimental group (Dex) contained 20 randomly chosen newborn piglets from 4 sows which were intramuscularly (i.m.) treated with dexamethasone in the dosage of 3 mg per sow every 2nd d during 24 last days of pregnancy. Sows, of Large Polish White breed, were kept under standard rearing conditions (temperature and humidity) with free access to fresh water and fed two times per day on standard commercial diets for pregnant sows. Three newborn piglets were chosen from every sow. The piglets were held with their respective mothers and fed
naturally sow’s milk during 14 days. The piglets were i.m. treated with 0.5 mg/kg b. w. of dexamethasone every 2nd d starting from the 2nd day of their life to the 14th d of the experiment. At the same time 4 pregnant sows assigned for mothers of control piglets were treated with physiological saline in the same way as the sows of Dex group and then, after the parturition, 3 newborn piglets were chosen from every sow. The control group contained 20 piglets which were given physiological saline in the same way and amount as the experimental piglets and held under the same conditions. Piglets from both groups were weighed just after their birth and on the 14th d of their life. At the end of the experiment piglets from control and experimental groups were euthanised with lethal doses of *natrium pentobarbitalum* (Morbital; Biowet Pulawy, Poland) and their left and right femora and humeri were isolated and stored at -25°C until further analyses. The room temperature was about 20°C during the tests.

**Volumetric bone density of cortical and trabecular bones.** Quantitative computed tomography (QCT) method and SOMATOM AR. T – SIEMENS apparatus supplied with VR 3 software were used for volumetric bone density determinations of the cortical and trabecular bones. Bone density was measured for cortical bone using 2 mm thick, cross sectional QCT scans in the middle of the shaft of the humeri and femora (C1) and 1 cm from this scan towards distal part of the bones. This second measure was referred as C2. Volumetric bone density for trabecular bone compartment was examined using the same method in the distal part of the bones. Cortical and trabecular bone density were calculated by automatic computation.

**Analysis of bone mineral density (BMD) and bone mineral content (BMC).** Dual-energy X-ray absorptiometry (DEXA) method and NORLAND XR 43 apparatus were used for the examination the bone density and mineral content. The parameters were measured for the whole bone samples and for both trabecular and cortical bone compartments.

**Analysis of mechanic and geometrical properties.** Mechanical properties of the investigated bones were estimated using three-point bending test, according to Ferretti’s *et al.* *(8)* method in INSTRON 4302 apparatus connected with a computer, registering the relationship between force acting perpendicularly to length of bone and resulting in displacement. These results were presented graphically and ultimate strength (Wy) were estimated. Through the measurement of horizontal and vertical diameters of cross section of the humerus and femur, both external and internal diameters and geometrical parameters such as cross-sectional area (A) and mean relative wall thickness (MRWT) were estimated (6-9).

**Statistical analysis.** All the data are presented as mean ± standard error (±S.E.). Statistical analyses were performed using Statistica 5.0 software. The Student’s *t*-test was used to determine statistical significance level of differences in variables between the investigated groups. A significance level of *P*≤0.05 was used for all comparisons.

**Results**

At the beginning of the experiment, just after birth, Dex piglets reached body weight of 1586 g (±85) and control piglets 1330 g (±83) and this difference was found to be statistically significant. At the end of the experiment, the results were opposite. The body weight of control piglets at the age of 14 d was higher (4420 g ±328) than in Dex group (2830 g ±220) and the difference was significant as well.

Both bones were longer in the control group than in Dex group although there were no statistically significant differences. The administration of dexamethasone showed the tendency to lower the length of the bones and this was considered as the catabolic effect on the development of the whole body (Table 1).

The mean value of the cross-sectional area (A) of the femur was significantly higher in the Dex group than in control. The obtained result in the humerus was the opposite. The administration of dexamethasone significantly decreased the mean value of cross-sectional area of the humerus in neonatal life (Table 1). The MRWT of both bones was lower in Dex group in comparison with the bones in control group and statistically significant differences were found for both bones (Table 1).

The volumetric bone density of trabecular part of the femur was significantly higher in the control than in the Dex piglets and the obtained values were 1.10 g/cm³ ±0.02 and 0.66 g/cm³ ±0.02, respectively. The density of cortical part of the femur in C1 was higher in the control (1.32 g/cm³ ±0.01) in comparison with the Dex group (1.26 g/cm³ ±0.04). The density of cortical part of the femur in C2 was not markedly higher in control group (1.51 g/cm³ ±0.02) in comparison with the Dex group (1.50 g/cm³ ±0.03). In the 1st and 2nd scans of cortical bone only the tendencies for lower values were observed. The volumetric bone density of trabecular part of the humerus was significantly lower in the Dex group (0.87 g/cm³ ±0.04) in comparison with control group (1.28 g/cm³ ±0.02). The density of cortical part of the humerus in C1 was higher in the control (1.58 g/cm³ ±0.05) in comparison with the Dex group (1.45 g/cm³ ±0.04). The density of cortical part of the humerus in C2 was significantly higher in control group (1.93 g/cm³ ±0.07) than in the Dex group (1.73 g/cm³ ±0.04).

Moreover, the BMD of the whole humerus and femur was significantly lower under the Dex administration when compared with the mean values in the control group. The BMC of the whole humerus and femur was significantly higher in the control group when compared with the Dex group.

The ultimate strength of the femur was significantly higher in control group in comparison with the mean value obtained in piglets under the influence of dexamethasone. The ultimate strength of the humerus was lowered in the Dex group in comparison with the control one, although it was observed only the tendency. In the Dex group the tendencies to lower the mean values of the maximum elastic strength of both bones were observed (Table 1).
Table 1

Properties of the humerus and femur in control and dexamethasone (Dex) treated piglets during their last 24 d of prenatal and 14 d of neonatal life and examined at the age of 14 d

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Humerus</th>
<th>Femur</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group</td>
<td>Dex group</td>
</tr>
<tr>
<td>Number of examined bones</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>6.96 (±0.09)</td>
<td>6.75 (±0.06)</td>
</tr>
<tr>
<td>Cross-sectional area (mm²)</td>
<td>42.66 (±1.72)</td>
<td>36.33 (±1.14)*</td>
</tr>
<tr>
<td>Mean relative wall thickness</td>
<td>1.02 (±0.11)</td>
<td>0.56 (±0.02)*</td>
</tr>
<tr>
<td>BMD of the whole bone (g/cm²)</td>
<td>0.284 (±0.01)</td>
<td>0.242 (±0.005)*</td>
</tr>
<tr>
<td>BMC of the whole bone (g)</td>
<td>0.889 (±0.007)</td>
<td>0.776 (±0.02)*</td>
</tr>
<tr>
<td>Maximum elastic strength (N)</td>
<td>383 (±21.4)</td>
<td>350 (±21.1)</td>
</tr>
<tr>
<td>Ultimate strength (N)</td>
<td>623 (±30.5)</td>
<td>544 (±23.3)</td>
</tr>
</tbody>
</table>

*P≤0.05

Discussion

Glucocorticoids show a dual metabolic effect on body weight depending on the used dose and long- or short-term therapy. The treatment with high dosage has a strong catabolic effect causing decrease in food intake and loss of body weight (4, 12, 15). The opposite action has a low dosage which induces the increase in food intake in humans and animals. They exhibit lean legs, abundant abdomen and very fat neck (1). Dexamethasone given in single dosage (1 mg/kg b.w.) to piglets within 1 h after their birth caused accelerated growth assessed on the 18th d of their life. Dex pigs were heavier than controls and the effect of drug on meat quality was minimal (4, 13). It is known that chronic long-term administration of dexamethasone as a potent synthetic steroid hinders the development of the whole organism including the skeletal system. General catabolic effect of dexamethasone on the whole growth and development was observed in neonatal rats and piglets (2, 3, 12). The investigations showed the highest decrease in protein synthesis and increase in protein degradation in the intestine and lower effect on skeletal muscles. Its influence on other tissues was not established.

Dexamethasone not only inhibits body weight gain and causes bone mass loss but it also acts directly on bone cells. Dexamethasone as well as other glucocorticoids inhibits the synthesis of collagen and proteins, decreasing the production of osteoid in the bone (1). The negative effect on geometric and mechanical parameters of bones was observed as well (7, 9, 11). Bone mineral density and longitudinal bone growth were disturbed and this was reported not only in our studies. Growing minipigs were treated with prednisolone and the changes of the trabecular architecture and bone chemical markers were described. There was obtained similar effect on mechanical parameters under the administration of prednisolone. Moreover, this effect was associated with marked reduction of turnover in bones (1, 11).

Our study showed the catabolic action of dexamethasone on longitudinal bone growth, whole bone development, and bone mineral density. Newborn piglets treated with dexamethasone during their prenatal and neonatal life had shorter femora and humeri in comparison with controls. Volumetric and mineral bone density, and mineral bone content were lowered under the influence of dexamethasone administered in dosage of 0.5 mg/kg b.w. every second day. The used steroid lowered the geometric parameters as well. It caused the skeletal system did not mature considering the values of the cross sectional area (A) and the mean relative wall thickness of the assessed femora and humeri. Dexamethasone altered these parameters and caused that the bones were thinner than in the control group. For this reason the strength needed to break them was lower.

In conclusion, i.m administration of dexamethasone diminished the skeletal system quality in piglets and had the detrimental effect on their bone density, similar to other results obtained in animals treated daily with higher doses (6, 9, 11). The results of these investigations proved additionally the possibility of using piglets before and after birth as an experimental animal model for testing the influence of some physiological, pharmacological and toxicological factors which can alter the processes of the growth, development, and mineralisation of the skeleton assessed on the model of the femur and humerus. Dexamethasone administered during perinatal period detrimentally influences the skeletal system and its...
development and the processes of its mineralisation together with the negative effect on the whole body mass as well.

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**References**