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The influence of prolonged administration of high doses of hydrocortisone and salmon calcitonin on cortical bone strength in young adult rats

Wpływ przedłużonego podawania wysokich dawek hydrokortyzonu i kalcytoniny łososiowej na wytrzymałość kości korowej szczurów

Administration of glicocorticosteroids remains unavoidable in several diseases of both adults and children. The most commonly reported conditions in which glicocorticosteroids are used are asthma, rheumatoid arthritis, auto-immunological diseases and kidney diseases (1). Children's skeletons are susceptible to the nociceptive effects of glicocorticosteroids (7). Osteoporosis and fractures of the vertebral bodies and of other locations may be related to prolonged glicocorticotherapy in children and adults (1,7,9).

Under clinical conditions it is usually very difficult to separate the negative influence of glicococorticosteroids on skeletal tissues from those related to the pathology of the diseases being treated. For instance, asthma could itself constitute a serious risk factor for osteoporosis due to disease-induced disturbances in the calcium metabolism, and rheumatoid arthritis disturbs bone metabolism and decreases mechanical stimuli. The influence of glicocorticosteroids on skeletal tissue has been observed in the laboratory on experimental animals.

Several studies have shown that the ultimate strength of bone is only partially related to its density. Variety of changes in the material composition or structural geometry of the skeleton can offset the effects of altered bone mineral content. Cellular activity may also have a role to play in the mechanical properties of bone and this aspect of bone metabolism has not been studied carefully (10). Mechanical testing of bone samples informs of the quality of bone tissue regarded as a ma-

terial whereas direct assessment of strength of whole bones remains the only mean to answer crucial questions concerning desired outcome of all treatment regimens to improve bone integrity and reduce the incidence of fractures (10). Obviously such studies can be exclusively performed in the laboratory on animals.

Rats are regarded as suitable animals for investigations on bone metabolism (14). It has been shown that prolonged administration of hydrocortisone or prednisolone may result in osteopenia and decrease of bone strength in the rat. Calcitonins have been recently shown to partially protect cancellous bone and its strength in ovariectomized female rats. Due to its antiresorptive (4,8) and osteogenic effects (11,12) this polipeptide hormone should have a protective effect on bones in glicocorticosteroid-induced osteoporosis. Experimental investigations concerning the protective influence of calcitonin on glicocorticosteroid-induced bone loss are rare (19) and we did not find in the literature any reports on biomechanics of bone under influence of these hormones administered concomitantly in supraphysiological doses.

The direct assessment of bone strength could demonstrate such biomechanical protection especially important in long-term studies. The strength of a single bone as a supportive organ depends largely on the material, i.e. tissue characteristics and is related to internal architecture of collagen bundles and hydroxyapatite crystals. Glicocorticosteroids impair formation both of collagen and mineral phase of bone and they cause myopathy that diminish mechanical stimuli on bones (2). Glicocorticosteroid-induced osteoporosis is multi-factoral and affects bone tissue in several direct (1) and possibly indirect (2,15) compound biochemical and biomechanical (2) mechanisms. Mechanical strength presents the most important parameter of bone physiological competency. We were unable to find studies on either cancellous or cortical bone strength in prolonged administration of both hydrocortisone and high doses of salmon calcitonin in relation to hydrocortisone-treated rats.

The aim of our investigations was to assess the combined effect of the prolonged administration of high doses of hydrocortisone together with high doses of salmon calcitonin on bone strength in sexually mature but still growing male rats. It has been shown by Ferretti et al. (13) that hydrocortisone acts biphasically on bone so our experiment was performed in two phases.

MATERIAL AND METHODS

In order to analyse the influence of hydrocortisone, both alone and in combination with salmon calcitonin on the strength of cortical bone when administered for two different periods of

time in sexually mature and still growing (i.e., young adult) male rats the experimental protocol was as follows. Six groups of male Wistar rats (mean weight 300 g) were kept under standard laboratory conditions. There were two control groups (groups K), (n=8) that received the appropriate volumina of vehicula. Rats in two of the other experimental groups were given hydrocortisone hemisuccinate, POLFA, Poland, (groups H), i.p. 10mg/kg bid (n=10), the animals of the resting two groups (n=10) received hydrocortisone as above and salmon calcitonin, Sandoz, Switzerland, (groups H-SC), s.c.15IU/kg bid. The animals were weighed at one week's intervals. The first phase of the experiment lasted 28 days, after which, under deep barbitural anaesthesia, one control (K) and two experimental (H and H-SC) groups were sacrificed by cardiocentesis. After another 28 days the three other groups were sacrificed in the same fashion.

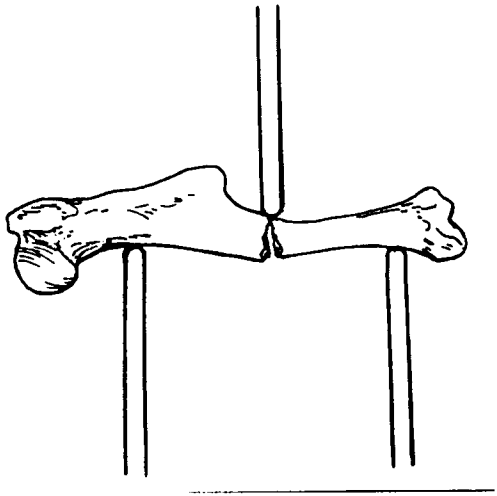

Fig. 1. The principle of three point bending test of the rat humerus

Our previous experience (16) has shown that the rat humerus is a suitable long bone for the simple and reliable three point bending test according to Burstein and Frankel (6). Both humeri of the rats were dissected free and they were stored at –20° C in plastic bags from which excess air was removed. Mechanical testing (Fig.1) was performed with the use of an Instron 6022 universal testing machine. The distance between the supports was 16mm, the thickness of rounded ends of the supports was 1.2 mm, the speed of transmission of the load was 20mm/min. During testing the humeri were kept wet with saline and at room temperature (20° C). A plotter registered load/deformation curves (Fig.2). Data of ultimate strength, energy and displacement were recorded.

The arithmetical mean of a pair of data for both humeri from the same animal was regarded as a single result. Based on the observations of others (13) and our own experience (16) the strength and energy were adjusted to the 100g of end body mass of the animals. The sets of data obtained (i.e., the end body mass, strength, strength adjusted to the end body mass, energy, energy adjusted to the end body mass and displacement of the loading head to the moment of

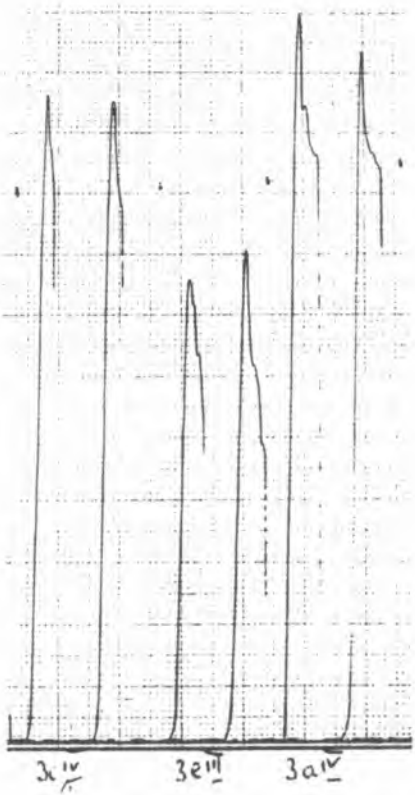

Fig. 2. Examples of load-deformation curves of three consecutive pairs of tests on rat humeri

fracture) were statistically analysed within the groups using appropriate parametric and non-parametric tests of analysis of variance (ANOVA or Kruskall-Wallis). Differences between means were compared using the Student's t-test or U-test of Mann-Whitney. $P<0.05$ was regarded as significant.

RESULTS

All the animals steadily gained weight during the experiment. One of the hydrocortisone-treated rats died of pneumonia (proved by autopsy) after 45 days. After both 28 and 56 days (phase I and II) significant differences in the end body mass were observed in the rats receiving both hydrocortisone and calcitonin. An unsignificant decrease of body mass was observed in the animals under hydrocortisone treatment alone (Tab. 1).

Table 1. End body mass of the rats (g) (standard deviations in brackets)

Experimental group	Controls	Group H	Group HS
After 28 days	350 (25) n=8	340 (55) n=10	310* (30) n=10
After 56 days	425 (40) n=8	395 (35) n=9	375 (20) n=10

* $p<0.05$.

MECHANICAL TEST PARAMETERS

After phase I (28 days) no significant differences in ultimate strength (Tab. 2), energy (Tab. 3) and displacement of the loading head to fracture (Tab. 6) was observed within the groups although in the rats on hydrocortisone-calcitonin treatment a decrease of 11% in strength was recorded in relation to the controls. When adjusted to end body mass the parameters of strength and energy appeared almost equal in the groups (Tab. 4 and 5) .

Table 2. Strength of midshaft rat humeri (N) (standard deviations in brackets)

Experimental group	Controls	Group H	Group H-S.C.
After 28 days	94.73 (11.05) n=8	92.14 (8.47) n=10	84.26 (10.42) n=10
After 56 days	120.76 (13.47) n=8	104.21* (12.51) n=9	109.22* (9.15) n=10

*$p< 0.05$.

Table 3. Energy of fracture of the midshaft of rat humeri (J) (standard deviations in brackets)

Experimental group	Controls	Group H	Group H-S.C.
After 28 days	0.0254 (0.0055) n=8	0.0258 (0.0061) n=10	0.0244 (0.0042) n=10
After 56 days	0.0380 (0.0093) n=8	0.0279* (0.0060) n=9	0.0334 (0.0061)

*$p<0.05$.

After phase II (56 days) there was a significant decrease in ultimate strength in both experimental groups, this being higher in those animals that received hydrocortisone alone (Tab. 2). A significant decrease was observed in the hydrocortisone-treated group in both energy and displacement of the loading head (Tab. 3 and 6). When adjusted to the end body mass of the rats the highest value of strength and energy was observed in hydrocortisone-calcitonin treated animals (Tab. 4 and 5).

Table 4. Strength of the midshaft of rat humeri adjusted to the end body mass (N/100g) (standard deviations in brackets)

Experimental group	Controls	Group H	Group H-S.C.
After 28 days	26.78 (2.47) n=8	27.43 (2.69) n=10	27.05 (3.02) n=10
After 56 days	28.4 (2.33 n=8	26.40 (3.48) n=9	29.28 (1.86) n=10

Table 5. Energy of fracture of the midshaft rat humeri adjusted to end body mass (J/100g) (standard deviations in brackets)

Experimental group	Controls	Group H	Group H-SC
After 28 days	0.0072 (0.0014) n=8	0.0076 (0.0011) n=10	0.0078 (0.0012) n=10
After 56 days	0.0089 (0.0015) n=8	0.0071* (0.0016) n=9	0.0090 (0.0016) n=10

* $p<0.05$.

Table 6. Displacement of the loading head to fracture of the midshaft rat humeri (mm) (standard deviations in brackets)

Experimental group	Controls	Group H	Group SC
After 28 days	0.653 (0.055) n=8	0.690 (0.125) n=10	0.707 (0.065) n=10
After 56 days	0.766 (0.114) n=8	0.632* (0.110) n=9	0.688 (0.098) n=10

* $p<0.05$.

DISCUSSION

It is well known that chronic administration of glicocorticosteroids inevitably reduces bone mass, particularly that of the cancellous tissue (15). Some authors support the thesis that cortical bone is not immune from glicocorticosteroid-induced osteoporosis (15). Many authors (1, 7, 15) are of the opinion that glicocorticosteroids exert their action on bone in several ways. It has been demonstrated *in vitro* that after a short period of administration they increase the metabolism and are later able to directly inhibit the metabolism of osteoblasts. Glicocorticosteroids impair the metabolism of vitamin D. They provoke an excessive loss of calcium through kidney and a lack of adequate absorption of this mineral in the gut. Relative hypocalcemia and direct stimulation of parathyroid glands are stimuli to excessive production of parathormon what has long been regarded as a major cause of glicocorticosteroid -induced osteopenia due to excessive stimulation of bone resorption. Prolonged administration of glicocorticosteroids can disturb the function of all organs including the digestive, reproductive, musculatory and nervous systems (1) thus indirectly encouraging bone loss.

Calcitonins show anti-resorptive action directly inhibiting osteoclasts via the receptor way in a very large range of applied concentrations and doses (3, 4, 8). They are regarded as non-toxic (4) and induced antibodies when present do not affect the anti-resorptive activity of calcitonins but may alter their pharmacokinetics. F a r l e y et al. (11,12) and have demonstrated that calcitonins exert stimulatory action on bone formation.

High doses of calcitonins administered to experimental animals produce relative anorexia and loss of weight. Body mass is regarded as an important mechanical factor influencing bone strength. B u r r and M a r t i n (5) have demonstrated in their experiments that loading increases bone mass. Osteoporosis is more common in thin people when compared to heavy and this observation is also true of children.

Although the reaction of human and animal skeletal tissues are similar in many basic respects, drawing direct conclusions from the results of animal studies must be done with extreme caution. Our data showed an absence of reaction of rat long bone to the influence of hydrocortisone administered in high doses after 28 days. Small doses of applied glicocorticosteroids seem not to be osteopenic in humans under certain conditions. The situation changes dramatically when the administration of glicocorticosteroids is prolonged and high doses are used. A decrease of bone quality inevitably results both in humans (9, 15) and animals (19).

P e a t et al. (18) have pointed to the clinical problem of prevention of glicocorticosteroid-induced osteporosis and recent epidemiological studies in the United Kingdom have shown that the risk of secondary osteoporosis due to use of glicocorticosteroids is practically neglected and in most people no prophylactic measures are taken.

Children, because they are physically active, are particularly prone to fractures of long bones. Protection of bone quality for this group is therefore important. In many clinical situations the use of glicocorticosteroids still remains a potent curative means of treatment. Clinical studies on bone loss are based above all on either metabolic or densitometric indices not necessarily reflecting biomechanical competence of the skeleton.

A clinical study by N i s h i o k a et al. (17) has shown protective influence of calcitonin on the bone density of the axial skeleton in glicocorticosteroid-dependent nephorisis in children. As the number of children with transplanted organs grows so, too, grow the orthopaedic problems related to the application of glicocorticosteroids. Although the hypothesis that calcitonin administered in high doses could be effective in reducing risk of long bone fractures due to glicocorticosteroid-induced cortical bone loss in children is attractive it can be proven only on the basis of prospective clinical studies. To the knowledge of the authors no such studies have been performed. Neither the protection of directly measured bone strength in different anti-osteopenic treatments in animal studies in corticosteriod-induced osteoporosis has been assessed. In this aspect sudies on bone-sparing glicocorticosteroid deflazacort which may possibly offer a new treatment less harmful to bones would be valuable.

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STRESZCZENIE

Szczurom płci męskiej o masie ciała średnio 300g podawano przez 28 i 56 dni pozaotrzewnowo wysokie dawki hydrokortyzonu (10mg/kg/d, i.p. bid) oraz hydrokortyzonu (10mg/kg/d, bid) i kalcytoniny łososiowej (15UI/kg/d, s.c. bid), zwierzęta grup kontrolnych otrzymywały *placebo*. Stwierdzono znamienne zmniejszenie wytrzymałości trzonów kości ramiennych zwierząt poddanych działaniu hydrokortyzonu przez 56 dni. Skorygowana do masy ciała wytrzymałość trzonów kości ramiennych była najwyższa w grupie zwierząt poddanych działaniu hydrokortyzonu i kalcytoniny łososiowej. Nie odnotowano istotnych zmian wytrzymałości kości ramiennych po 28 dniach trwania doświadczenia.

ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. LII SECTIO D 1997

9. Zygmunt Urbanowicz: Fascicular structure of terminal divisions of the brachial plexus posterior cord in man.
   Budowa pęczkowa końcowych części pęczka tylnego splotu ramiennego u człowieka.
10. Maria Matysek, Irena Królikowska-Prasał, Sławomir Gonciarz: Morphological picture of the big saphenous vein following cryoobliteration of varices.
    Obraz morfologiczny żyły odpiszczelowej dużej po krioobliteracji żylaków.
11. Ryszard Maciejewski, Andrzej Dąbrowski, Andrzej Sory, Zbigniew Wójtowicz, Wojciech Polkowski, Janusz Golan: The role of the oesophageal branches of aorta for the blood supply to the oesophageal remnant.
    Znaczenie gałęzi przełykowych aorty w ukrwieniu kikuta przełyku.
12. Andrzej Borzęcki, Zofia Święs, Halina Borzęcka, Marek Majewski: Urinary delta aminolevulinic acid excretion in children attending primary school in Kraśnik and Łęczna.
    Wydalanie kwasu delta aminolewulinowego w moczu u dzieci ze szkół podstawowych w Kraśniku i w Łęcznej.
13. Tomasz Karski, Maria Karska, Marta Tarczyńska: Orthopaedics in secondary prophylaxy and in the treatment of the cerebral palsy.
    Zadania ortopedii we wtórnej profilaktyce i leczeniu dzieci z mózgowym porażeniem.
14. Tomasz Karski: Hip abductor contracture as a biomechanical factor in the development of the so-called "idiopathic scoliosis". Explanation of the etiology.
    Przykurcz odwiedzeniowy biodra jako czynnik biomechaniczny w rozwoju tzw. „skolioz idiopatycznych". Wyjaśnienie etiologii wady.
15. Marta Tarczyńska, Tomasz Karski, Saleh Abobaker: Zwichnięcie biodra u dzieci z mózgowym porażeniem – wyłączny skutek choroby czy także wynik błędów leczenia.
    Paralytic hip luxation – the result of illness or also mistreatment.
16. Janusz Złomaniec, Witold Krupski, Stanisław Bryc, Marek Łosicki: Ocena zmian urazowych twarzoczaszki na rekonstrukcjach przestrzennych 3 D TK.
    Assessment of traumatic changes of the facial cranium in spatial 3D CT scans.

17. Witold Krupski, Janusz Złomaniec, Stanisław Bryc, Grażyna Złomaniec: Bone fragments imaging by means of 3D computerised tomography. Uwidocznianie fragmentów kostnych za pomocą 3D TK.
18. Barbara Drop, Anna Drop: Sto lat nauczania stomatologii w Polsce. One hundred years of teaching stomatology in Poland.
19. T. Katarzyna Różyło: New means of visualisation of dental caries. Nowe metody wizualizacji próchnicy zębów.
20. Witold Krupski, Janusz Złomaniec, Stanisław Bryc, Grażyna Złomaniec: Wartość badania TK w rozpoznawaniu wznowy pooperacyjnej raka jelita grubego z zastosowaniem wlewu dwukontrastowego i jednokontrastowego oraz powietrza.
The value of CT examination in recognising colon cancer postoperative recurrences with the use of bicontrast and monocontrast enema and the air.
21. Barbara Drop, Teresa Bachanek: Stan narządu żucia u 18-letniej młodzieży wiejskiej.
Stomatognathic system of eighteen year olds from the rural population.
22. Zofia Bieganowska-Klamut, Małgorzata Kostrzewa, Ewa Dybiec, Paweł Wieczorek: Przypadek zaburzonego rozwoju kory mózgowej z padaczką oporną na leczenie.
Developmental disorders of the cerebral cortex with epilepsy resistant to treatment: a case report.
23. Zofia Bieganowska-Klamut, Paweł Wieczorek, Ewa Dybiec, Małgorzata Kostrzewa: Porencefalia u niemowląt i dzieci w obrazie tomografii komputerowej.
Tomographic imaging of porencephaly in infants and children.
24. T. Katarzyna Różyło: The significance of the formaldehyde cycle in the pathology of hard tissues of teeth.
Znaczenie cyklu formaldehydowego w patologii twardych tkanek zęba.
25. Kazimierz Pasternak: The effectiveness of the bonding of amino acid by tRNA of livers of rats experimentally exposed to cadmium and barium.
Zdolność wiązania aminokwasów przez tRNA wątroby szczurów doświadczalnie narażonych na kadm i bar.
26. Andrzej Drop: Ultrasonograficzne środki kontrastowe.
Ultrasound contrast agents.
27. Lidia Bielak, Małgorzata Kiełczykowska, Stanisław Biliński: Równowaga kwasowo-zasadowa pochodnych hydrazonu 4-R-3-R_1-tiazol-2-onu.
II.[1] Stałe jonizacji α-metylo-β-(4-R-tiazol-2-ilo)- oraz α-metylo-β

(4-R-3-R_1-tiazol-2-ilideno)-hydrazydów kwasu octowego.
The acid-base equilibrium of 4-R-3-R_1-thiazol-2-one hydrazone derivatives.
II.[1] Ionization constants of α-methyl-β-(4-R-thiazol-2-yl)- and α-methyl-β-(4-R-3-R_1-thiazol-2-ylidene)-hydrazides of acetic acid.

28. Lidia Klukowska, Anna Nadulska, Stefan Dyba, Ingeborga Tychowska: The influence of cisplatinum on blond, some blond enzyme activities, magnesium level in rat serum and oxygen consumption in liver and kidneys.
Wpływ cisplatyny na krew obwodową, enzymy, poziom magnezu i oddychanie tkankowe wątroby i nerki u szczura.

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