

Evaluation of Bone Mineral Density in Rats Administered Methylphenidate

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ABSTRACT:

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Objective: Methylphenidate is a drug which is frequently prescribed in order to cope with the symptoms of attention-deficit hyperactivity disorder. One potential side effect of methylphenidate is its negative impact on the growth of children. Considering the association between linear growth and increase in bone mineral density, we aimed to evaluate the effects of methylphenidate on bone mineralization in rats.

Methods: Bone mineral density of methylphenidate administered rats (10 to 30 mg/kg/day, two months) was measured by dual photon absorptiometry and was compared with that of a control group.

Results: There were no significant differences in values of bone mineral density which were measured on both proximal and central femur regions between the study and control groups. There were also no significant differences in femur lengths between the study and control groups.

Conclusions: These results indicate that methylphenidate administration for two months' duration at 10 mg/kg and 30 mg/kg dosages seems to have no adverse effect on bone mineralization in rats.

Key words: methylphenidate, bone mineral density, growth, rats

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INTRODUCTION

Attention-deficit hyperactivity disorder (ADHD) is the most prevalent behavioral disorder among children. Methylphenidate is a central nervous system stimulant, which has been widely used since the 1960s to treat children with ADHD. Methylphenidate has been extensively studied, providing a rich background for use in children with ADHD (1). However, clinicians and parents still have concerns about the probable negative effects of methylphenidate on the growth of children. Whether growth alterations are a direct effect of methylphenidate (2-4) or related to ADHD itself (5) remains unsolved.

Various methods including direct comparisons of mean heights and frequency percentiles from standardized charts have been used in previous studies which were investigating growth retardation in children with ADHD. Of

all methods, there is only one bone mineral density (BMD) study, which evaluates bone mineralization and skeletal development in ADHD. The study by Lahat et al. (2000) concluded that there was no significant adverse effect of methylphenidate on bone mineral density in children with ADHD (6). In such studies on humans, however, it may not be possible to exclude many confounding factors, such as diet, exercise, and drug dosage. Therefore, we preferred rats in order to measure bone mineral density.

The purpose of our study was to investigate the bone mineral density of rats which were administered 10 mg/kg and 30 mg/kg dosages of methylphenidate by using dual-energy X-ray absorptiometry (DXA).

METHODS

Subjects

A total of 30 four-week-old Wistar male rats (120 ± 15 g) were housed in

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wire-topped plastic cages, as five animals per cage. Control and experimental rats received a standard diet of rodent chow (12-15 g/d) and water as they took. All rats were kept on an alternating 12-hour-light and 12-hour-dark cycle. The temperature inside the chambers was 22 °C (\pm 2) with relative humidity from 40 to 60%.

After the seven-day acclimatization period, the rats were randomly assigned to three groups of 10 rats per group. In group I, controls (sham) were administered distilled water; in group II, rats were administered 10 mg/kg/day methylphenidate; in group III, rats were administered 30 mg/kg/day methylphenidate.

All experiments were performed at the same time every day and in the light period (10:30-01.00 PM). The experiments performed in this study have been carried out according to the rules in the Guide for the Care and Use of Laboratory Animals adopted by National Institutes of Health (USA) and the Declaration of Helsinki. This study was approved by the Ethics Committee of Gulhane Military Medical Academy Research Center.

Drug Administration

Distilled water (0.5 ml) was given to each rat in Group I. The two different doses, either 10 mg/kg/day or 30 mg/kg/day, of methylphenidate were administered to group II and group III (study groups) respectively. Ten mg tablets of methylphenidate (Ritalin®) were dissolved in sterile distilled water via centrifugation providing 20 mg of methylphenidate per 10 ml. 0.5 ml of the solution (contains methylphenidate 1 mg) to each rat in group II, and 1.5 ml of the solution (contains methylphenidate 3 mg) to each rat in group III was administered for two months at 9 AM once a day, via orogastric entubation. The aim of entubation by using

with a software standardized for small animal research. The administration of distilled water or methylphenidate was discontinued at the end of the 60th day. All rats were killed with ether. For bone mineral density measurement, two regions, proximal and central femur of the right leg, were defined and analyzed. The reason for choosing the proximal femur instead of femoral neck for bone mineral density measurement was the difficult orientation to the femoral neck because of its relatively small size and unusual shape. Moreover, proximal femur represents the entire proximal epiphyseal and metaphyseal regions of femur. Bone mineral density determinations were expressed as grams per square centimeter. Femur length of the same leg was obtained during the bone mineral density measurement. All DXA measurements and analyses were performed by the same researcher (H.S. from in the department of radiology).

Statistical Analysis

Results were expressed as mean \pm standart error (of the mean) (SEM). Differences between the groups concerning length and bone mineral density were estimated using analysis of variance (ANOVA). A significance level of $p < 0.05$ was considered to be statistically significant.

RESULTS

No significant differences in bone mineral density values of both proximal and central femur regions were found among the three groups. There also was no significant difference in femur length among the three groups. The data of the three groups are presented in Table 1.

Table 1. Bone mineral density values and lengths of the right femur of rats in the control and study groups

Parameters	Group I (n=10)	Group II (n=10)	Group III (n=10)	P
Bone mineral density, g/cm ²				
Proximal femur	0.079 \pm 0.019	0.072 \pm 0.015	0.076 \pm 0.016	NS (P=0.61)
Central femur	0.077 \pm 0.008	0.076 \pm 0.005	0.078 \pm 0.006	NS (P=0.52)
Femoral Length, cm	4.65 \pm 0.65	4.64 \pm 0.57	4.76 \pm 0.37	NS (P=0.85)

Data represent means \pm S.E
NS = not significant

orogastric applicator was to prevent any drug losses due to uncontrollable reasons.

BMD Measurements

Bone mineral density was measured by DXA with Norland (Fort Atkinson, WI, USA) XR-36 densitometer,

DISCUSSION

This animal study was designed to investigate the possible effects of methylphenidate on bone mineral density values that might be related to bone development and growth retardation. We found no statistically significant difference in bone mineral

density values of rats with the two different dosages of Methylphenidate compared to the control group. There also was no negative effect of methylphenidate on femur length.

Stimulant-associated growth retardation in children with ADHD has been questioned. However, human studies which have examined height are not in accordance with whether the stimulant therapy has a suppressing effect on growth (7,8). In a review article, it was emphasized that 9 of 18 methylphenidate studies found initial height deficits (9). Recent studies suggest that it is possible to catch-up normal growth rates for children who have ADHD, without the cessation of stimulant treatment (5,10).

Bone mineral density is influenced by genetic, hormonal, and exogenous factors, such as physical activity, diet, certain medications, and exposure to sunlight. It is difficult to take control of these factors in human studies. In order to minimize the effects of these confounding factors, we used an animal model to investigate growth impairments. In our study, a homogeneous group of rats that are similar in age and phenotype in a tightly regulated environment with control of factors such as diet, exercise, drug dosage, and light-dark cycles were studied. However, the previous human clinical study on bone mineral density in children with ADHD (6) did not control these factors. Moreover, our study with rats also helped us to use methylphenidate at two different dosages. This animal experiment confirms the previous human study in which no effects of methylphenidate on bone mineral density measures were found.

Rats we included into the present study were at the age of one month. During two months of the study, they were in the periadolescence period as critical for bone mineralization and skeletal development. Pizzi et al. (1986) conducted a series of experiments designed to examine the effects of methylphenidate on growth and development in animals. In their study, methylphenidate (35 mg/kg, twice daily), when administered to neonatal rats on postnatal days 5-24, caused a reduction in femur length, along with a reduction in weights of many organs. However, when measured immediately following the drug cessation, a growth-rebound phenomenon was also observed; following a 30-day recovery period, the measures of femur lengths and many organ weights were not different in the study

and control animals (11). In the following study, Pizzi et al. (1987) extended their investigation on the growth suppressing effect of methylphenidate (35 mg/kg once a day) during the periadolescent period of development in rats (postnatal days 35-54). However, unlike neonatal rats, rats treated during the periadolescent period of development failed to show any growth impairment (12). Since the rats in our study were in periadolescent period, our findings are consistent with those of the second study by Pizzi et al.

It was suggested that the temporary effects on growth seemed to be related to stimulant dosage and to the presence or absence of drug holidays (13). In our study, both 10 mg/kg and 30 mg/kg doses of methylphenidate were given to the rats, and these methylphenidate doses had no adverse effect on the growth of the rats.

There were certain limitations in our study. First of all, administering methylphenidate intraperitoneally would be most appropriate route in these kind of investigations, but drug extract of methylphenidate to be applied intraperitoneally could not be obtained due to various administrative difficulties. Therefore, dissolved methylphenidate tablets within sterile distilled water was used orally as in another similar study (14). Other than this, determining blood levels or observing its effect on behaviors, such as locomotor activity to establish the effectivity of methylphenidate should have been done. Secondly, methylphenidate was administered in only four-week-old Wistar male rats (120 ± 15 g) and at 10 mg/day and 30 mg/day dosages for two months. So, our results are valuable for only conditions mentioned above and it should be kept in mind that different groups of rats or animal models under various conditions such as various dosages, administration routes, experimental conditions, and so forth, may give different results from each other. Lastly, we would like to remind that higher dosages or long term administrations of methylphenidate may cause unpredictable adverse effects (15) which were not seen at 10 mg/kg/d and 30 mg/kg/d dosages and for a period of two months.

The findings of our study indicated that methylphenidate administration for two months duration at both 10 mg/kg/d and 30 mg/kg/d dosages had no adverse effect on bone mineralization and did not compromise final height in rats.

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