Effect of shock waves upon the growth plate in rabbits

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SUMMARY

Objective: To evaluate the effects of shock waves upon the growth plate in rabbits by varying the energy and number of applied waves. Material and Methods: Six groups of five rabbits aged 5 weeks (Total: 30) were studied. OSSATRON (HMT) was used to generate shock waves. Animals were submitted to applications upon the proximal growth plate of the left tibia; the intensity (14, 21, 28kV) and number of waves varied (1,000 and 4,000). The right tibia was used as control. Animals were sacrificed after six weeks when measurements of tibia length were taken and the growth plate was microscopically studied. Statistical tests were used to compare measurements of the growth plate area and tibia length with those obtained in the control group, as well as between study groups. Results: In the present study, no statistically significant change was found in the measurements of the growth plate area and tibia length following application of shock waves in either group. A difference in the tibia growth tended to occur between the groups submitted to 1,000 impulses and the groups submitted to 4,000 impulses. However, this difference was not statistically significant. Hematomas were seen in the local of application in groups submitted to higher-energy waves and spontaneously subsided, special care being not required. Conclusions: The application of shock waves upon the proximal tibia in immature rabbits as described did not have any effect upon the growth plate, as shown by the evaluation methods used in the present study.

Keywords: Rabbits, Growth plate, High-energy shock waves.

INTRODUCTION

High-energy shock wave application has been widely used in Europe and United States for the last years in the management of orthopedic diseases. In the eighties this method became well known and shown to be effective in the treatment of renal calculi. Since then it has been the method of choice to treat this disease. In addition, its use has been extended to crush gallstones and salivary calculi. The main advantage of this method is its noninvasive nature, thus avoiding surgery and being associated with a fast recovery with much lower costs.

Several studies have reported the benefits of shock-wave therapy for musculoskeletal diseases. Orthopedic indications include chronic calcific tendonitis of the shoulder(5,7,17), lateral epicondyilitis of the elbow(4,10,15,16), plantar fasciitis(1,2,3,12,23,25), and delayed consolidation and pseudarthrosis(11,28,29).

The effects of shock waves were first documented during the Second World War (31). In addition, damage to lung tissue in shipwrecked persons caused by mine explosions was observed although no outer body damage occurred. Experiments using shock wave have been carried out since then. According to this author the first report of in-vitro renal calculi crushing induced by shock waves was published in 1971. In Munich (1980), the first patient with renal calculi was treated through this method. In the first prototypes, patients were kept immersed in water in a bathtub. In 1995 more than 2 millions of patients with nephrolithiasis had been treated worldwide. The use of this technology was then established and shock-wave therapy became the method of choice for nephrolithiasis therapy. From 1986 on, musculoskeletal tissue experiments have been carried out due to incidental findings found in experimental studies where osteoblastic responses were seen following the use of shock waves. The biological effects of shock waves were not fully elucidated. However, the use of shock waves in the treatment for pseudarthrosis, calcific tendonitis of the shoulder, epicondylitis, and plantar fasciitis, has been largely reported. In October 2000, FDA approved the use of shock waves only for plantar fasciitis. Other indications have been studied, such as review of the total hip replacement surgery, treatment for aseptic femoral head necrosis(18) as well as non-orthopedic diseases, including critical ischemia of limbs(8), degeneration and reinnervation of sensitive nerve fibers(26), and tumors, enhancing the penetration of chemotherapeutic...
agents within the cell and increasing the permeability of cell membrane.

The present study was carried out to evaluate the possible changes in bone growth following the application of shock waves, taking into account the importance of this therapeutic modality in immature patients.

**MATERIAL AND METHODS**

The present experimental study was carried out at the Federal University of São Paulo - Paulista School of Medicine, Service of Upper Limb and Hand Surgery of the Orthopedics and Traumatology Department. Six groups of five New Zealand white rabbits aged 6 weeks were studied with a total of 30 animals. Different energy amounts and shock numbers were used among the study groups.

Study Groups 1, 2, and 3 were submitted to the application of 1,000 waves of 14, 21, and 28 kV, respectively while study groups 4, 5, and 6 were submitted to the application of 4,000 waves of 14, 21, and 28 kV, respectively.

Animals were identified and kept under ideal conditions for the experiment. Study animals were anesthetized with ketamine (50 mg/kg) and diazepam (5 mg/kg). Shock waves were applied upon the left knee while the right knee was used as control. A gel was used to enhance the contact between the animal skin and the apparatus. The application site - proximal growth plate - was radioscopically identified, and the overlaying skin was marked with ink. Waves were applied upon the anterior face of the plate (Figure 1).

The apparatus used in the present study was the OSSATRON® developed by HMT (High Medical Technologies, Switzerland) specially for orthopedic use based upon the electrohydraulic principle for its generation, as shown by Figure 2.

It is provided with a rubber pouch coupled with the system of the electrode and the ellipsoid, which contains water and can be inflated or deflated by panel controls, thus allowing one to regulate the focus depth. The penetration depth of the focus can vary from zero to 100 mm. The amount of applied energy can be regulated, varying from 14 to 28 kV (40.6 and 71.9 MPa). The trigger frequency can vary from 0.5 to 4 per second (30, 60, 120, and 240 per minute) (Figure 3).

Applications are carried out by putting the pouch on the skin over the site to be treated. A gel is used between the pouch and the skin so as to assure as good a contact as possible between the media, thus avoiding interface since it would lead to reflectivity of part of the wave. The focus depth is regulated by placing the focus (Focus 2) over the site to be treated. The number and potency of triggers are defined and application can be started.

Six weeks following application of shock waves, animals were sacrificed by the gas chamber method (CO₂). They were put within cardboard boxes wrapped in plastic. After a completely airtight inner environment was obtained, a cannula was inserted into the box through an opening and gas was administered under low pressure for five minutes.

Tibiae were dissected and their length was measured with a caliper. Approximately 1-cmfragments distal to the proximal growth plate were collected after the proximal third of the tibial diaphysis was cross-sectionally cut. Tibial fragments were individually put into numbered flasks. Specimens were sent to the Pathology Department of the UNIFESP-EPM and submitted to fixation (10% formalin) and decalcification (10% nitric acid for three days). Specimens were then cut with a knife blade along the coronal plane. The posterior halves were immersed in an aqueous solution of 5% sodium bicarbonate, dehydrated with increasing concentrations of alcohol, cleared with xylol, and embedded in paraffin. Paraffin block was then sectioned and slides with 5-μm sections were submitted to hematoxylin and eosin staining.

The morphometric analysis was carried out through the Digital System of Analysis consisting of a microscope Olympus BX40 with plan-achromatic objectives coupled to a video camera Sony CCD-IRIS and a microcomputer Pentium 233 mm x with 64-MB RAM memory and image digitizing plate. The softwares Windows and Image Tool version 3.0 were used. Seven to 12 digitalized images under a magnification of 40X were required to evaluate the whole extension of the growth plate (Figure 4). Images were treated by using Adobe Photoshop 6.0, the rubber tool having been used to contour the outer border (Figure 5).

The area of the epiphyseal plate was measured with Image Tool 3.0, the values being expressed in square millimeters, as shown in the tables included in Results.

Measurements of the right (control) and left (shock-wave treated) growth plate areas were compared by nonparametric WILCOXON test for each group separately to compare possible differences. This method was also used to compare the tibial length measurements within each group. The variance analysis method was used to compare study groups; differences in tibial length and growth plate area values were used to compare control values with those submitted to shock waves.

The significance level (α-alpha) used in all cases was 0.05 (5%). The abbreviation NS was used to indicate non-significant result.

**RESULTS**

The results obtained in the present study were based upon the analysis of tibial length and growth plate area in coronal histological sections six weeks following shock wave application. Hematomas were seen in the application site in all study groups except study group I. Hematomas appeared soon after application and spontaneously recovered, special care being not needed. Measurements are shown in individual tables for each study group. Changes in the cellular aspect or bone bridge formation were not seen when growth plate areas were measured in coronal sections during microscopic examination.

As for tibial length and growth plate area nonsignificant (NS) results were found when Wilcoxon's test was used to compare values within the group (shock-wave versus control group) in any of the six study groups. No significant difference was found among the study groups when the growth plate area (p = 0.892) and tibial length (p = 0.189) values were compared by the variance analysis. Shorter growth values tended to oc-
cur in study groups 4, 5, and 6 submitted to 4,000 waves, as compared to study groups 1, 2, and 3 submitted to 1,000 waves.

The mean values of the growth plate areas in the control and the wave-treated side are shown in (Graph 1) for study groups 1 to 6. The mean values of the tibial length in the control and the wave-treated side are shown in (Graph 2) for study groups 1 to 6. The differences of the mean values of the growth plate areas for the control and the wave-treated side are shown in (Graph 3) for study groups 1 to 6. The differences of the mean values of the tibial length in the control and the wave-treated side are shown in (Graph 4) for study groups 1 to 6.

DISCUSSION

Extracorporeal shock wave therapy that is currently used as a routine therapy for renal calculi, is accepted for treatment of some musculoskeletal diseases in Europe and in the United States. The scientific background of this emerging technology is based upon clinical applications, with few investigational studies of the effects upon different tissues and mechanisms of action. The majority of studies found in literature retrospectively evaluated the results obtained following clinical applications. These facts justify our interest in carrying out the present study.

In the present study 30 female New Zealand white rabbits aged 6 months were studied. Although the number of animals was modest, it was considered enough for the present study. Investigational studies with similar casuistics are found in literature (19,21,27,30,32,35). The application period was based upon the growth curve of tibia in rabbits (20). Rabbits are small animals, which allows one to localize the growth plate and adequately apply the shock waves. Our experience was similar to that of other rabbits who also used rabbits in their studies (19,21,27,32).

As for the number of applied shock waves and the amount of energy, we chose values encompassed by the whole spectrum of the apparatus and simultaneously similar to those used in clinical practice.

With OSSATRON® (HMT) the number of applied waves can vary according to the operator. The manufacturer suggests 1,000 to 3,000 waves for treatment of enthesopathy and 4,000 to 6,000 waves for treatment of pseudarthrosis and delayed consolidation.

Manufacturer’s instructions for treatment of enthesopathy were not always followed in all studies reviewed. Applications varied from 1,000 to 9,000 waves, and no pattern of session numbers could be identified (4,9,10,15,16). This also applies to the studies of treatment of pseudarthrosis (14,28,29). This is one of the reasons why comparison of results is not always possible. In rabbits, we chose two ranges of shock waves: 1,000 and 4,000.

As for the energy used in each wave, the apparatus allows one to vary energy from 14 kV to 28 kV. The manufacturer suggests the use of 14 kV in enthesopathy, such as plantar fasciitis and epicondylitis of the elbow, and 26 to 28 kV in pseudarthrosis and delayed consolidation. The literature review showed that the energy ranges used in the treatment for different diseases are also different from those recommended by the manufacturer (9,15,16,28,29). The unit of energy comparison also changes according to the type of the apparatus used. In different studies units are expressed in kV or mJ/mm². There is no accurate relationship between the values; however, approximately 14 kV corresponds to 0.06 mJ/mm². This is another reason why the comparison of studies is difficult.

For the reasons mentioned above, we divided the animals in groups according to (Table 1). Two different values (1,000 and 4,000) of waves and three different values of energy amount (14, 21, 28 kV) were used in each application.

As for wave application we decided to study the proximal growth plate of the tibia because it is easily identified due to its superficial localization and relatively great size, as compared to the animal size. Other studies also used the tibial growth plate in the evaluation of shock-wave application (19,27).
Animals were sacrificed six weeks following shock-wave application. During this period animals were aged 12 weeks. At this age, approximately 80% of bone growth was completed and growth plates are still open; the period of six weeks following this age, approximately 80% of bone growth was completed and compaction. During this period animals were aged 12 weeks. At the tibia and also study histological sections of the growth plate. We used a caliper to measure the tibial length because a two-decimal precision of measurement is possible and also because the whole bone is secured, thus correcting the measurement failures that would occur with a normal ruler.

According to manufacturer’s instructions the focus where waves would be projected, is a 3-dimensional spatial area and not a point. This place of higher concentration of energy is an ellipse of approximately 1 cm/1 cm/3 cm where the longitudinal diameter measures 3 cm and corresponds to the wave propagation direction. Taking into account this information and the fact that the immature tibia diameter is approximately 2 cm in adult animals, we decided to histologically study median coronal sections of the growth plate. Therefore, any change induced by shock waves in the growth plate would be identified in this histological section. The growth plate height measurements or similar variables, such as means of several heights or standardization of a specific place for height measurement, were not taken into account due to irregular plate broadness in coronal sections. The measurement of growth plate area seemed to be the most sensitive method to detect small changes in the growth plate height or length. The results of the present study confirmed that high-energy shock waves did not lead to any change in the growth plate area, as compared to the untreated growth plate used as control, when different energy amounts and potencies were used in shock-wave application on the proximal tibia in immature rabbits. Therefore, no statistically significant change was found in bone growth or delayed bone growth through the evaluation methods adopted in the present study. The present study showed in an experimental model that no change in the growth plate was induced by the energy used.

However, grouping trends were found for study groups 4, 5, and 6 and study groups 1, 2, and 3. They were submitted to 4,000 and 1,000 shock waves, respectively. This trend suggests that tibiae submitted to 4,000 shock waves may have shown delayed growth whatever potency is used while increased growth was seen in tibiae submitted to 1,000 shock waves.

This trend does not mean necessarily that growth changes occurred. However, due to the small difference found in the present study, an evaluation in a greater number of animals can elucidate whether there is a true difference in the bone growth following shock wave application or not. Taking into account the results of the present study, the clinical use of shock waves in skeletally immature patients is likely to have no negative effects upon the bone growth. However, new studies should be carried out in order that beneficial and/or deleterious effects of this new technology be elucidated and also that it become a new tool in the therapeutic armamentarium for diseases difficult to treat. The latter challenge scientific developments and lead thousands of patients to withdraw from their occupation and labor activities.

**CONCLUSIONS**

1. The application of shock waves upon the growth plate area in rabbits, as described in the present study, did not cause any change in the bone growth and growth plate structure, as shown by the evaluation methods adopted in the present study, six weeks following application. However, a trend to different tibial length values was found among the study groups submitted to 1,000 and 4,000 shock waves.

**REFERENCES**