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The Use of Dual Energy X-ray Absorptiometry to Study the Effects of Low-Dose Methotrexate on Bone Density

Rubina Khaleel

Carroll College, Helena, MT

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The Use of Dual Energy X-ray Absorptiometry to Study the Effects of Low-Dose Methotrexate on Bone Density

Submitted in Partial Fulfillment of the Requirements for Graduation with Honors to the Department of Biology and Chemistry at Carroll College, Helena, Montana

Rubina S. Khaleel
April 12, 1996
This thesis for honors recognition has been approved for the Department of Biology and Chemistry by:

Dr. John Christenson, Advisor

Mr. Guido Bugni

Mrs. Joan Stottlemeyer
TABLE OF CONTENTS

ACKNOWLEDGMENTS ii i

ABSTRACT iii

LIST OF ILLUSTRATIONS iv v

INTRODUCTION AND LITERATURE REVIEW 1

MATERIALS AND METHODS 19

RESULTS 22

DISCUSSION AND CONCLUSIONS 36

LITERATURE CITED 41
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ABSTRACT

A study at Deaconess Research Institute was initiated to determine the contribution of low-dose Methotrexate to the development of osteopenia. Findings are based upon analysis of Dual Energy X-ray Absorptiometry (DEXA) scans for bone density and bone mass. Scans were performed 18 to 24 mo apart for 5 of 11 patients currently undergoing low-dose Methotrexate therapy for the treatment of rheumatoid arthritis. The results suggest there is no correlation between low-dosage Methotrexate and osteopenia/osteoporosis and that therapeutic doses seem to pose little threat of significant bone loss.
<table>
<thead>
<tr>
<th>Fig.</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig. 1</td>
<td>DEXA Scan for Patient 1</td>
<td>23</td>
</tr>
<tr>
<td>Fig. 2</td>
<td>DEXA Scan for Patient 2</td>
<td>24</td>
</tr>
<tr>
<td>Fig. 3</td>
<td>DEXA Scan for Patient 3</td>
<td>25</td>
</tr>
<tr>
<td>Fig. 4</td>
<td>DEXA Scan for Patient 4</td>
<td>26</td>
</tr>
<tr>
<td>Fig. 5</td>
<td>DEXA Scan for Patient 5</td>
<td>27</td>
</tr>
<tr>
<td>Fig. 6</td>
<td>DEXA Scan for Patient 6</td>
<td>28</td>
</tr>
<tr>
<td>Fig. 7</td>
<td>DEXA Scan for Patient 7</td>
<td>29</td>
</tr>
<tr>
<td>Fig. 8</td>
<td>DEXA Scan for Patient 8</td>
<td>30</td>
</tr>
<tr>
<td>Fig. 9</td>
<td>DEXA Scan for Patient 9</td>
<td>31</td>
</tr>
<tr>
<td>Fig. 10</td>
<td>DEXA Scan for Patient 10</td>
<td>32</td>
</tr>
<tr>
<td>Fig. 11</td>
<td>DEXA Scan for Patient 11</td>
<td>33</td>
</tr>
<tr>
<td>Fig. 12</td>
<td>DEXA Scan for Female Control</td>
<td>34</td>
</tr>
<tr>
<td>Fig. 13</td>
<td>DEXA Scan for Male Control</td>
<td>35</td>
</tr>
</tbody>
</table>
INTRODUCTION AND LITERATURE REVIEW

Methotrexate is a folic acid analog which acts as a competitive inhibitor of the enzyme folic acid reductase. This inhibition results in an impaired ability of the cells to metabolize folic acid (1, 29). Hindered folic acid metabolism often affects DNA synthesis and cell division. Based upon its profound effects on cell division and cell product synthesis, Methotrexate is often grouped in the category of anti-metabolic drugs. Nonetheless, Methotrexate is one of the most widely used anti-metabolites in cancer chemotherapy (10). First used in 1948, Methotrexate is still an essential mode of therapy in the treatment of a variety of malignancies. Childhood lymphocytic leukemia, non-Hodgkin's lymphoma, osteosarcoma, choriocarcinoma, head and neck cancers, and breast cancers are just a few examples of the broad scope of application for Methotrexate chemotherapy (3, 10, 23, 27). Of late, clinicians have discovered many more applications of Methotrexate, for example, in the treatment of psoriasis, the suppression of graft rejection after bone marrow transplantation, as well as experimental uses in the treatment of various rheumatic diseases (3, 10, 27). Maintenance of relatively high doses of Methotrexate over extended time periods is often necessary in the treatment of these conditions. More often than not, patients taking large doses of Methotrexate have been known to develop various osteodeficiencies, mainly osteoporosis. Therefore, researchers have theorized a direct correlation between chronic high-dose Methotrexate therapy and osteoporosis (1, 23).
A theory regarding high-dose Methotrexate and osteoporosis has been formulated and researched; however, low-dose Methotrexate therapy also has wide applications in the treatment regimes of many rheumatic bone diseases. Taking this fact into account, studies on the effects of low-dose Methotrexate-induced metabolic bone diseases have been undertaken, but are yet to produce conclusive results. One previous study addressed the effects of weekly low-dose Methotrexate, as in the treatment of rheumatoid arthritis, on human bone density. This study involved a single bone mineral density measurement on patients with rheumatoid arthritis who, at the time of the investigation, were receiving weekly doses of Methotrexate. These patients were then compared with a control group which was not subject to Methotrexate therapy. The results of the study showed no significant difference between the two groups; however, the nature of this study was such that its authors rendered it inconclusive (5).

Methotrexate has now become a common drug used in the treatment of rheumatoid arthritis and other inflammatory arthritides. It acts as both an anti-inflammatory and an immunosuppressive agent. Predisposition of a patient to the development of osteopenia may be based on a number of factors including gender, post menopausal state, use of glucocorticoids, immobility, and the inflammatory joint disease itself. If low-dose Methotrexate is also a contributing factor to the development of osteopenia, an increased incidence of traumatic fractures, stress fractures, and osteonecrosis will result (6).
Based upon this assumption and the hypothesis that low-dose Methotrexate treatment may contribute to the development of osteoporosis, Dr. Susan C. English of the Deaconess Research Institute initiated a study to determine the effects of low-dose Methotrexate on the bone density of 11 patients with chronic rheumatoid arthritis. Taking into account many external factors that may also impact bone density, English and I attempted to determine the contribution of low-dose Methotrexate to the development of osteopenia/osteoporosis in the experimental participants.

Osteoporosis is thought of as one of the most, if not the most, significant disorders associated with aging (20). It is a metabolic bone disease in which the amount of normally mineralized bone, also known as bone mass, becomes reduced to the point of being at an abnormally high risk level for fractures (11). Reduced bone mass is the most significant and preventable cause of fractures (20). Bone loss begins in many different parts of the skeleton, and it also occurs at different rates (4). Areas of cancellous bone, generally found in the spinal column and at the ends of long bones, are usual sites of osteoporosis related fractures (20). During the course of a lifetime, women are believed to lose approximately 50% of their cancellous/trabecular bone and approximately 30% of their cortical/compact bone. Men are thought to lose 30 and 20% of each type of bone, respectively. This variable rate of loss is attributed to the calciotropic hormone fluctuations that occur with increasing age (4).
The terms osteopenia and osteoporosis allow for a distinction between two very similar yet different clinical conditions. The more traditional term "osteoporosis" is reserved for the clinical syndrome involving a decrease in bone mass. It is the clinical condition of reduced bone density with impending fractures sustained in the hip, vertebrae, radius and humerus. Osteoporosis is often thought of as a complication of osteopenia. Osteopenia is a reduction in the amount of bone per unit volume relative to that expected for the age and gender of a subject, or a reduction in bone mass below that of the peak adult bone mass value or below the theoretical fracture threshold (4, 11). In other words, patients with osteopenia have osteoporosis, but are yet to sustain a fracture. Osteoporosis is also defined by the fact that while bone mass is reduced, the mineral-to-collagen ratio in the bone is normal. This is also a method of distinguishing osteoporosis from osteomalacia. In osteomalacia, normal, elevated, or decreased bone mass may be present; however, the bone tissue itself is always relatively mineral deficient (11).

During childhood and adolescence, bone growth progresses at a very rapid rate. During this time, bones increase in size, strength, and mineral content while also changing in shape. This period of rapid growth and change is called bone modeling. Skeletal growth continues into the early twenties. After growth is completed, bone tissue ages and degrades; thus, a process called bone remodeling is necessary to regulate the continual renewal of bone. During the renewal period, old bone is removed and new bone is deposited in its place (4, 11, 22). On the cellular level, bone remodeling occurs at
discrete focal points in the skeleton called bone remodeling units. Each unit is composed of the cofactors and cells necessary to remove and redeposit bone. Bone remodeling cycles begin with an activation phase. This stage is initiated by the systemic hormones, parathyroid hormones, and 1,25-dihydroxyvitamin D3 which are necessary in the stimulation and recruitment of osteoclasts (4, 22). During this period, the cells that line the bone are replaced by osteoclasts. Over a period of a few weeks, each osteoclast excavates a lacuna, on the surface of the cancellous bone or a cavity within cortical bone. After completion of their task, the osteoclasts are gradually replaced by osteoblasts which work to synthesize new bone tissue and refill the space. Bone turnover is the rate at which bone is removed and replaced. Bone turnover rate is dependent on the total number of active remodeling units in the skeleton. The remodeling balance is the relative amount of bone that is resorbed and formed at each bone remodeling unit. The impact of this process at the tissue level is determined by the rate of bone turnover and the remodeling balance. Bone loss occurs when osteoclasts create an especially deep cavity, osteoblasts fail to sufficiently refill a normal resorption cavity, or when both of these events occur (20). Osteoporosis is caused by a deficiency in the bone remodeling process. Bone remodeling disorders can result from a variety of factors ranging from insufficient diet and exercise programs to drug and/or chemical use.

Rheumatoid arthritis is a systemic autoimmune disorder of unknown etiology. It is typically characterized by chronic, symmetric, and erosive inflammation of the peripheral joints.
leading to the ultimate destruction of the joint (6, 22). The majority of patients diagnosed with rheumatoid arthritis also have elevated serum rheumatoid factor levels as well. The severity of the disease has been known to fluctuate over time; however, the general result is the progressive development of various degrees of joint destruction, deformity, and disability (6, 22). This form of arthritis has been attributed to many different factors ranging from genetics and gender to microorganism and viral invasion.

Although data are somewhat limited, the primary pathological event associated with the disease seems to involve the activation and/or injury of synovial microvascular endothelial cells. This evidence suggests that the trigger event or agent is carried to the synovial fluid of the joint by way of the bloodstream. The activated/injured endothelial cells become swollen and gaps appear between them. The lumens of these blood vessels typically become blocked by platelet, leukocyte, and fibrin clots. Consequently, plasma leaks out of the vessels and deposits into the subsynovial lining tissue of the joint causing the development of a characteristic edema. Activation of the cells lining the joint cavity results in their marked proliferation. This pattern continues with some fluctuation in severity throughout the course of the disease. As the arthritis progresses into its later stages, the synovium becomes increasingly hypertrophic and edematous. Numerous villi-like synovial tissue projections also begin to invade the joint cavity. The synovial inflammatory process is accompanied by a large tumor-like expansion and activation of the number of connective tissue stromal cells. These abnormal cells actively invade and destroy the
periarticular bone and cartilage at the joint margins where the synovium and bone are attached. This new growth is referred to as *pannus*. As the condition progresses, periarticular bone and cartilage are eroded and destroyed while the joint capsule becomes enlarged and ruptures. In conjunction, increased osteoclast activity is also prevalent, thus causing subchondral bone loss as well (6, 22).

Rheumatoid arthritis is most commonly associated with periarticular and generalized bone loss. This is possibly due to enhanced cytokine production. Although it is still unclear, the observed retardation of joint damage by Methotrexate therapy is thought to be the result of a counteraction of increased cytokine levels in the cartilage and subchondral bone. Thus, low-dose Methotrexate therapy is thought to be potentially beneficial to cartilage and subchondral bone. In contrast, high-dose Methotrexate regimens, such as those used in chemotherapy, are known to cause severe reductions in bone turnover and osteoblastic activity, thus resulting in an increased risk of osteoporosis and consequently fractures (22).

In order to completely understand any treatment regimens for rheumatoid arthritis, it is necessary to backtrack and discuss the different aspects of bone loss. Bone loss caused by steroid therapy is indisputably one of the most common skeletal complications. Steroids, especially glucocorticoids, have both anti-inflammatory and immunosuppressive effects. These are positive attributes, however, high levels of glucocorticoids are known to interfere with bone remodeling. Treatment with high concentrations of steroids usually results in a significant decrease in blood calcium levels.
Glucocorticoids decrease the amount of calcium absorbed from food and increase the amount excreted through the urine. This process causes a decrease in blood calcium levels which, in turn, stimulates an increase in parathyroid hormone in order to re-establish the normal blood calcium concentration. In order to elevate blood calcium, parathyroid hormone removes the needed calcium from bone tissue. Glucocorticoids also exert direct effects on the cellular level by causing a marked decrease in osteoblastic bone formation accompanied by a concomitant increase in osteoclastic activity (16). Steroid-induced osteoporosis was originally described by Cushing in 1932 (16). Since his discovery, Cushing's disease, as it is now called, is a recognized characteristic result of chronic treatment with pharmacological doses of glucocorticoids. It is known to produce a severe osteopenia which bears a resemblance to clinical osteoporosis. With increased glucocorticoid therapy, the rate of bone degeneration is such that fractures can occur in less than one year. Although the actual mechanism is not well understood, the rapid bone loss caused by steroid therapy appears to be the result of a combination of factors such as a direct suppression of osteoblastic activity by glucocorticoids and an increase in osteoclastic activity caused by an increase in parathyroid hormone secretion (6). Bone loss increases in proportion to the dosage and duration of glucocorticoid therapy. When it is administered in bursts for short periods of time, osteoporotic effects can be somewhat minimized and controlled. In fact, glucocorticoids such as Prednisone have proved somewhat valuable in the treatment of almost all forms of inflammatory rheumatic disease (16).
Long term supraphysiological glucocorticoid therapy, on the other hand, is known to result in skeletal damage. This is particularly the case in patients suffering from rheumatoid arthritis. The resultant skeletal damage typically manifests itself as four clinical phenomena which may occur in isolation or simultaneously: inhibition of bone growth, delayed union of fractures, osteonecrosis, and steroid-induced osteoporosis (16).

Glucocorticoids act by promoting a negative calcium balance by reducing intestinal calcium absorption, thus producing a compensatory increase in parathyroid hormone levels, increasing bone resorption rates, and decreasing the rate of bone formation (12). Further evidence suggests that the increase in osteoclastic activity associated with steroid therapy is due in part to secondary hyperparathyroidism. The decreased intestinal calcium absorption and increased urinary calcium define the term negative balance (16).

Glucocorticoids have a definite impact on the loss of trabecular bone concentrated in the axial skeleton and on the ends of long bones. These effects can be easily quantified by measuring bone mineral density, ionized calcium levels, intact PTH levels, and examination of biochemical markers of bone formation such as osteocalcine levels (12). Older, immobilized, and post menopausal patients already display relatively low bone mass. This condition predisposes such patients to a rapid development of osteopenia. Younger individuals, however, are characterized by higher bone turnover rates which allow for more rapid bone loss. Biochemical changes in patients with glucocorticoid-induced osteopenia are generally inconspicuous. Fasting serum calcium, phosphate, and
vitamin D metabolite levels are generally within the normal range. The serum immunoreactive PTH concentration may range from a normal to a slightly elevated level. After initiation of therapy, serum alkaline phosphatase and osteocalcin levels show a progressive decline. These factors reflect the decline in osteoblastic bone formation. Urinary calcium excretion often increases during the initial years of steroid therapy. This is despite the fact that intestinal calcium absorption is reduced. After several years of therapy, however, urinary calcium levels usually decline and return to normal range (6).

Bone loss as a result of excess glucocorticoid levels is thought to follow two mechanisms: suppression of osteoblast function and inhibition of intestinal calcium absorption leading to secondary hyperparathyroidism and increased osteoclast activity. Glucocorticoids are also known to promote bone loss by directly stimulating renal excretion of calcium and also possibly stimulating PTH secretion (6).

Methotrexate was originally developed as and still is one of the best chemotherapeutic agents in the treatment of malignancies. It has an antiprolific property which is based on its ability to inhibit DNA synthesis (22). Methotrexate's ability to kill rapidly proliferating cells accounts for its use in the treatment of tumors. However, as we have recently found out, Methotrexate lacks the ability to distinguish between malignant and benign growth; thus it has been recognized that Methotrexate kills any rapidly replicating cell. This is evidenced in Methotrexate's effect on bone marrow stem cells, epithelial cells, hair follicles, and bone cells (5, 22).
When used in relatively low doses, Methotrexate is considered to be a highly favorable therapeutic approach for rheumatoid arthritis patients. It is known to be able to significantly retard the radiological deterioration of the joints, as well as alleviate joint inflammation, stiffness, and pain. Methotrexate's mechanism of action in the rapid improvement of arthritis-affected joints has yet to be clarified; however, it has been proposed that low doses of Methotrexate behave as immuno- and/or inflammatory suppressers. This is thought to be achieved through interference with cytokine production and leukocyte activity. This function of the drug is in contrast to its normal role as a cytostatic agent (22).

Methotrexate, previously known as Amethopterin, is 4-amino-4-deoxy-N\(^{10}\)-methylfolic acid. It is included in a unique class of molecules collectively referred to as folate antagonists (3, 22, 29). The folate vitamins are a class of essential cofactors that carries one-carbon groups that are necessary for the synthesis of purines and thymidylic acid, both of which are essential for DNA synthesis and cell division. Therefore, folic acid is a logical target in the design of a DNA inhibitor. Physiological folate cofactors all share certain structural features such as a multi-ring pteridine group linked to para-aminobenzoic acid. This in turn connects with a terminal glutamate residue. Folates found in the blood have a single terminal glutamate while most intracellular folates have multiple glutamate groups linked by gamma-peptide bonds. These intracellular folates are also referred to as polyglutamates. The polyglutamate forms of folic acid possess many unique properties that allow for them to be preferentially retained within the cell, and
they are generally more efficient cofactors than monoglutamated compounds. Another important feature of the folate vitamins is that they must be reduced to the tetrahydro form in order to be enzymatically active. The enzyme responsible for this activation is inhibited by Methotrexate. Methotrexate enters cells throughout the active transport system also used by the physiologic circulating leucovorin and folinic acid (10). After entering the cell, Methotrexate binds reversibly, yet tightly, to dihydrofolate reductase and inhibits the conversion of dihydrofolic acid to the active tetrahydrofolic acid (22, 29). This inhibition obstructs the generation of thymidalate, which is an essential ingredient for the production of DNA from desoxyuridylic acid, and ultimately DNA and RNA synthesis are also blocked (4, 22, 29). This inhibition is cell-cycle dependent since Methotrexate is most active during the S-phase of cell maturation. Methotrexate also acts by inhibiting protein synthesis. This inhibition is achieved by prevention of glycine production from the amino acid serine. It also prevents the generation of the amino acid methionine from homocysteine (29). These capabilities of Methotrexate are the key to its uses as a chemotherapeutic agent against malignancies (10).

It seems apparent that Methotrexate is a drug which is capable of inhibiting osteogenesis by interfering with protein and calcium metabolism in growing bone (1, 23). When tested on experimental animals, Methotrexate has been found to have negative repercussions on skeleton and ectopic bone formation whereby the number of osteoblastic cells appear to be significantly reduced. It has also been suggested that low-dose administration of Methotrexate for
prolonged periods of time may adversely affect bone mass. It is also speculated that low-dose Methotrexate induced bone loss is most prevalent in post-menopausal women. As yet, there are no positive findings to this effect (29).

In order to determine the direct effects of varying levels of Methotrexate on osteoblast growth and function, Gallagher (4) used osteoblast-like cells derived from adult trabecular bone tissue. These samples were then cultured and treated with Methotrexate and the active metabolite vitamin D3. 1,25-Dihydroxyvitamin D3 is a potent inducer of osteoblast proliferation and differentiation, alkaline phosphatase activity, osteocalcin synthesis, and it plays a central role in bone mineralization. Vitamin D3 is also thought to modulate the effects of Methotrexate on human bone cells. The results of the experiment show that the predominant effect of Methotrexate is to inhibit osteoblast proliferation at all concentrations including the low-dose range. In addition to this trial, Methotrexate was also tested in a dose concentration range that overlapped the average serum and synovial fluid concentrations of the drug in rheumatoid arthritis patients on low-dose therapy. From the results of this trial it can be concluded that Methotrexate has a dose-dependent inhibitory effect on osteoblast proliferation. Phenotypic characteristics of the osteoblast such as alkaline phosphatase activity and osteocalcin production were absolutely unaffected under the influence of Methotrexate. This indicates that the antiprolific action of Methotrexate is not interfering with the more mature, non-proliferative cells that are characterized by alkaline phosphatase activity and osteocalcin production. It appears
that Methotrexate is selectively targeting the proliferating cell fractions while leaving mature osteoblast populations unaffected (22).

Another study conducted by May et al. attempted to ascertain the effects of low-dose Methotrexate on bone metabolism and histomorphometry in rats. In this trial, 6-month old, female, Sprague-Dawley rats were divided into four groups: intraperitoneal Methotrexate injections with ovariectomy (MTX/OVX), intraperitoneal Methotrexate injections without ovariectomy (MTX/SHAM), intraperitoneal saline injections with ovariectomy (CTL/OVX) and without ovariectomy (CTL/SHAM). Injections were administered for 16 wk with the Methotrexate dose that would yield a similar serum Methotrexate level, 0.6 +/- 0.1µmol, as that for a rheumatoid arthritis patient. Bone formation, assessed by serum alkaline phosphatase and osteocalcin levels and histomorphometry, was significantly reduced and bone resorption, determined by urinary hydroxyproline levels and histomorphometry, was significantly increased in the Methotrexate groups. Therefore, it was concluded that prolonged administration of low-dose Methotrexate in rats causes suppression of osteoblast activity and stimulation of osteoclast recruitment resulting in diminished bone mass and osteopenia (15).

For this study, senescent female rats were used because, when ovariectomized, these rats have characteristics similar to those seen in women with post-menopausal osteoporosis. The effects of prolonged administration of weekly low-dose Methotrexate in these rats caused decreases in serum alkaline phosphatase, mineral
apposition rates, and osteocalcin levels. These data support the contention that Methotrexate does indeed cause decreases in bone formation. Since the osteoblast surfaces appear to be normal, decreased bone synthesis can be attributed to decreased bone matrix formation by osteoblasts rather than a decline in the osteoblast proliferation and differentiation. The increased count of osteoclast cells in the tibiae and vertebrae of the test subjects indicate a significant increase in osteoclast recruitment and activity. This finding was further substantiated by a marked increase in urinary hydroxyproline levels for the MTX/OVX group. The similarity in this parameter, low urinary hydroxyproline levels, in the MTX/SHAM and CTL groups suggests that estrogen may exert a protective effect. Thus, it can be concluded by histomorphometric and biochemical data that low-dose Methotrexate does indeed significantly decrease cancellous bone formation and increase bone resorption resulting in cancellous osteopenia in vertebrae and tibiae. However, the similarity in the results of urinary hydroxyproline levels in the control and experimental group without ovariectomy suggests that estrogen may have a somewhat protective effect. Ovariectomy seemed to have little effect on bone metabolism and histomorphometric features, except a possible protection against increased urinary hydroxyproline levels, on the experimental rats regardless of Methotrexate treatment. The lack of effect of ovariectomy impedes any further comment on whether estrogen may mitigate the effect of Methotrexate on bone (15).

A previous study has addressed the effect of weekly low-dose Methotrexate on bone density in humans. This study involved a
single determination of bone mineral density in rheumatoid arthritis patients who were either treated (experimental group) or not treated (control group) with Methotrexate. The two groups seemed to show similar bone mineral density patterns allowing the researchers to conclude that low-dose Methotrexate regimens, such as those used in rheumatoid arthritis therapy, do not cause a decrease in bone mineral density. However, because of the combination of the low total dose of Methotrexate (625 mg), the cross sectional nature, and the small number of participants these findings have been rendered inconclusive (15).

A team of researchers from the Istituto Ortopedico Rizzoli in Bologna, Italy also conducted an experimental trial with varying doses of Methotrexate. This group conducted trials based on the assumption that Methotrexate may cause an "osteopathy" characterized by severe pain in the extremities, osteoporosis, and even fractures. These side effects were more frequently manifested in children suffering from leukemia who are treated for prolonged periods, 2 to 5 yrs, with low doses of Methotrexate administered on a daily or weekly basis. The purpose of this trial was to evaluate the action of Methotrexate on the bone mineral content in a group of 59 young patients with osteosarcoma who were treated with adjuvent therapy including Methotrexate. The study is based on two dosage protocols administered intermittently over 8 mo (5).

Patients were divided into groups and bone density measurements were taken for two groups of patients with osteosarcoma who had been treated with both high and low doses of Methotrexate respectively. Data from these groups were compared
with the data collected from a third group of healthy individuals of the same age. Bone density was measured in the radius at the midpoint and at the trabecular distal point. In patients treated with low doses, differences in bone density were observed when compared with the controls. In the high-dose group, bone mineral content values were significantly lower than control values at the trabecular distal point. No decrease was observed at the midpoint. The significant reduction in the high-dose group bone mineral content indicated that the osteopenic effect of Methotrexate is dose dependent. The decrease in density at only the trabecular distal point proves Methotrexate's main action to be at the level of this type of bone. It has been proven that trabecular bone possesses a greater sensitivity to the action of osteopenic agents (5).

Results showed that subjects treated with low doses of Methotrexate had bone mineral density values which were greater at both measurement points. The group treated with high doses of the drug had values that were significantly lower than those of the controls. No patients treated with Methotrexate displayed fracture or pain which could be attributed to osteoporosis. Therefore, it is concluded that low doses of Methotrexate administered at intermittent cycles do not seem to interfere with bone metabolism. Obvious osteopenic effect was seen in bone mineral density values taken at predominant trabecular bone sites, such as the modified distal point of the radius, in the high-dose group. This exclusive trabecular localization of bone loss is in complete agreement with the greater sensitivity of trabecular bone to numerous osteopenic factors such as osteogenic deficit and cortisones (5).
In conclusion, the Bologna investigators believe that osteopathy due to Methotrexate is dose dependent. Higher doses, even when administered intermittently, are capable of causing bone loss which cannot be completely recovered from before the onset of the next treatment cycle. Thus, intermittent bursts can cause prolonged osteogenesis inhibition. For these reasons, it is believed that high-dose Methotrexate therapy in elderly subjects, in whom physiological osteopenia as the result of aging is common, may result in a greater incidence of osteoporosis and consequently, spontaneous fractures. As reflected by observations made on juvenile leukemia patients treated with Methotrexate, the data collected supports the contention that even low doses of Methotrexate administered intermittently over long periods of time may result in osteopenic abnormalities (5).
MATERIALS AND METHODS

The present study was a continuation of that initiated by English referred to above and was brought to completion. I began my task by seeking out participants who would enlarge the experimental group and bring more validity to the results.

A computerized chart search of the Deaconess-Billings Clinic medical records department was conducted in order to find study candidates. With the aid of the Deaconess Hospital Information Services department, a computer database was created from the total patient population. This database was comprised of both male and female patients ages 18 to 45 who are currently undergoing drug therapy for the treatment of an osteogenic disorder. This database consisted of approximately 310 potential candidates.

After database assembly, each patient chart was examined in order to isolate a group of subjects who best fit the study protocol. Those patients diagnosed with rheumatoid arthritis who are currently undergoing Methotrexate and Prednisone therapy were of special interest to us for use in the study. English and I selected participants currently using Methotrexate without adjuvent Prednisone therapy for the treatment of rheumatoid arthritis, who were capable of understanding the nature of the study and who were willing and able to adhere to the protocol requests throughout the 3-yr study, and who were willing to give signed written consent for the study. Patients were excluded on the following bases: current therapy with Prednisone, biphosphonate or sodium flouride preparations, androgens, anabolic steroids or other drugs that could
affect bone density, medical illness known to cause bone loss or deterioration, calcitonin therapy for 2 mo prior to the study, history of drug or alcohol abuse, cigarette smoking (>1 pack/day), and Paget's disease of the bone.

The study design included an experimental group consisting of patients who were currently taking Methotrexate without Prednisone and a control group of patients who were currently taking similar doses of Prednisone and/or other disease modifying agents such as Gold, Imuran, Plaquenil, or Sulfasalazine. Medication dosage was carefully monitored and recorded for both groups in the study, and a preliminary and concluding DEXA scan was also run with an 18-to-24-mo interval between scans. Information such as estrogen treatment, birth control pills, non-steroidal anti-inflammatory medication, dietary history, and life style were also monitored and recorded.

After candidates were selected, they were contacted by mail and invited to participate. Patients/candidates were sent a letter outlining the general goals of the study and the reasons why and how they were chosen to participate. A pamphlet regarding the bone density scan or DEXA scan was included with the letter. Candidates who were interested in participating were asked to call with any questions regarding the study or for an appointment. Those individuals with positive responses were then scheduled to receive a bone density scan or DEXA scan. I also contacted the patients who had already begun to participate in the trial and requested that they undergo a second scan for comparisons to determine the amount of bone loss that occurred.
In order to accurately assess predisposition to osteoporosis, I devised a questionnaire based on the common risk factors associated with this disease. This questionnaire was administered in order to obtain lifestyle and other information that would contribute to the development of osteoporosis. The data from the DEXA scan(s) were correlated and analyzed in conjunction with the candidate's age, sex, and lifestyle information. After preliminary analysis, the data were compared with the total amount of Methotrexate that the patient had received until the day of the scan. For some patients, a second DEXA scan was taken 18 to 24 mo after the initial screening. When a candidate arrived for screening, he/she was asked to complete the questionnaire regarding lifestyle and dietary calcium intake. The questionnaire for dietary calcium intake was a standard questionnaire provided by Deaconess Research Institute.
RESULTS

Eleven patients participated in the study, chosen from a pool of 200 potential candidates. Those selected received initial DEXA scans and must wait for the approximate 18-to-24 mo interval for the second scan (fig 6-11). Five of the eleven received a second scan 18 to 24 mo after their first scan.

The approximate amount of Methotrexate received by each candidate over the duration of the study ranged from 380 to 2650 mg. All patients were receiving weekly doses of Methotrexate that are considered to be within the low-dose weekly range of 2.5 to 37.5 mg/wk. Patients who had two DEXA scans showed changes in bone mass over the scan interval (Fig. 1-5). Out of the 11 participants, only 2 were male (Fig. 3, 5). All of the females of the study group developed signs of mild osteopenia in the wrist and, in one case, the lumbar spine. The overall trend for the females receiving two scans was a slight decrease in bone density. There were no significant bone density changes observed between the two scans for either of the male participants.
Fig. 1. DEXA Scan for Patient One

Female
53 yrs
5' 4"
201 lbs
Minimal exercise

Bone Mineral Density (g/cm²)

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Fig. 2. DEXA Scan for Patient Two

Female
51 yrs
5' 6"
127 lbs
Minimal exercise
Fig. 3. DEXA Scan for Patient Three

Male
36 yrs
6' 0"
175 lbs
Regular
Exercise

Bone Mineral Density (g/cm²)
Fig. 4. DEXA Scan for Patient Four

Female
35 yrs
5'2"
173 lbs
Regular exercise
Family history

Bone mineral density (g/cm²)

L1
L2
L3
L4 Region
Neck
Wards
Troch
Fig. 5. DEXA Scan for Patient Five

Bone Mineral Density (g/cm²)
Fig. 6. DEXA Scan for Patient Six

- Female
- 44 yrs
- 5'1"
- 149 lbs
- Low Dietary Calcium

Bone Mineral Density (g/cm²)
Fig. 7. DEXA Scan for Patient Seven

- Female
- 60 yrs
- 5' 5"
- 140 lbs
- Regular exercise
- Jun-95

Bone Mineral Density (g/cm²)
Fig. 8. DEXA Scan for Patient Eight

- Female
- 48 yrs
- 5' 8"
- 135 lbs
- Regular exercise

Bone Mineral Density (g/cm²)
Fig. 9. DEXA Scan for Patient Nine

Female
55 yrs
5'4"
150 lbs

Nov-93

Bone Mineral Density (g/cm²)

Neck Region
L1
L2
L3
L4
Troch
Radius
Ulna
Wards

1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Fig. 11. DEXA Scan for Patient Eleven

- Female
- 55 yrs
- 5'4"
- 132 lbs

Bone Mineral Density (g/cm²)

- Radius
- Troch
- Wards
- Neck
- L4 Region
- L3
- L2
- L1
Fig. 12. DEXA Scan for Female Control

Female
53 yrs
5' 4''
190 lbs

Bone Mineral Density (g/cm²)

L1  L2  L3  L4  Neck  Wards  Troch

Region
Fig. 13. DEXA Scan for Male Control

Male
40 yrs
6' 0"
180 lbs
DISCUSSION

Dr. English, in conjunction with the Deaconess Research Institute, developed a study to investigate the effects of low-dose Methotrexate therapy on bone density in patients currently under treatment for rheumatoid arthritis. This decision was based upon the common use of Methotrexate to treat rheumatoid arthritis and the many inconclusive studies (5, 10, 16, 23) attempting to establish a link between the drug and osteopenia/osteoporosis.

Skeletal development, like any other building process, is only as good as its components. The entire process is dependant upon the interplay between bony, cartilagenous, fibrous, and hematopoietic tissues. Normal skeletal maturation is not only necessary to meet structural demands, but it is also essential in order to maintain the systematic mineral homeostasis that is an integral part of life.

Bone can be categorized as compact or cortical, and trabecullar or cancellous. Corticol bone is responsible for the mechanical integrity of the skeleton. Approximately 80% of the appendicular skeleton is comprised of corticol bone. The axial skeleton, on the other hand, is mainly trabecular bone which is responsive to any skeletal metabolic demands. The structural features of each type of bone ultimately determines an individual’s predisposition towards a specific bone disorder such as osteoporosis (11, 17).

The osteoporosis risk factor is four times greater for women than it is for men. This descrepency can be attributed to the fact
that women have longer life spans, smaller body frames, and large hormonal changes (17).

The longer the lifespan, the greater the likelihood of developing osteoporosis. Age has been associated with osteoporosis for one main reason: after peak bone mass is achieved, it gradually declines with time. In other words, the more years that pass, the greater the loss of bone. Some bone tissue loss occurs naturally with age. However, in patients with osteoporosis, an abnormally rapid loss of bone tissue is known to occur (17).

There is an increased incidence of bone loss in petite women as opposed to larger women. This is simply due to the fact that petite women have less bone to lose than big-boned women. Thin women are also at greater risk for the disease than heavier women.

Estrogen seems to have a protective effect against bone loss. Women who are pre-menopausal are exposed to healthy levels of estrogen on a regular basis. At menopause, however, the amount of naturally produced estrogen markedly declines. Early menopause, either natural or surgically induced, can increase a woman's likelihood of developing osteoporosis because the protective effects of estrogen are lost suddenly at an earlier age (11, 17).

A candidate's lifestyle is also an important consideration in analysis of osteoporosis risk. Personal and lifestyle information are key to calculating a possible risk of developing osteoporosis. Therefore, in order to ensure that Methotrexate may be the causitive agent for patients developing osteoporosis/osteopenia, an assessment of the patient's predisposition to osteoporosis is necessary. Osteoporosis risk is greatly increased for both men and
women who are calcium deficient, inactive, alcoholic, or under
treatment with certain medications (8, 11, 17). Race and heredity
are also important factors that should not be overlooked when
determining any predisposition to osteoporosis.

After calculating possible risk, a bone density scan is
necessary to determine whether significant bone loss has or has not
occurred. Such scans are obtained through the use of Dual Energy X-
ray Absorptiometry or DEXA. DEXA is a very helpful tool in
determining bone mineral content and density. In fact, it is
essential for the study of mineral metabolism and osteogenic
diseases (7). Bone mineral density is defined as the bone mineral
content divided by the area of interest. This quantity is then
expressed in grams/cm². There are many available techniques for
the determination of bone mineral density. Most require the use of
some type of radioactive isotope such as I¹²⁵ or gadolinium-153
(19). The DEXA method is advantageous over other scanning
techniques because it does not employ the use of radioactive
elements. Instead, it uses an X-ray tube to achieve a higher proton
flux, thus yielding faster scanning times, a higher degree of
resolution, greater precision, and improved isotope counting
statistics (13, 19). One of the best reasons to use an x-ray tube
instead of a radioisotope is the greater intensity. An average x-ray
tube is capable of producing a proton flux 500 to 1000 times greater
than a 1-curie gadolinium-153 source (21). All in all, the DEXA scan
measurement is one of the fastest, safest, and most accurate
noninvasive methods known for determining bone density in the hip,
spine, vertebrae and forearm (13). Measurements are then
translated into computerized images of the areas, resembling an X-ray, and a graphical interpretation of the results.

In my study, comparison of the patients' initial and second scans (Fig. 1-5) shows any loss in bone density that may have occurred during the interval period. Data from the DEXA scan(s) of each patient were converted to bar graph form and compared with a standard. By comparing graphs of the scans, points of bone loss could be readily detected and the amount of bone lost could be accurately calculated. It is important to note that DEXA scan results are printed and interpreted by comparing the bone mineral density of the patient to the average bone density of a young adult and then of a normal age matched candidate. Significant osteopenia is thought to exist if bone mineral density is two standard deviations below the young/normal population. In the female subjects with two DEXA scans, observable bone loss occurred to varying degrees in either the vertebrae, wrist, hip and/or any combination thereof. This bone loss could well be attributed to age and lifestyle. These data correlate with the higher rate of natural bone density decrease characteristic of most small build, post-menopausal women.

The data from patients who received a single DEXA scan could also be correlated with the weekly dose of Methotrexate and the duration of therapy. These data can then be compared with the respective female/male control graph (Fig. 12, 13), in order to determine whether bone loss has or has not occurred. Our study shows that some bone loss did occur in the female patients; however there was no decrease in bone density observed in the male patients.
Trials similar to those reported here have been conducted at other institutions around the country (Sems et al., University of Iowa, Buckley et al., Medical College of Virginia, Richmond, West, et al. Fitzsimmons AMC, Christophidis et al., Monash University, Australia, Cited in abstract proceedings of ACR, 1995). Although the sample used here is small, the data obtained fit favorably with that obtained by others. Therefore, the results obtained by other researchers and in the recent study allow us to conclude that low-dose Methotrexate, as used in rheumatoid arthritis therapy, is not associated with a decreased bone mineral density or any change in bone mineral density that could significantly contribute to the development of osteopenia and/or osteoporosis.


5. Gnudi, S. et al. The Effects of Methotrexate on Bone; A Densiometric Study Conducted on 59 Patients with Methotrexate Administered at Different Doses, 227-231.


