Ipriflavone: An Important Bone-Building Isoflavone

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Abstract

Ipriflavone, an isoflavone synthesized from the soy isoflavone daidzein, holds great promise in the prevention and treatment of osteoporosis and other metabolic bone diseases. It has been widely studied in humans and found effective for inhibiting bone resorption and enhancing bone formation, the net result being an increase in bone density and a decrease in fracture rates in osteoporotic women. While ipriflavone appears to enhance estrogen's effect, it does not possess intrinsic estrogenic activity, making it an attractive adjunct or alternative to conventional hormone replacement therapy. Preliminary studies have also found ipriflavone effective in preventing bone loss associated with chronic steroid use, immobility, ovariectomy, renal osteodystrophy, and gonadotrophin hormone-releasing hormone agonists. In addition, it holds promise for the treatment of other metabolic diseases affecting the bones, including Paget's disease of the bone, hyperparathyroidism, and tinnitus caused by otosclerosis.
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Introduction

Ipriflavone (chemical structure: 7-isopropoxyisoflavone), derived from the soy isoflavone, daidzein, holds great promise for osteoporosis prevention and treatment (see Figure 1). Ipriflavone (IP) was discovered in the 1930s but has only recently begun to be embraced by the medical community in this country. Over 150 studies on safety and effectiveness, both animal and human, have been conducted in Italy, Hungary, and Japan. As of 1997, 2,769 patients had been treated a total of 3,132 patient years.[1]

Pharmacokinetics

IP is metabolized mainly in the liver and excreted in the urine. Food appears to enhance its absorption. When given to healthy male volunteers, 80 percent of a 200 mg dose of IP was absorbed when taken after breakfast.[2] IP appears to be extensively metabolized. In dogs and rats, seven metabolites were identified in the plasma, labeled MI-MVII. In humans, however, only MI, MII (daidzein), MIII, and MV seem to predominate. The mean excretion half-life in healthy human volunteers was 9.8 hours for ipriflavone and ranged from 2.7-16.1 hours for its metabolites. Ipriflavone metabolism was not found to be significantly different in elderly osteoporotic or mild kidney failure patients than in younger, healthy subjects.[3] Studies using

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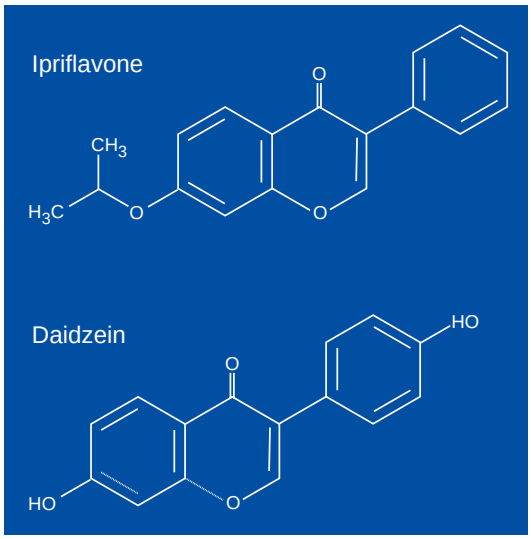
Figure 1. Chemical structure of Ipriflavone and Daidzein.



labeled IP in rats found it concentrated primarily in the gastrointestinal tract, liver, kidneys, bones, and adrenal glands.[3]

Review Of Bone Remodeling

Bone is subject to continual remodeling; i.e., the bone is renewed through a process of resorption of old bone by osteoclasts and formation of new bone by osteoblasts. Osteoclastic activity is stimulated by parathyroid hormone when serum calcium levels are low. Conversely, calcitonin is secreted from the thyroid in response to hypercalcemia, and antagonizes the bone-resorptive effects of parathyroid hormone. This process occurs in discrete sections called basic multicellular units (BMUs). This interaction between osteoclasts and osteoblasts is a coupled process.

Mechanisms of Action

Ipriflavone appears to have several mechanisms of action, all of which enhance bone density, making IP seemingly superior to many of the other treatments available for osteoporosis prevention and treatment. While it has been popular to label osteoporosis drugs as primarily either anti-resorptive or bone-forming, this does not take into account the fact these two processes are coupled. Because of this coupling, substances which have a beneficial effect on prevention of bone resorption by osteoclasts may also prevent osteoblastic activity when taken long-term. Treatments which are primarily anti-resorptive include estrogen, calcium, bisphosphonates, and calcitonin, while sodium fluoride, anabolic fragments of parathyroid hormone, and insulin-like growth factor demonstrate mainly bone forming activity.[4-5] While IP is considered to be primarily an anti-resorptive, it also possesses bone forming properties.

Anti-resorptive mechanisms: An animal study found IP inhibited parathyroid hormone-, vitamin D-, PGE2- and interleukin 1ß-stimulated bone resorption.[6] Bonnuci et al found parathyroid-stimulated osteoclastic activity and resulting hypercalcemia were inhibited in a dose-dependent manner by IP supplementation in rats.[7]

Ipriflavone metabolites have also been found to inhibit bone resorption. An *in vitro* study on fetal rat long bones found all metabolites capable of inhibiting parathyroid-stimulated bone resorption.[8] MIII was the strongest inhibitor, approximately three times more potent than MII; MI and MV were the least potent.

Azria et al observed no inhibition of bone resorption of incubated bone slices or changes in rat osteoclast motility at IP concentrations greater than 100 times peak blood concentrations after a standard therapeutic dose.[9]

On the contrary, Notoya et al found ipriflavone to inhibit bone resorption by mouse osteoclasts. The mechanisms involved included inhibition of both the activation of mature osteoclasts and the formation of new osteoclasts.[10] When IP was combined with vitamin K in cell media, an additive inhibition of bone resorption was noted. In this respect, vitamin K and ipriflavone appear to have similar mechanisms of action. However, ipriflavone, but not vitamin K, was found to stimulate alkaline phosphatase activity, an

indicator of new bone formation. The authors concluded the inhibitory effects of IP on bone resorption are similar to those of vitamin K, while mechanisms for osteoblastic activity are different.[11]

Other *in vitro* studies of isolated osteoclasts using bone resorption assays and measurements of intraosteoclastic calcium found ipriflavone inhibited osteoclastic activity (motility and resorptive activity) by modulating intracellular free calcium. These results were achieved at concentrations mimicking the plasma concentrations reached from typical oral IP dosages *in vivo*.[12] Other researchers confirmed the effect of ipriflavone on calcium influx in chicken, rat, and rabbit osteoclasts and preosteoclasts.[13] The effect of calcium influx into osteoclasts has not been clearly elucidated. Miyauchi et al found IP increased intracellular calcium in osteoclasts and pre-osteoclasts, and that osteoclast maturation was inhibited. These findings suggest the high calcium concentration in precursor cells inhibit osteoclastic maturation.

Bone-forming mechanisms: An *in vitro* examination of the osteoblastic effect of IP and its metabolites resulted in some interesting findings. Ipriflavone and metabolite II stimulated cell proliferation of an osteoblast-like cell line (UMR-106a – a cell line often used to determine the effect of various hormones and drugs on bone metabolism). IP and metabolite I increased alkaline phosphatase activity, metabolite V enhanced collagen formation, and IP alone inhibited parathyroid hormone activity.[14]

Bone marrow osteoprogenitor cells and trabecular bone osteoblasts were isolated from human donors and incubated with IP and its metabolites. These substances were found to regulate osteoblastic differentiation by enhancing the expression of important bone-matrix proteins and facilitating mineralization.[15]

Further evidence of ipriflavone's direct action on osteoblastic activity was provided by Sortino et al, who found IP to affect intracellular messenger systems in UMR-106a cells by inhibiting both calcium influx into osteoblasts and phosphoinositide hydrolysis. Both calcitonin and estrogen act to preserve bone in a similar manner.[16]

Bonucci et al found *in vitro* IP applications stimulated osteoblast-like cell proliferation and inhibited both parathyroid-induced bone degeneration and preosteoclastic cell proliferation. The researchers concluded the inhibition of resorption may be an indirect effect, mediated by osteoblasts.[17]

Effect on Advanced Glycation End Products (AGE): AGE (proteins nonenzymatically reacted with sugar) have been implicated in a number of chronic degenerative conditions especially related to diabetes and aging. AGE have also been implicated in bone resorption around amyloid deposits in dialysis-related amyloidosis. Both ipriflavone and calcitonin were found, *in vitro*, to inhibit this AGE-associated bone resorption.[18] This may have implications for age- and diabetes-related osteoporosis as well.

Lack of Estrogen Effect: One of the benefits of ipriflavone in the treatment of osteoporosis is its lack of estrogenic effect. Melis et al administered ipriflavone or placebo to a group of 15 postmenopausal women. Leutinizing hormone, follicle-stimulating hormone, prolactin, and estradiol were measured after a single oral dose of 600 or 1000 mg, and after 7, 14, and 21 days of treatment with 600 or 1000 mg doses. No differences in endocrine effect were noted between the ipriflavone and placebo groups. To examine the neuroendocrine effect, the women received a naloxone infusion (to block the opioid effect of estrogen) before and after 21 days of treatment with ipriflavone, conjugated estrogens (0.625 mg/day), or placebo. There was

no evidence of central nervous system opioid effect with IP or placebo; whereas, estrogen therapy restored the opioidergic activity, with a decrease in climacteric symptoms. Vaginal cytology was unchanged after 21 days of IP or placebo compared to a significant increase in superficial vaginal cells in the estrogen group.[19]

In vitro investigation of the interaction between ipriflavone and preosteoclastic cell lines found it was not mediated by direct interaction with estrogen receptors.[20] Instead, unique binding cites for ipriflavone were identified in the nucleus of preosteoclastic cells. The presence of IP binding sites was confirmed by Miyauchi et al. They identified two classes of binding sites in chicken osteoclasts and their precursors.[13] Similar IP binding sites have been identified in human leukemic cells, a line with similar characteristics to osteoclast precursors.

IP metabolites were also tested and the only one which exhibited any affinity for estrogen receptor binding, although weak, was metabolite II (daidzein, a known soy isoflavone phytoestrogen). Daidzein's effect was not strong enough to influence growth or functional characteristics of the preosteoclastic cell line.[20]

While IP does not have a directly estrogenic effect, it appears to potentiate estrogen's effect. Calcitonin secretion is modulated by estrogen, the levels of calcitonin significantly dropping in ovariectomized rats. Estrogen replacement returned calcitonin levels to normal after three weeks. While ipriflavone alone did not enhance calcitonin levels, it acted synergistically with estrogen, necessitating lower doses of estrogen to achieve normal calcitonin secretion. It appears IP increases the sensitivity of the thyroid gland to estrogen-stimulated calcitonin secretion.[21]

Cecchini et al found ipriflavone inhibited bone resorption, in a manner similar to estrogen, in both intact and ovariectomized rats, without a uterotropic effect.[22] The compound appears to have a selective effect on bone but not reproductive tissue, suggesting it may behave as a selective estrogen receptor modulator, similar to raloxifene and droloxifene, without the potential harmful effects associated with this new class of drugs (See Table 1).

In another animal study, ipriflavone was found to have a uterotropic effect on intact but not ovariectomized rats. However, when administered simultaneously with estrone and estradiol to the ovariectomized animals, it potentiated the effect of the estrogens. This seems to again point to the lack of direct estrogenic effect of IP while augmenting existing estrogenic effects.[23]

Effect on Crystalline Structure: Certain osteoporosis medications, such as sodium fluoride, increase bone density but change the crystalline structure, making the bone actually more fragile.[24] A study using high doses of ipriflavone (200-400 mg/kg/day) in rats for 12 weeks found no change in the crystalline structure of the bone. The researchers concluded "the positive effect of ipriflavone on bone mineral density appears to be associated with an increased apatite crystal formation rather than an increase of crystal size."[25] A study on rat long bones found ipriflavone increased the resistance to fracture by 50 percent without changing mineral composition or bone crystallinity.[26]

Ipriflavone and Osteoporosis: The Clinical Evidence

In the last decade there have been over 60 human studies – many double-blind and placebo-controlled – on the use of ipriflavone for the prevention and reversal of bone loss. An overview of these studies follows.

A two-year, double-blind, placebo-controlled trial was conducted in nine Italian centers. Postmenopausal women (n=196

Table 1. Osteoporosis therapies, mechanisms, benefits, and risks.

Medication	Primary Mechanism	Benefits	Risks
Conventional ERT	Inhibits bone resorption	Improves vasomotor symptoms of menopause	Increased risk of estrogen-related cancer, venous thrombosis, hypertension, gallbladder disease
Calcitonin	Inhibits bone resorption	No estrogenic effect; analgesic effect on bone pain	Secondary hyperparathyroidism; anti-CT antibody production Injectable: nausea, vomiting, vertigo Intranasal: rhinitis, nasal irritation
Bisphosphonates	Inhibits bone resorption	No estrogenic effect	Gastric erosion, thyroid adenoma (rats); Safety of Tx. > 4 years not known
Sodium Fluoride	Stimulates osteoblasts (bone formation)	Increases bone mineral density for up to 5 years	Abnormal bone crystallinity; no decrease in hip fracture rate; decrease in cortical bone in favor of trabecular bone; acute GI nausea, vomiting, and hemorrhage
Selective Estrogen Receptor Modulators (Raloxifene)	Inhibits bone resorption	Estrogen effect on bone and lipids; not on breast or uterus	Increased risk of venous thrombosis, pulmonary embolism; fatal liver toxicity; ovarian tumors and hepatic cancer (animals)
Ipriflavone	Inhibits osteoclast and enhances osteoblast activity	No estrogen effect; fewer side effects; analgesic effect on bone pain	GI upset

completers) aged 50-65 with established primary osteoporosis were randomly assigned to receive either ipriflavone (200 mg TID with meals) or placebo; subjects in both groups also received one gram calcium daily (in the forms of gluconolactate and carbonate). Inclusion criteria included a bone mineral density (BMD) of the distal radius at least one standard deviation below the mean and x-ray evidence of osteopenia. BMD was measured by dual photon absorptiometry (DPA). After two years the IP-treated group had demonstrated insignificant increases in BMD while the placebo group experienced a decline in bone mineral density, with an average difference between the placebo and IP groups of 3.5 percent.[27]

A similarly designed double-blind study evaluated 453 postmenopausal women age 50-65 with either radial (measured by DPA) or lumbar vertebral bone density (determined by dual x-ray absorptiometry – DEXA) at least one standard deviation below the mean and x-ray evidence of osteopenia.

They were randomly assigned to receive either ipriflavone (200 mg TID) plus one gram calcium or placebo plus calcium. At the end of the two-year study, those women on ipriflavone maintained bone mass in both the spine, which is primarily trabecular bone, and the distal radius, where cortical bone predominates. While density of the hip and pelvis were not evaluated, since they are a combination of cortical and trabecular bone, it is not unreasonable to assume protection in this area as well. A significant decrease in BMD was noted in the placebo group. Metabolic markers of bone loss were also affected by ipriflavone. Serum bone Gla-protein (BGP) and urinary hydroxyproline/creatinine (HPO/Cr), signs of bone turnover, were measured every six months during the study and found to be significantly elevated after one year in the placebo group. The IP group had no change in BGP and a decrease in HPO/Cr.[28]

In addition to helping prevent bone loss, IP can also contribute to increased bone density. A study of 198 women, designed exactly like the two studies cited above, found a one percent increase in vertebral bone density after two years on ipriflavone, while the placebo group experienced significant bone loss.[29]

A double-blind study on 40 women, using the same protocol, found similar results. After 12 months the placebo group experienced a 2.2-percent decrease in bone density in the spine and 1.2-percent decrease in the forearm, while BMD increased in the IP group by 1.2 percent in the spine and 3 percent in the forearm.[30]

An interesting Hungarian study was conducted on 91 postmenopausal women age 47-70 who were given either IP (200 mg TID) or placebo; both groups received calcium. For analysis the researchers divided the groups into an early menopause group (menopause < 5 years) and a late menopause group (> 5 years). There were no statistically significant differences between the placebo and treatment groups in the early menopause group; however, the late menopause group and the total study population had a statistically significant increase in BMD at the lumbar spine after six months compared to the placebo group who experienced a decrease. While both the placebo and IP groups experienced an initial increase followed by a decrease in bone density at the femoral neck, the decrease reached statistical significance only in the placebo group. Interestingly, the peak effect of ipriflavone in this study was reached after six months of treatment. Thereafter, significant differences between the two groups were not observed. This led the researchers to speculate whether the most positive clinical results might be achieved with intermittent IP therapy.[31] A cyclic approach to treatment with ipriflavone remains to be investigated.

It appears ipriflavone may be particularly effective for treatment of so-called "senile osteoporosis" (osteoporosis in women or men over age 65) as evidenced by the results of two studies in seven Italian centers. In one double-blind, two-year study of 28 elderly (age 65-79) osteoporotic women with x-ray evidence of at least one vertebral fracture, subjects received either 200 mg ipriflavone three times daily or placebo, plus one gram calcium. The IP treated group demonstrated a significant increase in BMD (6 percent after one year). The placebo group experienced a small but statistically insignificant decrease. In addition, urinary hydroxyproline was significantly decreased in the IP group, suggesting a decrease in bone turnover. Subjective reports of decreased bone pain and use of analgesics were noted.[32]

Another study, designed exactly as the one above, found similar results. In 84 subjects a 4-percent increase in radial bone density was noted after two years in the IP group and a statistically significant 3-percent decrease in the placebo (calcium only) group. The most clinically relevant finding was a decrease in

fracture rates in the IP group (2 of 41 patients experienced fractures in the IP group, whereas 11 of 43 experienced fractures in the placebo group).[1]

Ipriflavone Combined with Other Nutrients or Medications

Some studies have combined ipriflavone with other bone-preserving supplements or medications. A Japanese study examining the effect of combining ipriflavone with 1α vitamin D (a form commonly used in Japan for osteoporosis) found a decrease in vertebral bone density in the vitamin D (1 mcg/day), ipriflavone (600 mg/day) and placebo groups, but a maintenance of bone density in the combined group.[33]

A number of studies have examined the effect of ipriflavone and estrogen for the treatment of osteoporosis. While low doses of conjugated estrogen (0.15-0.30 mg/day) typically are high enough to prevent hot flashes and other neurovegetative symptoms of menopause, a somewhat higher dose (0.625 mg/day or higher) is generally necessary for bone protection. Some studies, however, found when combining ipriflavone and estrogen, lower doses of estrogen afford protection.

An Italian study examined 133 healthy postmenopausal women at risk for developing osteoporosis because of family history, smoking, low calcium intake, etc. Subjects, all receiving one gram calcium daily, were divided into five groups: 1) placebo; 2) placebo plus conjugated estrogen (CE) (0.15 mg/day); 3) placebo plus CE (0.30 mg/day; 4) 600 mg/day ipriflavone plus CE (0.15 mg/day); or 5) 600 mg IP plus CE (0.30 mg/day). After 12 months insignificant bone loss was noted in the placebo and both estrogen-plus-placebo groups. By contrast, an increase in BMD was reported in both estrogen-plus-IP groups, reaching statistical significance only in the IP-plus-0.30 mg CE. Symptoms of hot flashes were relieved in all groups except the placebo control group.[34]

Gambacciani et al studied 80 menopausal women (age 40-49) randomly divided into four groups, with 52 subjects completing the two-year study: 1) 500 mg/day calcium; 2) ipriflavone 600 mg/day plus 500 mg calcium; 3) 0.30 mg/day conjugated estrogens plus 500 mg calcium; 4) lower dose IP (400 mg/day), CE (0.3 mg/day) plus 500 mg calcium. Both the control and CE-treated groups experienced statistically significant decreases in vertebral bone density at 24 months (average of -3.7 percent in the control group and -2.2 percent in the CE group), while both the IP and IP-plus-CE groups experienced a small but significant ($P<0.05$) increase of 1.2 percent in both groups after 24 months.[35]

In another double-blind, placebo-controlled one-year study, 83 postmenopausal women were divided into three groups: 1) double placebo; 2) placebo plus CE (0.3 mg/day); or 3) CE (0.3 mg/day) plus IP (600 mg/day). After 12 months, those in the double placebo group demonstrated a progressive decrease in bone density; those in the CE group maintained their BMD for six months, but showed a 1.4 percent bone loss at the end of 12 months; and those in the CE-plus-IP group showed a significant increase in BMD after one year (+5.6 %; $p<0.01$).[36]

Not all studies have found ipriflavone protective from bone loss when combined with low dose estrogen. In a study comparing several protocols: 1) 500 mg calcium (controls); 2) 25 mcg transdermal estradiol plus five mg medroxyprogesterone (12 days); 3) 50 mcg transdermal estradiol plus five mg medroxyprogesterone (12 days); 4) 600 mg IP; or 5) 600 mg IP, 25 mcg transdermal estradiol, and 5 mg medroxyprogesterone, only the group taking the higher estrogen dose showed any significant increase in bone density (+1.84%). The IP group showed slightly improved bone density (+0.11%), while the

IP-plus-25 mcg estradiol group actually experienced a slight decrease (-0.22%).[37]

Many practitioners in their search for safer forms of estrogen replacement have turned to the weaker estrogen, estriol. However, its use for the prevention of osteoporosis remains controversial.[38] A Japanese study compared the use of ipriflavone alone or with estriol.[39] Seventy-nine postmenopausal women receiving ipriflavone (600 mg/day) alone or in combination with 1 mg estriol daily were compared to controls who received nothing. After one year, the controls demonstrated a 4-5 percent decrease in bone density. Both the IP and the IP-plus-estriol groups maintained bone density over the course of the study, with no significant difference between the latter two groups. This study points to the efficacy of ipriflavone but not low-dose estriol in bone preservation. It is possible a higher dose of estriol would prove more efficacious.

An open, controlled 12-month trial compared ipriflavone with salmon calcitonin in 40 postmenopausal women. Significant increases in bone density were observed in both groups after 12 months: a 4.3-percent increase in BMD in the ipriflavone group and a 1.9-percent increase in the calcitonin group. Markers of bone loss (serum osteocalcin, alkaline phosphatase, urinary calcium, and hydroxyproline/calcium ratio) were significantly reduced in both groups.[40]

Ipriflavone in the Prevention of Surgical or Drug-Induced Osteoporosis

Gonadotropin hormone-releasing hormone agonists (GnRH-A) such as Lupron, are used to induce hypogonadism, for the treatment of such conditions as uterine fibroids and endometriosis. These drugs induce a temporary menopause-like condition characterized by rapid bone loss as well as hot flashes and other symptoms of menopause. Researchers examined the effect of ipriflavone in restraining bone loss induced by these drugs. In a double-blind, placebo-controlled trial, 78 women treated with GnRH-A (3.75 mg leuproreline every 30 days for six months) were randomly assigned to receive either ipriflavone (600 mg/day) or placebo; both groups received 500 mg calcium daily. In placebo subjects, markers of bone turnover (urinary hydroxyproline and plasma bone Gla) were significantly elevated while BMD decreased significantly after six months. Conversely, there were no changes in BMD or bone markers in the ipriflavone-treated group. Although BMD improved in the placebo group after withdrawal of leuproreline, it was still below baseline values at 12 months (six months after discontinuation of the drug).[41]

Typically an ovariectomy results in rapid bone loss. In order to examine the effect of ipriflavone in the prevention of this bone loss, 32 recently ovariectomized women received either 500 mg calcium or 600 mg ipriflavone in addition to the calcium for 12 months. In the calcium-only group, markers of bone loss (urinary hydroxyproline, serum alkaline phosphatase, and plasma bone Gla) increased significantly and BMD significantly decreased six months after surgery. On the other hand, radial bone density and biochemical markers in the ipriflavone group showed no significant changes, indicating ipriflavone appeared to protect women from the sudden bone loss often experienced after ovariectomy.[42]

Researchers examined the effect of a combination of ipriflavone and conjugated estrogen in preventing rapid bone loss after ovariectomy. Estrogen had been previously tested (at a dose of 0.625 mg/day), and was found to be ineffective in this population for preventing acute post-surgical bone loss. Women (n=116), post-ovariectomy, were divided into four groups: 1) placebo; 2) CE (0.625 mg/day); 3) 600 mg ipriflavone; or 4)

CE plus IP. Vertebral bone density was measured by the DEXA method and two biochemical markers of bone turnover, urinary pyridinoline and serum osteocalcin, were measured before, 24, and 48 weeks after beginning treatment. BMD was reduced in all groups after 48 weeks of treatment (6.1, 3.9, and 5.1 % in groups 1-3, but only 1.2 % in group 4 – the estrogen-plus-ipriflavone group). In this study, concomitant use of estrogen plus ipriflavone significantly slowed bone loss.[43]

Ipriflavone may be effective in preventing osteoporosis associated with long-term steroid use. An animal study found ipriflavone, administered orally to rats with steroid-induced osteoporosis, was able to increase bone density and mechanical strength of the tibia and femur. Human studies in this population are warranted.[44]

Osteoporosis may occur as a result of long-term immobilization of a limb. Two rat studies have found ipriflavone to either increase bone density[45] or slow bone loss[46] in this population. Studies on human populations are indicated.

Ipriflavone in the Treatment of Other Conditions

Paget's Disease: Several other pathological conditions involving bone may be helped by ipriflavone. Paget's disease of the bone is characterized by specific areas of rapid bone turnover with both increased osteoclastic and osteoblastic activity. This results in abnormal bone, increased fracture rate, and perhaps most distressingly, bone pain which can be quite severe. A small study of 16 patients with Paget's disease randomly allocated subjects to one of two cross-over regimes, either 600 mg or 1200 mg IP daily for 30 days with a 15-day washout period between each regime. Serum alkaline phosphatase and urinary hydroxyproline/creatinine, generally elevated in Paget's disease, were reduced during both sequences, alkaline phosphatase by an average of 31.5 percent and HOP/Cr by an average of 25 percent. Bone pain scores were reduced in both treatment groups with the most significant decrease in the 1200/600 mg daily regime.[47]

Hyperparathyroidism: Because *in vitro* studies have found ipriflavone to inhibit parathyroid-stimulated bone resorption, a small preliminary study tested its effectiveness in inhibiting bone loss associated with hyperparathyroidism. Nine patients with primary hyperparathyroidism, six females and three males age 34-72, were treated for 21 days with 1200 mg daily ipriflavone in three divided doses. In five patients the treatment was prolonged for 42 days. Statistically significant reductions in markers of bone turnover (urinary Ca/Cr and HOP/Cr) were observed in all patients after 21 days. By day 42 there was a trend toward increases in alkaline phosphatase and serum osteocalcin. The researchers explained this phenomenon as a positive uncoupling of osteoclastic and osteoblastic activity, since bone formation seemed not to be affected by the treatment. In other words, they postulated the increase in alkaline phosphatase was a result of increased bone formation rather than due to bone resorption.[48] The study was quite small and short-term, bearing further investigation.

Otosclerosis: Tinnitus, predominantly low tone, is a common symptom of otosclerosis. A small, double-blind study of 16 patients tested the effectiveness of ipriflavone or placebo in combination with stapedectomy in the treatment of tinnitus due to otosclerosis. Subjects were treated for three months preoperatively and three months postoperatively with 200 mg ipriflavone or placebo four times daily. During the preoperative phase, while ipriflavone resulted in no improvement in hearing loss, tinnitus was arrested in four of nine patients. One of seven in the placebo group experienced relief of tinnitus. Postoperatively, all patients in the ipriflavone group but only 50 percent of the

patients in the placebo group experienced relief of tinnitus.[49] The exact reason for ipriflavone's benefit in otosclerosis remains to be determined.

Renal Osteodystrophy: Chronic renal failure results in abnormalities of calcium, phosphorus, vitamin D, and parathyroid metabolism. The eventual outcome is a decrease in bone mineralization. Twenty-three hemodialysis patients with decreased bone mineralization due to renal failure (renal osteodystrophy) were administered ipriflavone (400-600 mg daily) and observed for a period of 1-9 months. Alkaline phosphatase levels significantly decreased with IP treatment, while calcitonin was significantly increased after one month compared with levels prior to treatment. Serum IP levels before and after hemodialysis were not much greater than for patients with normal kidney function. Ipriflavone increased serum calcitonin levels to a greater extent in these patients than in patients with normally functioning kidneys. There were no instances of adverse effects, indicating that, while this report is preliminary, ipriflavone may be a safe, effective supplement for patients in renal failure suffering from osteodystrophy.[50]

Oxygen-sparing: Experimental studies on the cardiological effects of ipriflavone in rabbits, dogs, and rats have found IP decreases cardiac oxygen consumption, a phenomenon which was more pronounced in anoxic conditions. Significant decreases in lactic acid concentrations in myocardial tissue, especially in areas of ischemia, were also observed. Ipriflavone also counteracted calcium accumulation in the mitochondria induced by coronary ligation. Overall, ipriflavone seemed to have an oxygen-sparing effect, positively influencing mitochondrial energetics.[51]

Safety of Ipriflavone

In general, ipriflavone appears to be quite safe and well tolerated. As of 1997, long-term safety of ipriflavone (for periods ranging from 6-96 months) had been assessed in 2,769 patients for a total of 3,132 patient years in 60 human studies in Hungary, Japan, and Italy.[1] The incidence of adverse reactions in the IP-treated patients was 14.5 percent, while the incidence in the placebo groups was 16.1 percent. Side-effects were mainly gastrointestinal (GI). Since the placebo groups in most studies received calcium, it is not unreasonable to assume calcium may have as much to do with GI effects as ipriflavone. Other symptoms observed to a lesser extent included skin

Table 2. Ipriflavone has limited effects on lab values.

Lab Test	# of patients tested	# of patients out of range	Percent out of range
Erythrocytes	1258	15	1.19
Leukocytes	1258	46	3.66
Hemoglobin	1244	8	0.64
Prothrombin time	108	1	0.92
Lymphocytes	749	22	2.94
AST	1086	8	0.74
ALT	1190	11	0.92
LDH	132	4	3.03
Gamma GT	700	10	1.43
Total bilirubin	1014	6	0.59
Total proteins	1184	5	0.42
Albumin	799	7	0.88
Gamma globulin	293	8	2.73
BUN	1121	18	1.61
Creatinine	1149	11	0.96
Creatinine clearance	141	2	1.42
Blood sugar	838	17	2.01
Total cholesterol	664	7	1.05
Triglycerides	467	7	1.50

Adapted from Angusdei D, Bufalino L. Efficacy of ipriflavone in established osteoporosis and long-term safety. *Calcif Tissue Int* 1997;61:26

rashes, headache, depression, drowsiness, and tachycardia. Minor transient abnormalities in liver, kidney, and hematological parameters were documented in a small percent of subjects (see Table 2).

A reduction in theophylline metabolism and increased serum theophylline was observed in a patient being treated with ipriflavone.[52] Animal studies indicated this may be due to inhibition of certain cytochrome p450 enzymes, resulting in diminished elimination of the drug via the liver.[53-54]

While ipriflavone was found to have potential for treatment of renal osteodystrophy and short-term use was without side-effects, pharmacokinetic studies have revealed elevated levels of ipriflavone and its metabolites in the serum of patients with moderate to severe renal failure.[55] Patients with mild renal disease seem to tolerate ipriflavone at doses similar to those of healthy subjects. Researchers recommend lower doses (200-400 mg/day) in patients with more advanced renal failure. Further study of its safety in this population is warranted.

Conclusion

The therapeutic benefits of ipriflavone in the prevention and treatment of osteoporosis have been well researched. IP appears to restrain bone loss in postmenopausal women and in some cases, particularly in elderly populations, stimulates new bone growth and decreases fracture rates. It has also been found to enhance the effect of low-dose estrogen on bone preservation. Ipriflavone appears to be effective in prevention of acute bone loss after surgery or GnHR-As, and may protect from steroid-induced osteoporosis as well. Preliminary studies have pointed to its effectiveness in the treatment of other conditions involving bone pathology, including Paget's disease, hyperparathyroidism, renal osteodystrophy, and tinnitus due to otosclerosis. Ipriflavone appears to exert its bone protective effects by inhibition of osteoclastic and enhancement of osteoblastic activity without having a direct estrogenic effect. While fracture rate was decreased by about 50 percent in some preliminary trials, longer term studies are indicated, particularly to evaluate ipriflavone's effectiveness in decreasing hip fracture rate. The Ipriflavone Multicenter European Fracture Study began in 1997; results will not be available until 2001.

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