Treatment of Osteoarthritis with Tiaprofenic Acid and Indomethacin
Use of the Pond-Nuki Canine Model for Biochemical and Histological Studies

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Summary
Cage-matched control and experimental greyhounds were sacrificed 12 weeks after production of instability in the right knee, due to anterior cruciate ligament cut. Eroded osteoarthritis and normal articular cartilage were used for the analyses. In these tissue samples, significant histological protection against cartilage breakdown was afforded by orally administered tiaprofenic acid 5 mg/kg bodyweight but not by orally administered indomethacin 1 mg/kg bodyweight. Tiaprofenic acid caused preservation of fast sedimenting proteoglycan aggregates, as well as retention of hyaluronate content and favourable proteoglycan aggregate S value levels. Biochemical data on indomethacin remain to be reported.

Medicinal chondroprotection in human osteoarthritis, although not proven, is a concept encouraging investigative efforts and has recently been reviewed (Altman & Gottlieb 1987). In establishing this research area, most progress has been made by the use of in vitro cell cultures, explant cultures and animal model studies. In this way, a growing list of pharmaceutical agents has been evaluated, and positive chondroprotective properties have emerged for certain drugs (Howell 1989). Increasingly, the nonsteroidal anti-inflammatory drugs (NSAIDs) in common use are being tested for either deleterious or favourable effects on cartilage metabolism, structure, degradative enzymes, etc. Although precise differences between these agents and their negative or positive effects on cartilage metabolism may be meaningful, results in humans cannot be inferred or predicted from data in animal models. Differences in multiple variables indicate the need for further investigations in human cartilage systems (Rainsford 1990).

Tiaprofenic acid has shown evidence of chondroprotective properties in animal models. Tiaprofenic acid is a 5-benzoyl-2 thienyl-propionic acid and is one of a series of alkyl derivatives of thienyl acetic acid (Sorkin & Brogden 1985). Indomethacin is a chlorobenzoyl indole-3-acetic acid (Rainsford 1990). Data on these 2 drugs suggest that their primary action is on the cyclo-oxygenase pathway, which is thought to be responsible for the oxygenation of free arachidonic acid to the unstable intermediate endoperoxides prostaglandin (PG) G2 and PGH2 (Rainsford 1990; Sorkin & Brogden 1985). Both drugs have strong anti-inflammatory actions in synovial membrane in both human and animal disease (Rainsford 1990; Sorkin & Brogden 1985). In addition to being a potent inhibitor of PG formation (Burkhardt & Ghosh 1987), tiaprofenic acid
has been shown to decrease proteoglycan degradation in human osteoarthritic cartilage explants; to decrease proteoglycanase and collagenase release in unstimulated cultures in human osteoarthritic cartilage; and to reduce interleukin-1 (IL-1) stimulation of proteoglycanase release in chondrocyte cultures (Pelletier & Martel Pelletier 1989; Shinmei et al. 1987). Vignon et al. (1989a) studied human femoral articular cartilage from osteoarthritic femoral head cartilage before surgery for total joint replacement, with respect to treatment of explant cartilage with tiaprofenic acid. In this protocol, patients with osteoarthritis were compared with normal controls. Phospholipase A2, proteoglycanase and collagenase activities were elevated in the osteoarthritis-positive control cartilages (Vignon et al. 1989a). When the cartilage explants were exposed to tiaprofenic acid in a similar dose response study, there was a reduction in proteoglycanase and phospholipase A2, but not collagenase (Vignon et al. 1989a). In a study involving synovial membrane and fluids taken from patients with rheumatoid arthritis, Vignon et al. (1989b) showed that tiaprofenic acid was a potent inhibitor of PGE2 (dinoprostone), PGF2α, and thromboxane B2. In another model (the 4-week immobilised rabbit knee joint), Ghosh (1989) also observed the specific conservative effects of tiaprofenic acid. There was evidence of reduced proteoglycan loss from articular cartilage and reduced turnover of proteoglycans measured in vitro in cartilage samples from a low dose tiaprofenic acid-treated group compared with controls. Indomethacin, a well established NSAID, has been used extensively as a comparator drug when assessing tiaprofenic acid. Anti-inflammatory and other properties show a close similarity with respect to a variety of parameters, except in relation to osteoarthritis. To our knowledge, no studies have been carried out on the effects of indomethacin or on structural proteoglycan in canine osteoarthritis models. Accordingly, certain novel parameters of proteoglycan structure have been used in our own laboratory to assess tiaprofenic acid and indomethacin in the Pond-Nuki dog model of osteoarthritis (Manicourt et al. 1988, 1989).

1. Materials and Methods

Tiaprofenic acid was administered in capsule form to greyhounds (bodyweight 20 to 25kg), previously examined for normal joints and subjected to surgical resection of the anterior cruciate ligament of the right hind leg. Seven dogs received tiaprofenic acid 5 mg/kg bodyweight for 11 consecutive weeks, commencing 1 week postsurgery. Three dogs received indomethacin 1.2 mg/kg bodyweight. Concurrently, 4 dogs were used as positive osteoarthritis controls and 4 cage-matched unoperated dogs comprised the negative controls. Starting after surgery and throughout the period of therapy, the dogs were walked daily for 2 hours as part of a mild exercise programme. After sacrifice at the twelfth week, the tibial plateaux of the operated joints were dissected and examined histologically and the femoral condyles were subjected to biochemical analysis. Full thickness samples of cartilage from presumed weight-bearing sites were used. Fragments of tibial plateau and femoral condyles were saved as dry weight and used in the denominators for subsequent measurements.

Histological sections of cartilage were stained, embedded by conventional techniques and graded according to the Mankin scoring system (Mankin et al. 1971). Biochemical analyses were made after papain digestion of approximately 100mg of wet cartilage tissue in order to determine the total hexuronate (PG) and hydroxyproline (collagen) contents in the cartilage (Manicourt et al. 1988, 1989), and also to establish the relative proportions of chondroitin-6-sulphate (C6S), chondroitin-4-sulphate (C4S), nonsulphated chondroitin (OS), and hyaluronate (HA) [Takemoto et al. 1985; Zebrower et al. 1986]. Transport ultracentrifugal analyses were made after collagenase digestion of approximately 300mg of wet cartilage tissue and subsequent purification in a Cs2SO4 density gradient. The g(S) polydispersity distribution function was obtained for each sample using an ultracentrifuge operating at 20°C, with a PG concentration of about 0.3 mg/ml in a solution containing a number of protease inhibitors. (For more detailed information on the