The Effects of Disodium Ethane-1-Hydroxy-1, 1-Diphosphonate on Adjuvant Induced Arthritis in Rats

Marion D. Francis*, Lawrence Flora, and William R. King
The Procter & Gamble Company, Miami Valley Laboratories, P.O. Box 39175, Cincinnati, Ohio 45239

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Freund's Adjuvant, consisting of dead Mycobacterium butyricum suspended in mineral oil, produces an arthritic response in rats which resembles certain forms of arthritis in man. The arthritic response consists of soft tissue inflammation, pannus formation and two separate osteopathies; accelerated bone resorption and abnormal periarticular bone formation. Disodium etidronate administered at 4 mg/kg/day subcutaneously from the time of adjuvant injection markedly inhibited bone resorption, pannus formation, inflammatory erosion of cartilage, and the pathologic bone formation associated with the adjuvant model. When the disodium etidronate treatments were discontinued, the pathologic bone formation became radiologically visible within two weeks after cessation of treatment. The role of disodium etidronate in controlling bone resorption and surrounding tissue concentration of calcium and phosphate and the relation to arthritic processes in this rat model are discussed. The data suggest a potential use of disodium etidronate in some forms of human arthritis.

Key words: Arthritis — Phosphonates — Bone — Resorption — Pathology — Calcification.

Freund's Adjuvant, de MYCOBACTERIUM BUTYRICUM tué, suspendu dans de l'huile minérale, provoque une arthrite chez les rats qui rappelle certaines formes d'arthrites chez l'Homme. L'arthrite comporte une inflammation de tissu mou, une formation de pannus et deux ostéopathies distinctes, une résorption osseuse accélérée et une formation anormale d'os périarticulaire. L'étidronate disodique, administré à raison de 4 mg/kg/jour par voie cutanée, à partir de l'injection de l'adjuvant, inhibe nettement la résorption osseuse, la formation de pannus, l'érosion inflammatoire de cartilage et la formation d'os pathologique, en rapport avec l'adjuvant. Après arrêt du traitement à l'étidronate disodique, la formation d'os pathologique devient visible radiographiquement deux semaines après l'arrêt du traitement. Le rôle de l'étidronate disodique sur la régulation de la résorption osseuse et sur la concentration du calcium et du phosphate, dans les tissus environnants, ainsi que les rapports des phénomènes arthritiques avec ce type d'expérience sont envisagés. Les résultats suggèrent la possibilité de l'emploi de l'étidronate disodique dans certaines formes d'arthrites humaines.

Freund's Adjuvans, das aus abgetötetem und in Mineralöl suspendiertem Mycobacterium butyricum besteht, verursacht bei Ratten eine arthritische Reaktion, welche gewissen Arthritiformen beim Menschen ähnlich ist. Die arthritische Reaktion besteht in einer Entzündung der Weichteile, einer Pannusbildung und zwei verschiedenen Osteopathien, ferner in einer beschleunigten Knochenresorption und einer abnormalen periartikulären Knochenbildung. Wird gleichzeitig mit der Verabreichung des Adjuvans Dinatriumetidronat in einer Dosis von 4 mg/kg/Tag subcutan gegeben, so werden sowohl die Knochenresorption, als auch die Pannusbildung, die entzündliche Knorpelerosion und die mit der Adjuvansgabe verbundene pathologische Knochenbildung merklich gehemmt. Wurde die Dinatriumetidronat-Behandlung abgebrochen, so wurde die pathologische Knochenbildung innerhalb 2 Wochen

For reprints: Dr. Marion D. Francis, FAIC, the Procter & Gamble Company, Miami Valley Laboratories, P.O. Box 39175, Cincinnati, Ohio 45239, U.S.A.
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Arthritic responses have been induced in a number of different animal species [11] including rats [14, 19, 21], pigs [23], guinea pigs [12] and rabbits [2, 12, 13]. Arthritis induced by Freund's Adjuvant [10] in rats produces lesions which resemble those observed in rheumatoid arthritis and rheumatoid spondylitis in man [15, 19, 20]. Although the exact mechanism of adjuvant-induced arthritis is unknown, certain characteristics of the disease suggest that it is caused by an immunologic response to constituents of the Mycobacteria capsule [15, 20]. The disease is apparently systemic, exhibiting not only polyarthritis but in some cases other manifestations such as splenic capsulitis, conjunctivitis, iritis, and the presence of cutaneous nodules [20]. The similarity of the changes which occur in the adjuvant model to those occurring in man, is further manifested by resorption of bone [1-3, 5, 9, 14, 20] and by subsequent calcification of inflamed tissue leading to bony ankylosis of the joints [1, 3, 24].

Phosphonate compounds such as disodium ethane-1-hydroxy-1,1-diphosphonate (disodium etidronate) have been shown to be effective crystal growth inhibitors of hydroxyapatite, the main constituent of bone, and to function through a mechanism resembling chemisorption [8]. Disodium etidronate and other phosphonates have been suggested for possible use in the treatment of osteoporosis [8] and have been shown to prevent bone resorption both in vitro [6] and in vivo [6, 7, 17]. Since the progression of some forms of arthritis regularly involves bone resorption and sometimes abnormal deposition of bone, it was of interest to study the effects of phosphonates such as disodium etidronate in the adjuvant model of arthritis.

Methods and Materials

Female Wistar rats (Camn Research Institute, Wayne, New Jersey) weighing 250-350 g were randomly allocated into five experimental groups and given treatments as outlined in Table 1, Part I. The modified Freund's Adjuvant (MFA) and mineral oil treatments were given on the first day only by means of a single 0.1 ml subcutaneous injection in the right rear foot pad. The MFA treated animals were given either disodium etidronate daily (MFA + disodium etidronate group) or saline daily (MFA + saline group) by subcutaneous injection. Whole-body radiologic examination was made initially and at 2-5 week intervals thereafter. When pathologic calcification became apparent radiologically in the adjuvant-injected limbs of the saline-treated animals, five animals were killed from each group. The MFA-injected feet and other areas where the arthritic response was apparent were removed and placed in ethanol. The tissues were demineralized using either 5% nitric or 5% acetic acid, embedded in paraffin, and 5 μ sections were stained with hematoxylin and eosin (HE) for histologic examination. The remaining animals in the mineral oil injected group were then maintained with no change of treatment for an additional 10 weeks, while animals in the MFA + disodium etidronate and MFA + saline groups were allocated into several additional groups and received treatments as shown in Table 1, Part II.

Radiologic examination was carried out on a dental X-ray unit (Oralix Super 50, model 412, North American Phillips Company, New York, New York) and 11"x14" nonscreen X-ray