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

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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 Adjuvans, das aus abgetötetem und in Mineralöl suspendiertem Mycobacterium butyricum besteht, verursacht bei Ratten eine arthritische Reaktion, welche gewissen Arthritisformen 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 abnormen 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 Knochenresorption und die mit der Adjuvansgabe verbundene pathologische Knochenbildung merklich gehemmt. Wurde die Dinatriumetidronat-Behandlung abgebrochen, so wurde die pathologische Knochenbildung innerhalb 2 Wochen.

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nach Behandlungsabbruch röntgenologisch sichtbar. Die Kontrollfunktion des Dinatrium- 
etidronates bei der Knochenresorption und bei der Calcium- und Phosphatkonzentration in 
den umliegenden Geweben, sowie die Beziehung dieser Substanz zu den arthritischen Prozessen, 
wie sie bei diesem Rattenversuch vorliegen, werden besprochen. Auf Grund dieser Resultate 
ist eine potentielle Anwendung des Dinatriumetidronates bei gewissen Formen der mensch- 
lichen Arthritis denkbar.

Introduction

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 resorp-
tion 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 phos-
phonates 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 (Cam 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 (Orafix Super 50, model 412, 
North American Phillips Company, New York, New York) and 11" × 14" nonscreen X-ray