治療薬としてのPTH

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abstract

副甲状腺ホルモン（PTH）は，骨形成作用を有する骨粗鬆症治療薬であり，骨折閾値以下にまで低下した骨塩量を増加させうる能力を有している．大規模臨床試験で，骨塩量の増加および脊椎圧迫骨折や非脊椎骨折の予防効果があることが証明されており，米国では2002年より，骨粗鬆症治療薬として認可されている．さらに，ステロイド性の骨粗鬆症や男性の骨粗鬆症に対しても骨塩量増加をエンドポイントとして，その効果が確認されている．ただし，皮質骨量に対する作用は十分ではない．

おもしろいことに，PTH治療に引き続くビスフォスフォネート治療は治療効果が相加されるが，併用療法では単独療法以上の効果は期待できない．動物実験において，高用量長期のPTH投与により骨肉腫の発生を認めたため，治験は一時中止されていた．しかし，今後再開の見通しが立ち，その場合には海外の標準である連日皮下投与ではない方法が採用される可能性が高い．

I はじめに

現在の骨粗鬆症治療薬は，ビスフォスフォネート・カルシトニン・女性ホルモンなど骨吸収抑制剤が主流である．代表的薬剤であるビスフォスフォネートは，強力に骨吸収を抑制し，骨塩量を増加させる．しかし，その増加は初年度には著明であるが，その後の増加には鈍化傾向が認められる．これまでの大規模臨床試験[1]の報告でも，3年で10%程度の増加にとどまる．

したがって，骨折閾値以下まで低下した骨量を増加させうる骨形成促進剤は非常に魅力的な存在である．副甲状腺ホルモン（PTH）は，骨形成を促進し骨量を増加させることが知られており[2]，米国では2002年に骨粗鬆症治療薬として認可されている．

II PTHが骨量に与える影響

Finkelsteinら[3]は，子宮内膜症の治療のためにGnRHアゴニストの投与を受け偽閉経状態となった女性患者を無作為に2群に分け，一方にヒトPTH（hPTH）（1-34）の40μg連日皮下投与を行った．その結果，PTH非投与群では12カ月で4～5%の骨塩量低下が腰椎・大腿骨に認められ，全身骨塩量でも2%の低下が認められた．ところが，同時にPTHを投与された群では，腰椎で逆に2%の骨塩量増加を認め，大腿骨では治療6カ月の時点まではPTH非投与群と同様に減少傾向が認められたが，12カ月時点では開始時の骨塩量に復していた．

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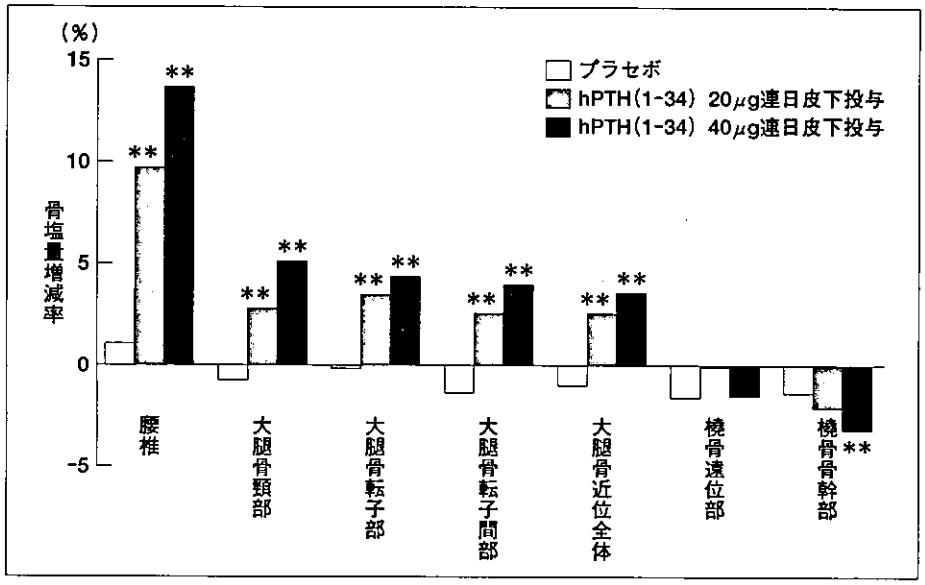

図1
PTH投与後の身体部位別骨塩量変化率
脊椎圧迫骨折を有する閉経後女性を各群500名前後で平均21カ月の観察．開始時の骨塩量に対する変化率で表示．
**：$p<0.001$ vs プラセボ
〔文献4)の表より作成〕

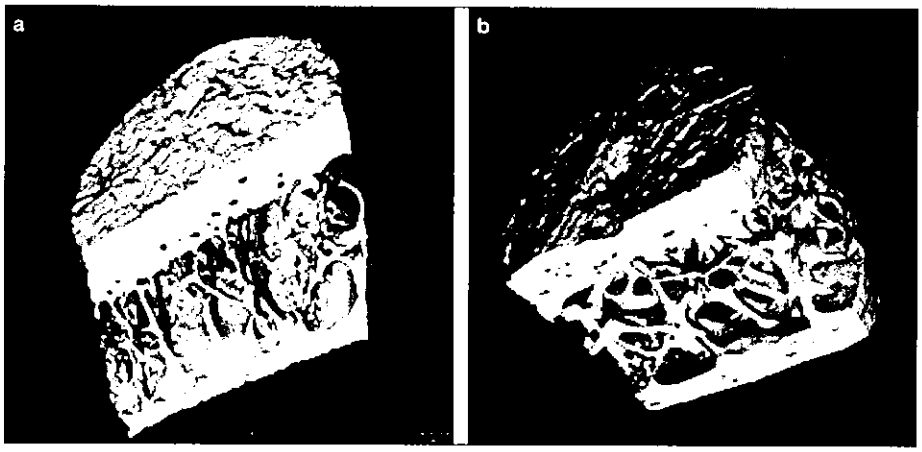

図2
PTH投与前後の腸骨骨梁構造の変化
65歳の女性にテリパラチド20μgを21カ月間投与し，投与前後で腸骨生検を行い，3Dμ CTイメージに再構築した．骨梁幅や皮質骨との連結を観察することができる．
a：投与前，b：投与後
〔文献8)より引用〕

この結果は，PTH投与が閉経後骨粗鬆症の治療薬として非常に有望であることを示している．実際その後に，Finkelsteinの同僚であるNeerを中心に行われた大規模臨床試験[4]により，その効果は確認された．彼らは1,637名の脊椎圧迫骨折を有する閉経後女性を3群に分け，1群にはプラセボを，残り2群にはhPTH(1-34)の20あるいは40μg連日皮下投与を平均21カ月間行った．40μg投与群では腰椎で13.7%，大腿骨頸部でも5.1%の増加を認めた．図1に示すように他の部位でも，有意な骨塩量増加が認められたが，橈骨骨幹部においては逆にPTH投与群で有意な減少を認めた．

PTH投与は，閉経後骨粗鬆症に限らず，ステロイド性骨粗鬆症[5]や男性における骨粗鬆症[6]に対しても有意な骨塩量増加をもたらした．これら海外でのPTHの投与方法は，すべて連日の自己皮下投与であるが，わが国での臨床研究では別の投与方法が採用された．Fujitaら[7]は，骨粗鬆症女性220例に対して，hPTH(1-34)（50，100，200U）を週1回の皮下注で投与し，1年後に最大用量群で腰椎骨塩量が8.1%増加したと報告している．

III PTHが皮質骨および骨構造に与える影響

先に述べたNeerらの研究[4]では，橈骨骨幹部の骨塩量はPTHの投与により逆に減少を示した．他の研究[3),5)~7)]でも，PTHの投与により橈骨骨幹部

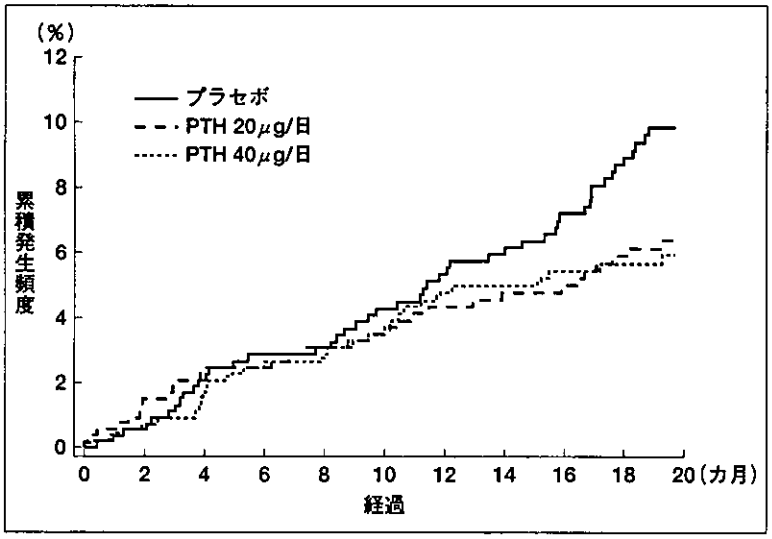

図3
非脊椎骨折の累積発生頻度
既存脊椎骨折を有する女性を3群（プラセボ，PTH 20μg/日，PTH 40μg/日）に分け，20カ月間追跡し，非脊椎骨折を起こした症例の累積割合を示す．開始時には3群間に差を認めないが，9～12カ月後にはPTH投与群よりもプラセボ群で非脊椎骨折を有する女性の割合が増加した．
〔文献4)より引用改変〕

などの皮質骨骨塩量を増加しうるという証拠は得られていない．これはおそらく，まず皮質骨の代謝が促進され，海綿骨の骨塩量増加の基礎になっているためだと考えられている．

一方，PTH投与後の骨梁構造変化に関しては，骨生検を用いた最近の報告[8]がある．Jiangらは，51人の脊椎圧迫骨折を有する閉経後5年以上を経過した女性に対して，無作為にテリパラチド〔rhPTH(1-34)〕の連日自己皮下投与を行った．群分けは，プラセボ（n＝19），PTH 20μg（n＝18），PTH 40μg（n＝14）であり，各症例において投与前後に腸骨骨生検を行った．PTH投与群では，**図2**で明らかなように海綿骨の容積・海綿骨間の結合性・皮質骨幅の増大が認められた．この図からも明らかなように，皮質骨に対するPTHの効果はそれほど強力ではない．

Ⅳ PTHの骨折抑制効果

骨塩量は確かに増加するが，骨折抑制に関する効果はどうであろうか．最も大きな臨床試験[4]では，1つ以上の新脊椎圧迫骨折発生率がPTH投与群で，65(20μg群)～69(40μg群)%抑制された．また，非脊椎骨折の累積発生頻度においても，PTH投与群では40%の抑制が認められた（**図3**）．ホルモン補充療法（HRT）をすでに受けている閉経後骨粗鬆症患者にPTHを3年間投与した場合にも，脊椎圧迫骨折は非投与群と比較して有意に抑制された[9]．

Ⅴ PTHとビスフォスフォネート

ともに強力な骨量増加作用を示すPTHとビスフォスフォネートであるが，どちらがより強力なのであろうか．Bodyら[10]は，この2つの薬剤の効果を閉経後骨粗鬆症患者を対象として，同一臨床試験において二重盲検にて比較した．その結果，PTH（テリパラチド40μg連日皮下投与）は，腰椎・大腿骨頸部・全身の骨塩量をビスフォスフォネート（アレンドロネート10mg/日）よりも有意に増加させ，非椎体骨折の発生抑制効果もアレンドロネートより優れていた．ただし，橈骨遠位1/3骨塩量はアレンドロネートに比較して有意に低下した．

2剤を連続して投与すれば，その効果は増加するであろうか，あるいは減弱されるであろうか．Rittmasterら[11]の結果を**図4**に示す．まず，PTH〔rhPTH(1-84) 50～100μg連日皮下〕投与により，1年で腰椎骨塩量は各群平均7.1%の増加を示した(対照群は1.3%)．次の1年は全員に10mg/日のアレンドロネートを投与したところ，1年目にPTH投与を受けた群では平均13.4%の腰椎骨塩量の増加を認め，1年目が対照群であった群は7.1%の増加を示した．この結果から，PTHに引き続きビスフォスフォネートを投与する方法は，単独投与よりもより効

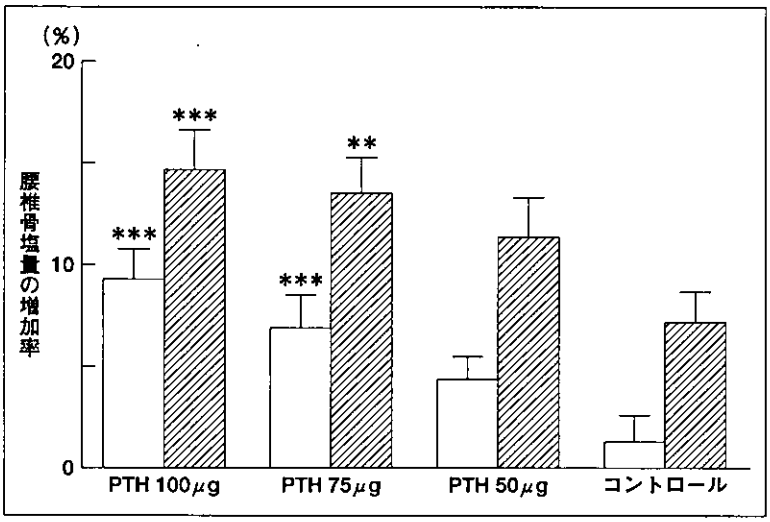

図4
PTHに引き続きアレンドロネートを投与した場合の腰椎骨塩量変化
閉経後骨粗鬆症患者66名を4群〔rhPTH(1-84) 50μg, 75μg, 100μgおよびプラセボ投与〕に分け，1年間経過観察．この時点での腰椎骨塩量増加を白抜きカラムで示す．次の1年はPTH投与を中止し，全員にアレンドロネート10mg/日を投与した．最終（2年後）時点での腰椎骨塩量増加率を斜線カラムで示す．
**：$p<0.01$ vs プラセボ/アレンドロネート群
***：$p<0.001$ vs プラセボ/アレンドロネート群
〔文献11)より引用改変〕

果的であることが判明した．

では，併用療法でも相加的効果を示すであろうか．この命題に挑戦した研究は，2003年の米国骨ミネラル代謝学会でmost outstanding awardを受賞するとともに，すぐにN Engl J Medにて公表された[12]．結果は否定的で，テリパラチドとアレンドロネートの併用は1年の前向き調査で，単独投与群よりも優れてはいなかった．PTH作用が発揮されるには，骨代謝回転が上がる必要があるのかもしれない．

Ⅵ PTHの日本における現状

前述のように，海外でのPTHの投与方法は連日の自己皮下注射が基本である．しかし，わが国では自己注射がなかなか認められないこともあり，臨床試験[7]で採用された週1回の間欠的皮下投与が標準となるものと思われる．

わが国の臨床試験は，その後，ラットへの高用量かつ長期のPTHの投与により，骨肉腫発生頻度が上昇することが判明し，中止された．しかしこの件に関して，米国食品医薬品局（FDA）がヒトにおける治療においては，期間および用量とも当てはまらないと判断し，骨粗鬆症治療薬として認可したため，再びわが国においても臨床試験が計画されている．現時点で3つの企業が臨床治験を予定している．すべてhPTH(1-34)であるが，2社はリコンビナントで残り1社が合成ペプチド．また，投与方法も2社は皮下注射であるが，残り1社は経鼻投与を考えている．骨粗鬆症治療のさらなる進歩はそこまできている．

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Use of Local Electroporation Enhances Methotrexate Effects With Minimum Dose in Adjuvant-Induced Arthritis

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Objective. **To investigate the effects of electrical pulses on the ability of methotrexate (MTX) to attenuate inflammation and subsequent joint destruction in rats with adjuvant-induced arthritis (AIA).**

Methods. **Rats in the experimental group received an intraperitoneal injection of MTX (0.125 mg/kg body weight), followed 30 minutes later by application of direct electrical pulses (50V, 8 Hz) to their left hind paws with an electroporation apparatus (M+/E+ group; n = 8). The procedure was repeated twice weekly for 3 weeks. Three control groups received the following treatments, respectively: MTX without electrical treatment (M+/E− group; n = 9), electrical treatment but no MTX (M−/E+ group; n = 10), or no electrical treatment and no MTX (M−/E− group; n = 9). Progression of AIA was monitored by joint swelling and radiologic and histologic changes in the ankle joint.**

Results. **Three weeks after injection of the adjuvant, and at the height of the arthritic reaction, the swelling and radiologic and histologic changes in the left hind paws in the M+/E+ rats were significantly reduced, as compared with changes observed in the control groups.**

Conclusion. **These results demonstrate that application of electrical pulses in combination with use of systemic low-dose MTX can ameliorate local arthritic reactions. This response probably occurs because electrical stimulation promotes transient passage of MTX through pores in the cell membranes, with a resultant local increase in the concentration of the drug within the cells. These results point to a potential use of electrochemotherapy to increase the efficacy of MTX or other drugs in an arthritic joint that is refractory to treatment, without increasing the dose of the drug.**

Although new biologic agents (1) can ameliorate inflammatory reactions and consequently protect the joints of patients with rheumatoid disease from progressive damage (2), methotrexate (MTX) remains one of the most effective and widely used disease-modifying antirheumatic drugs (DMARDs) (3). However, chronic inflammation often persists in isolated joints even after effective systemic MTX treatment, presumably as a result of an inadequate concentration of MTX in the joint that is refractory to treatment. In patients with persistent inflammation, synovectomy is often indicated for symptomatic relief, although data on the long-term clinical effectiveness of this approach are limited (4). Another option is an additional dose of MTX, but this increases the risk of adverse events. Because MTX has weak cell permeability, and the pharmacologic effects of this drug depend upon its intracellular concentration, any method for increasing intracellular MTX levels in the joint may be effective in attenuating the inflammatory response.

Electroporation has been used to facilitate the transport of nonpermeable molecules into cells. Transient cell membrane pores, generated electrically, allow nonpermeable molecules, including genes and drugs, to enter into the cells (5). Electroporation systems are now available for clinical use to deliver anticancer drugs into malignant solid tumor cells (6–8) as electrochemotherapy. Encouraging clinical results have been reported for the treatment of malignancies, in terms of efficacy, safety, and cost (9). This suggests that electroporation may be useful for the local treatment of rheumatoid arthritis (RA) that is refractory to conventional therapy.

We used electroporation to enhance the effect of

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low-dose MTX treatment on the progression to severe arthritis and associated joint destruction in a rat model of adjuvant-induced arthritis (AIA) (10–12).

MATERIALS AND METHODS

Animals. Inbred 7-week-old male Lewis rats were purchased from Charles River Japan (Kanagawa, Japan) and housed with free access to standard laboratory chow and water, under 12-hour dark/light cycles in conditioned air.

Induction of arthritis. The adjuvant mixture was prepared by mixing dried heat-killed *Mycobacterium butyricum* (Difco, Detroit, MI) in paraffin oil (Wako, Tokyo, Japan) at a concentration of 5 mg/ml. To induce systemic arthritis, 0.2 ml of the preparation was injected into the tail bases of 8-week-old rats that had received anesthesia via ethyl ether inhalation.

Pulsed electrical stimulation for electroporation. For electrical stimulation to generate transient pores in cell membranes at the target tissue site, we used an electroporation apparatus (CUY-21; Gene System, Osaka, Japan). Direct-current electrical pulses (8 Hz, 75 msec pulse duration, 50 volts/cm electrode distance) of 1-second duration were delivered 6 times during a single procedure. Each of the six 1-second pulses was applied by 2 parallel stainless steel electrodes that were moved between each pulse through 60° in a plane perpendicular to the long axis of the left hind paws, 30 minutes after an intraperitoneal injection of MTX or saline. We used electrode paste (Gelaid; Nihon Koden, Tokyo, Japan) to prevent skin burns.

Experimental protocol. The animals were assigned to an experimental group or to 1 of 3 control groups, as follows: MTX injection with electroporation (M+/E+ [experimental] group; n = 8), MTX without electroporation (M+/E− group; n = 9), electroporation with saline (M−/E+ group; n = 10), or no treatment (M−/E− group; n = 9).

MTX was provided by Wyeth-Pharmaceutical (Tokyo, Japan). The dose of MTX was set to 0.125 mg/kg body weight, based on preliminary experimental data indicating that no significant systemic antiarthritic changes were recognized at this dose. The drug was administered intraperitoneally twice weekly for 3 weeks, and the animals were killed by asphyxia in carbon dioxide (for radiologic and histologic examination).

These experimental protocols were in accordance with institutional regulations for animal care and were approved by the Institutional Committee for Animal Care of Osaka City University.

Gross inspection and radiologic evaluation. Twice weekly, the animals were weighed using an electronic balance, and hind paw thickness was measured with digital calipers. Three weeks after the adjuvant was injected, the animals were killed using CO_2 asphyxiation, and both hind limbs were harvested and fixed by perfusing cold 4% paraformaldehyde through the left ventricle, followed by immersion in cold 4% paraformaldehyde solution. Soft x-ray images of the hind paws were obtained with a soft x-ray apparatus (DCS-600EX; Aloka, Tokyo, Japan) using settings of 45 kV, 4 mA, and 30 seconds of exposure time. Destructive changes in hind paw bones seen on radiographs were evaluated by criteria previously described by Clark et al (13), with some modifications. Briefly, radiographic

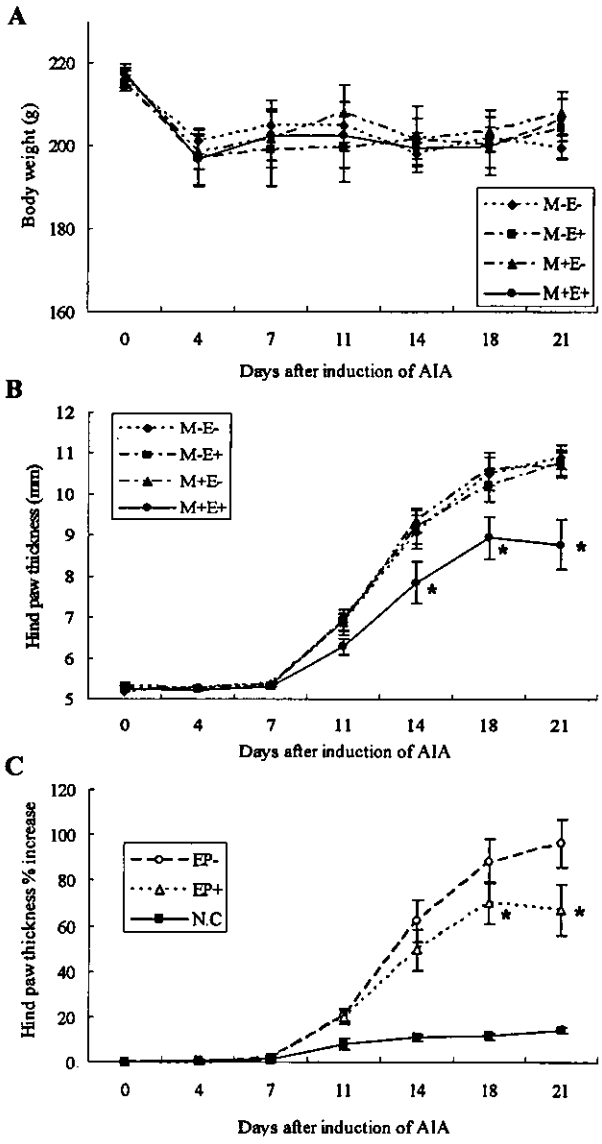

Figure 1. Effects of electrochemotherapy with methotrexate (MTX) on body weight and paw swelling in rats with adjuvant-induced arthritis (AIA). **A,** Weight loss was observed in all groups on day 4. There was no significant weight difference between the 4 groups throughout the entire study period. **B,** Left hind paw thickness, as measured by digital calipers, was maximal on day 21 in the M−/E− (no treatment; n = 9), M−/E+ (electroporation with saline; n = 10), and M+/E− (MTX without electroporation; n = 9) groups. The thickness of the left hind paw treated with electrical pulses after administration of MTX, 0.125 mg/kg/week (M+/E+; n = 8) was significantly decreased when compared with the other groups. * = $P < 0.05$ versus the M−/E−, M−/E+, and M+/E− groups. **C,** Effects of electrical pulses on paw swelling in the M+/E+ group. Electrical pulses were applied to the left hind paw only (electrically treated [EP+]) (n = 8), not the right paw (not electrically treated [EP−]) (n = 8). Application of electrical pulses after administration of low-dose MTX significantly inhibited hind paw swelling on days 18 and 21, as assessed by paw thickness and when compared with EP− paws. NC = negative control (non–adjuvant-injected model) (n = 5). * = $P < 0.05$ versus EP−. Bars show the mean ± SEM.

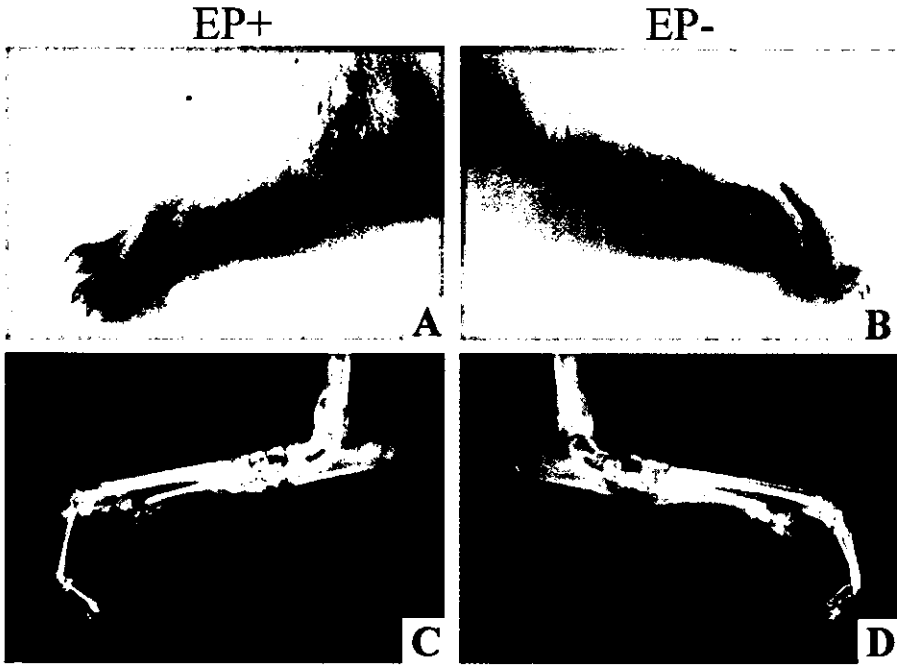

Figure 2. Gross appearance and radiographs of the hind paws of the same rat in the M+/E+ group on day 21. Following administration of MTX (0.125 mg/kg/week), electrical pulses were applied to the left hind paw only (EP+) (**A** and **C**). Note the obvious difference in the degree of swelling and joint damage between the left paw (EP+) and right paw (EP−) in gross appearance (**A** and **B**), as well as on soft x-ray (**C** and **D**). See Figure 1 for definitions.

changes in terms of radiodensity, subchondral bone erosion, periosteal reaction, and cartilage space were evaluated under blinded conditions by 2 rheumatologists (KI and TK) and graded on a 0–3 scale (where 0 = normal and 3 = severely damaged).

Histologic sections. Both hind paws were harvested from the animals for histopathologic examination. After the removal of skin, bones in the hind paws were decalcified in a neutral buffered 14% solution of EDTA/10% formalin, dehydrated in a graded ethanol series, embedded in paraffin, sectioned sagittally into 4-μm sections, and stained with hematoxylin and eosin or toluidine blue. Pathologic changes were evaluated by 2 observers according to a previously reported rating system (14), as follows: grade 0 = normal synovium, cartilage, and bone; grade 1 = hypertrophic synovium with cellular infiltration without pathologic change in bone and cartilage; grade 2 = pannus formation and cartilage erosion in addition to the hypertrophic synovium; grade 3 = additional severe erosion of cartilage and subchondral bone; grade 4 = loss of joint integrity and ankylosis.

In order to identify and count osteoclastic cells, sections were stained for tartrate-resistant acid phosphate (TRAP) using a staining kit (Sigma-Aldrich, St. Louis, MO). TRAP-positive multinucleated cells were counted in 11 selected fields (8 fields in the distal tibia and 3 fields in the talus), all at 100× magnification.

Statistical analysis. Body weight and hind paw thickness were evaluated by repeated analysis of variance and Fisher's protected least significant difference test. Pairwise comparisons were made using Wilcoxon's signed rank tests among groups. All statistical analyses were carried out using StatView software version 5.0 (SAS Institute, Cary, NC). P values less than or equal to 0.05 were considered significant.

RESULTS

Effects of electrochemotherapy on progression of AIA. No significant difference in body weight was noted between the 4 groups during the course of this experiment (Figure 1A), indicating that low-dose MTX, with or without electroporation, had little effect on the systemic physical condition of the rats with AIA.

The thickness of the hind paws in all rats was

Table 1. Radiologic and histologic scores and osteoclast numbers in rat AIA, 21 days after injection of adjuvant*

Group	Radiologic score (n = 8)	Histologic score (n = 8)	Osteoclast number (n = 5)
Right hind paw, EP-negative	3.8 ± 4.5	2.5 ± 1.2	77.6 ± 10.2
Left hind paw, EP-positive†	1.8 ± 2.2	1.3 ± 0.5	22.0 ± 2.4

* Values are the mean ± SD. AIA = adjuvant-induced arthritis; EP = electroporation.
† For all comparisons, $P < 0.05$ versus EP-negative.

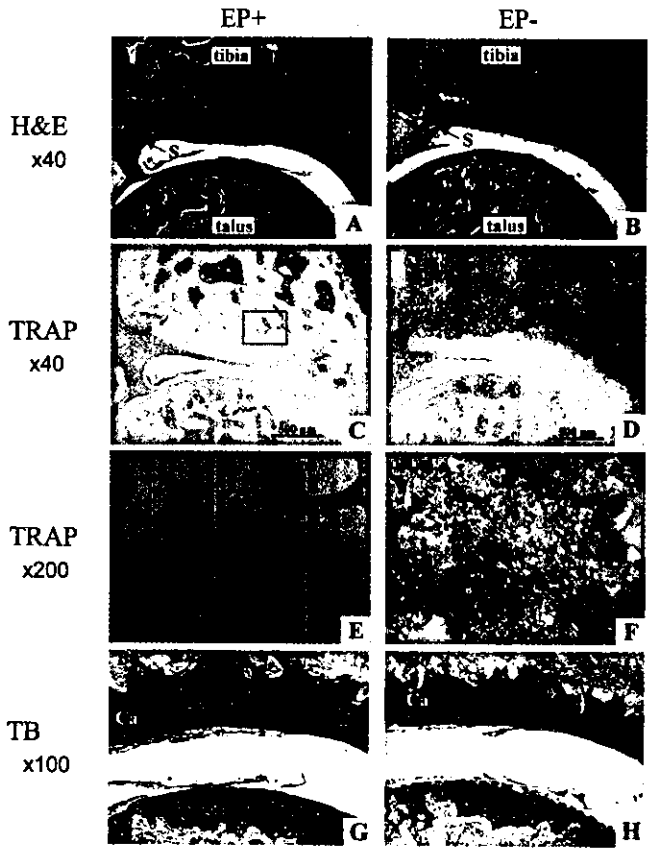

Figure 3. Histologic analysis of the ankle joints of the same rat in the M+/E+ group on day 21. **A** and **B**, Staining with hematoxylin and eosin (H&E). **C, D, E,** and **F**, Staining with tartrate-resistant acid phosphate (TRAP). **G** and **H**, Staining with toluidine blue (TB). The electroporation procedure was applied to the left ankle joint only (EP+) (**A, C, E,** and **G**). No inflammatory synovial tissue erosion into subchondral bone was observed with application of electroporation (**A**) compared with MTX only (**B**). Inflamed synovium infiltrated with lymphocytes was found to contain abundant osteoclastic multinucleated cells on TRAP staining (**D** and **F**). However, there was no difference in metachromasia of articular cartilage in the left and right hind paws. **E**, Higher-magnification view of the boxed area in **C**. **F**, Higher-magnification view of the boxed area in **D**. Bo = subchondral bone; Ca = cartilage; Pa = pannus; S = synovial tissue (see Figure 1 for other definitions).

significantly and consistently increased from day 11 until the end of the experiment. However, in the M+/E+ group, swelling of the left hind paw was significantly suppressed on days 14, 18, and 21 (Figure 1B) when compared with the 3 control groups (M+/E−, M−/E+, and M−/E−). The gross appearance of the hind paws is shown in Figures 2A and B. Thus, application of electrical pulses appeared to prevent the hind paw joints from progressing to advanced AIA. The degree of swelling differed significantly between the left (electrically treated [EP+]) and right (EP−) paws of the same rat in the M+/E+ group (Figure 1C).

Radiologic evaluation of bones and joints. Radiologic analysis revealed that the hind paw joints were severely damaged in the M−/E−, M−/E+, and M+/E− groups at 21 days after injection of the adjuvant. Therefore, at a dose of 0.125 mg/kg body weight, MTX did not prevent the joint damage (Figure 2D) or local swelling (Figure 2B) caused by progression of arthritis. In contrast, the radiologic damage score was significantly lower in the electrically treated left (EP+) hind paws in the M+/E+ group (Figures 2A and C and Table 1).

Histologic analyses. In the M+/E+ group, the histologic scores were significantly lower in the left hind paws (EP+) than in the right hind paws (EP−) (Figures 3A and B and Table 1). Inflamed synovial tissues with abundant lymphocytes were observed to erode into subchondral bone (Figure 3B). In sections of these joints, the population of TRAP-positive multinucleated osteoclastic cells was significantly lower in the bones of the left hind paw (EP+) than in those of the right hind paw (EP−) (Figures 3C, D, E, and F and Table 1). Toluidine blue staining revealed no degenerative changes of cartilage tissue, including irregularity of articular surface, disorganization of tidemark, and alternation of metachromasia, in either hind paw (Figures 3G and H).

DISCUSSION

These results indicate positive effects of pulsed electrical stimulation for attenuating arthritis by enhancing the antiarthritic effect of MTX. We believe that this is attributable to micropores created by the electrical pulses in the cytoplasmic membranes of cells in the synovium or other inflamed cells. The subsequent passive influx of MTX into the cells would attenuate the inflammatory responses that led to the AIA, although this study did not provide direct evidence of MTX influx. In this preliminary study, we could not identify the cells targeted by electrochemotherapy, and MTX-negative synovial cells, inflammatory cells, or both, may be targets for the drug.

The effects of electrical fields on living cells have been investigated since the 1960s, and high-voltage electrical pulses have been reported to generate transient and reversible pores in cell membranes. This phenomenon has been termed electroporation and is currently used to transfer genes or drugs into cells (6). Electrochemotherapy involves electroporation with drugs, and this methodology is used for the treatment of malignant tumors (5–9). The use of electrochemotherapy to introduce anticancer drugs into malignant tumors has been reported, e.g., bleomycin

for melanoma, basal cell carcinoma, Kaposi's sarcoma, squamous cell carcinoma (6), or chondrosarcoma (15). However, electrochemotherapy with MTX for the treatment of RA has not been reported, although the less-permeable character of MTX and its use as a DMARD in RA would make it an ideal candidate for this approach. Because the effect of pulsed electrical stimulation is expected only at the local site, this method might be applicable for an isolated joint with arthritis that is refractory to systemic chemotherapy or in the early stages of RA involving a limited number of joints without significant joint-destructive changes.

Clinical application of this therapy should not affect normal tissues. Using TUNEL staining, we did not observed any difference in the number of apoptotic cells between the M+/E+ and M+/E− groups (data not shown). We also confirmed in the pilot study that electrical pulses, used under the same conditions as those used in this experiment, did not influence the normal tissues of inbred 9-week-old male Lewis rats. In this pilot study, no inflammatory reactions were observed on histologic examination of the area treated with the electrical pulses, suggesting that electroporation under these conditions did not cause any damage to normal tissue, including cartilage, bone, muscle, and blood vessels (results not shown). However, the clinical application of electrochemotherapy requires further study, including the dose of MTX and the parameters of the electrical pulses.

This experimental study is limited in 2 key areas. First, electrochemotherapy was not applied to joints with established arthritis, and the effect of electrochemotherapy was estimated based on the progression of arthritis. This differs from the clinical situation, in which, as indicated previously (10,11), the inflammatory phase in this AIA model is self-limiting. Therefore, the efficacy of electrochemotherapy for the treatment of established chronic arthritis is difficult to determine in this model. Second, optimization of the application of pulsed electrical current may not be sufficient to obtain maximum delivery of MTX into cells and to achieve maximal antiinflammatory effect in RA. The conditions that enable the efficacy of electrical stimulation in electrochemotherapy may be quite different from the conditions used in the clinical treatment of malignancies that were reference sources for the present study. The potential value of electrochemotherapy for the treatment of RA has been illustrated by these studies, and further work is required to optimize electrochemotherapy to control disease in joints with RA refractory to treatment.

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