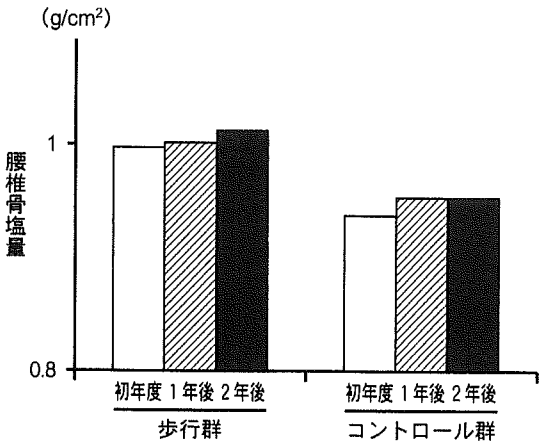

図2　歩行が腰椎骨塩量に与える影響

過去2年間に上肢の骨折を罹患した女性165名を無作為に歩行群とコントロール群（上肢の訓練のみ）に分け，2年間観察。初期の値には両群間に差を認めず，初年度から1年後，さらに2年後と，歩行群では軽度増加傾向を示すが有意な変化ではない。コントロール群でも腰椎骨塩量に変動を認めなかった。

（文献17より作成）

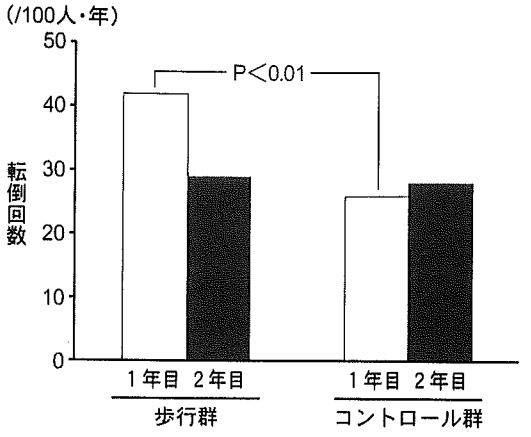

図3　歩行が転倒に与える影響

図2と同じ研究。観察期間中に生じた転倒回数を100人が1年間に転倒する回数に換算して比較。1年目で，歩行群に有意に多い転倒が観察された。

（文献17より作成）

転倒防止などを介した骨折予防であることを示している。

歩行と骨粗鬆症

歩行は最も簡単な運動であり，工夫をすれば継続して行える最も安価な運動療法である。これまでにも歩行が骨量増加に与える研究はなされており，Eibrahimらは，歩行の奨励により骨塩量の低下は抑制可能ではあるが，同時に転倒の危険性が増加する可能性があると指摘している（図2，図3）[17]。しかも，彼らの研究は上肢に骨折を生じた女性を対象としたもので，研究参加へのモチベーションは高いと考えられるが，2年間での脱落率は41%と高いものであった。現在のところ，歩行だけによる転倒予防を目的とした研究は行われていない。

転倒を防止できる運動

高齢者における運動の効果が骨折予防に結びつくとすれば，転倒予防を介しての効果であると考えられる。転倒危険因子に関しても多くの研究があり，それらから得られた結果をもとにして，多くの転倒予防プログラムが考案されている。多くの研究は，介入法として運動プログラムを用いているが，その効果は完全には証明されていない。

Robertsonらによるメタアナリシス[18]では，筋力強化とバランス改善プログラムにより転倒は35%減少したが，重度外傷発生に関しては効果がなかったと結論している。骨折を生じる転倒は全転倒の10%程度にすぎないと考えられ，骨折抑制を目標とした介入試験で有意な結果は出にくいと思われる。

単独の運動様式で効果が確認されているのは，太極拳である。Journal of American Geriatrics Societyの1990年代におけるベスト論文に選ばれたWolfらの報告[19]では，バランスの各種指標に太極拳参加が影響を与えなかったにも関わらず，転倒リスクを47.5%低下させたという。彼らは7年後に同様の研究成果を報告[20]しているが，このときは太極拳による転倒リスク減少率は25%で

コントロール群との間に有意差を認めなかった。しかし，単一の運動様式で25%も転倒リスクを減少できたことは十分に評価できるのではないだろうか。ただし，現実問題としてどれだけの人が太極拳を継続して実施できるのかという懸念は残る。

おわりに

骨粗鬆症の治療あるいは予防の観点から運動療法を考えるならば，骨量増加を運動療法の直接的な目的とすべきではないだろう。あくまでも目的は，転倒そして骨折の予防である。その目的を達成するためには，運動はもちろんのこと，薬物療法・環境改善・視力調整・装具など，様々な戦略が考えられる。その中で運動療法は，最も研究対象となりやすい方法と思われるが，転倒の増減をどのように評価するのか，効果判定をバランス改善や筋力増強で行って良いのかなど，解決すべき問題は多い。

さらに，運動療法の対象者は中高齢者である。ならば骨粗鬆症の運動療法は，簡単で継続性があり，しかも楽しんでできるものであることが望まれる。現時点では，単一でこの条件を満たす運動療法は見つかっていない。

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臨床分子内分泌学3

—甲状腺・副甲状腺・骨内分泌代謝系—

IV. BMPs

薬理作用と生理作用

骨形成促進作用

小池達也　　高岡邦夫

IV. BMPs

薬理作用と生理作用

骨形成促進作用

Stimulation of bone formation

小池達也[1]　高岡邦夫[2]

Key words：骨形成蛋白，Smad，骨粗鬆症，脊椎手術

はじめに

骨形成蛋白(bone morphogenetic protein: BMP)は，骨基質抽出物質が異所性に骨を誘導する現象から，1960年代にその存在が明らかとなった．その後，1988年にWozneyら[1]が最初のBMPを同定したのを皮切りに，現在までに15種類以上のBMPが同定され，TGF(transforming growth factor)-βスーパーファミリーに属することが明らかにされている．

異所性骨形成を指標に精製が進められたBMPであるが，すべてが骨形成促進作用を示すわけではなく，その後の発生学分野の研究により様々な組織の形成にBMPが関与していることが明らかにされている[2]．

1. BMPの骨形成促進作用

骨形成が要求される場としては，以下の3つの場合が考えられる．まず，発生段階で骨組織が形成されるときであり，生後では外傷などにより，骨組織の破断あるいは欠損を生じた場合の修復時と骨のリモデリングの場である．

a. 発生過程

脊椎動物の形態を規定するものは文字どおり骨格であり，我々の骨格の大部分は肢芽間充織などに由来する軟骨をもとにして，軟骨内骨化により形成される．この発生過程におけるBMP mRNAの局在は精力的に研究されており，各BMPの働きを考えるうえで重要な情報を提供している[3]．

例えば，BMP-5 mRNAは軟骨形成前の間葉系細胞に発現する一方で，BMP-2，4，7は軟骨を取り囲む間葉系細胞に発現している．また，BMPファミリーの一つであるGDF-5は関節が将来形成される部分に強く発現しており，その欠損マウスでは関節形成異常が生じる．つまり，軟骨の増殖分化のみならず，関節形成にGDF-5が重要な働きをしていることが明らかになっている[4]．

しかし，遺伝子操作マウスで遺伝子機能が明らかになる例はむしろまれで，BMP-2やBMP-4のノックアウトマウスは胎生期に死亡し，BMP-7のノックアウトマウスは生後すぐに死亡し，しかも骨における異常は顕著ではない．これらの結果は，BMPが胎生期の臓器形成に重要な働きを示すことだけでなく，他のBMP群との間で機能の補完作用が存在することを示している．幾つかのBMP因子の骨形成における役割は，自然発生変異体の解析からも明らかになっている．BMP-5はマウスの耳軟骨や肋骨形成異常を示すshort earの，GDF-5はbrachypodismの原因遺伝子であることが判明して

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いる[5].

b. 骨折治癒過程[6]

骨折治癒過程は多くの細胞やサイトカインが関与する複雑な系でコントロールされている．動物実験において，実験的に作製した骨折部にBMP-2，4やBMP受容体が発現することが報告されている．これらのシグナルは骨折を生じていない骨組織には検出されず，骨折部に出現する未分化間葉系細胞に弱いBMPシグナルが認められる．その後に，間葉系細胞のみならず新たに出現した軟骨細胞にもBMPが強く発現するようになる．これらの過程で，各BMPは発現強度も部位も完全には一致せず，それぞれが異なる作用を発揮して骨折治癒過程を押し進めていると思われる．

c. 骨量増加

骨組織においては，骨吸収が生じた部位に骨芽細胞が新しい骨を添加することが繰り返され，動的な平衡状態が保たれている．骨粗鬆症では，骨吸収を担う破骨細胞機能の亢進が大きな影響を有しているが，骨形成系細胞の機能低下も当然ながら骨量低下に関与すると考えられる．骨量測定値は正規分布を示し，これは特定の一部の因子により骨量が規定されていないことを示しており，骨粗鬆症は遺伝的背景に限っても多因子により制御されている疾患ととらえることができる．

しかし，疾患そのものあるいは治療を考えていくうえで，BMPと骨粗鬆症の関連を示す報告が幾つかある．osteogeninとして精製されたBMP-3は成人の骨に最も多く含まれる分子であるが，驚くべきことにBMP-3(−/−)マウスは野生型よりも高い骨量を示し，BMP-3は他のBMPの生理的な抑制因子であることが明らかとなっている[7].

また，アイスランド人を対象とした遺伝子解析により，骨折や骨量減少に関与する領域が20番染色体のp12.3に存在することが報告された[8]．ここにはまさにBMP-2遺伝子が存在しており，更に詳細な塩基配列解析により，欠失を含む3種類の変異が見いだされた．この結果はデンマークでの閉経後骨粗鬆症コホートにおいても確認された．病態としては，これらの変異により生成されるBMPは蛋白質としての安定性や輸送などに問題を生じるのではないかと考えられている．

一方，治療に向けての実験では，エストロジェン欠乏および加齢によるマウスの骨粗鬆症モデルにおいて，腹腔内にrh(recombinant human)BMP-2を投与すると，骨量が回復することが報告されている[9].

2. 骨形成促進におけるシグナル伝達

TGF-βスーパーファミリーに属する因子は，細胞表面に存在するI型とII型と呼ばれる構造が類似した2種類のセリン/スレオニンキナーゼ型受容体を介して細胞内にシグナルを伝達する．細胞内に入ったシグナルは，転写調節因子であるSmad系を介して，核に到達する．この一連の経路に様々な抑制因子あるいは促進因子が存在することが知られている．

a. Smad系[10] **(図1)**

II型受容体のキナーゼがI型受容体をリン酸化し，I型受容体が活性化される．この活性化されたI型受容体キナーゼの最も重要な基質がSmad群である．ほ乳類では8種類のSmadが同定されており，その機能から3つに分類されている．受容体からのシグナルを受け取った特異型Smad(receptor regulated Smad: R-Smad)は共有型Smad(common mediator Smad: Co-Smad)と複合体を形成して核内へ移行し，他の転写制御因子とともに標的遺伝子の発現を調整する．抑制型Smad(inhibitory Smad: I-Smad)はリガンド刺激により発現が誘導され，I型受容体と安定に結合することにより，R-Smadと受容体との結合を阻害し，シグナル伝達を抑制する(negative feedback).

b. 抑制因子

BMPのシグナル伝達の中心であるSmad系にも抑制因子(I-Smads)が存在するが，それ以外にも幾つかの抑制因子が報告されている．細胞外の因子としては，**図2**に示す因子群がBMPと複合体を形成し，リガンドと受容体の結合を阻害して，アンタゴニストとして作用する．例

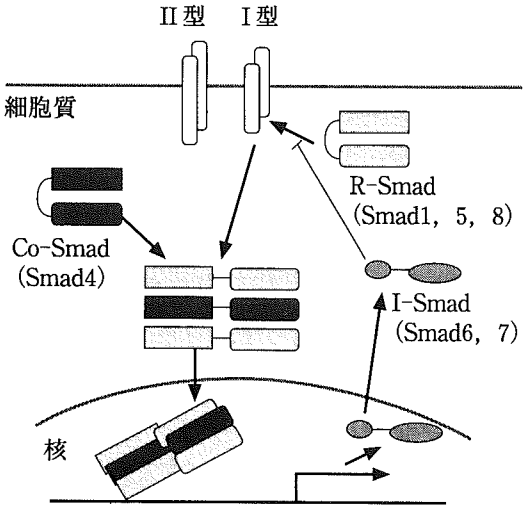

図1 BMPのシグナル伝達系

細胞表面に存在するI型とII型と呼ばれる構造が類似した2種類のセリン/スレオニンキナーゼ型受容体にリガンドが結合すると，II型受容体のキナーゼがI型受容体をリン酸化し，I型受容体が活性化される．この活性化されたI型受容体キナーゼは特異型Smad(receptor regulated Smad: R-Smad，BMPの場合はSmad1，5，8)をリン酸化し，共有型Smad(common mediator Smad: Co-Smad，Smad4)と複合体を形成して核内へ移行し，他の転写制御因子とともに標的遺伝子の発現を調整する．抑制型Smad(inhibitory Smad: I-Smad，Smad6，7)はリガンド刺激により発現が誘導され，I型受容体と安定に結合することにより，R-Smadと受容体との結合を阻害し，シグナル伝達を抑制する．

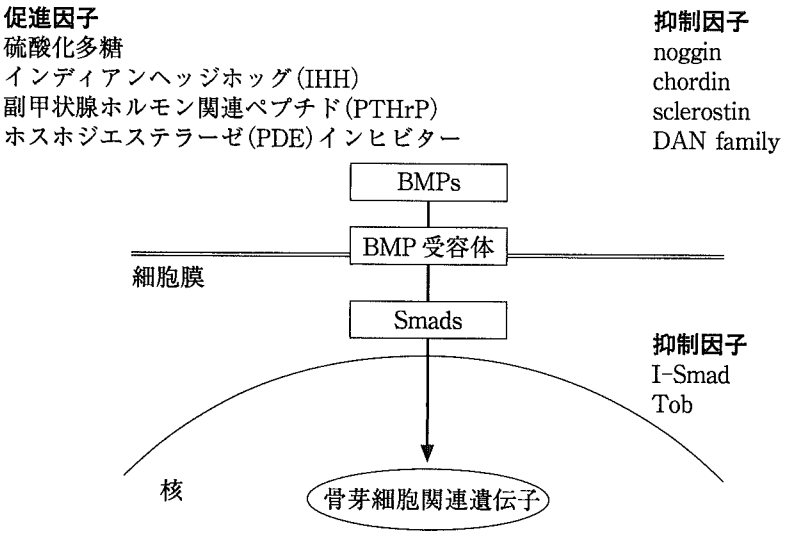

図2 BMP活性の抑制因子および促進因子

細胞内外に存在する抑制および促進因子を示す．

えば，nogginはBMP-7の受容体結合部位を覆い，その作用を阻害する．疾患との関連では，頭蓋骨癒合症の病態がnogginの存在にて説明することができる．通常，nogginはFGF(fibroblast growth factor)シグナルにより負に制御されているが，FGF受容体が持続的に活性化されている頭蓋骨癒合症ではnogginの発現が強力に抑制され，BMP活性が異常に高くなることで頭蓋骨癒合が生じると考えられる[11]．

細胞内の因子としては，I-Smads以外にSmad結合蛋白であるTobがある．増殖抑制遺伝子である*tob*を欠損したマウスは，骨芽細胞におけるBMPシグナルが亢進し，骨量が増加することが報告されている．その作用は，核内でTobがSmadに結合することにより達成されていると考えられている[12]．

c. 促進因子

BMPシグナルを抑制する因子が多数存在するにもかかわらず，促進する因子に関する報告は少ない．Indian hedgehogや副甲状腺ホルモン関連ペプチドがBMP活性を促進すると報告されているが，Takadaら[13]はヘパリンやヘパラン硫酸のような硫酸化多糖がBMP活性を増強することを証明した．詳細な機序はまだ解明されていないが，BMPがヘパリンカラムクロマトグラフィーで粗精製できること，および生体

内の多糖供給源であるプロテオグリカンの一種をノックアウトしたマウスが骨粗鬆症を呈することなどを考え合わせると，非常に興味深い報告である．

一方，著者らはcAMP分解酵素であるphosphodiesterase(PDE)の阻害剤がBMPの活性を増強することを報告してきた[14]．単独では全く作用を示さないことから，BMPのシグナル伝達経路に影響を与えていると考え，現在解明を進めている．

3. 臨床応用

現時点では，臨床応用に供されているのはrhBMP-2とrhBMP-7(OP-1)であり，アメリカ合衆国ではFDAにより認可を受けているが，我が国では許可されていない．BMPを局所へ投与するにはキャリアが必要で，キャリアは時間とともに分解され，BMPの局所濃度を保ち，骨形成の足場を提供し，過剰な骨形成を防ぐ性質をもっていることが望ましい．この目的のために，無機物・合成ポリマー・天然ポリマーなどが利用されている．

a. 脊椎手術

脊椎外科において脊椎固定術は頻用される手技であるが，多くの場合に骨移植を必要とする．異種同種の区別なく，骨移植には様々な合併症が伴うことが多く，骨移植を回避する方法の開発が望まれる．腰椎の1椎間の前方固定に適応があるのが，Medtronic Sofamor Danek社のINFUSE Bone Graft/LT-Cage Lumbar Tapered Fusion Device(rhBMP-2)である．ケージ内に自家骨を封入する群とrhBMP-2を添加した群で比較したところ，癒合率・手術時間・出血量・入院期間などで，BMP群の方が優れていた．ただし，使用BMP-2量は20mgである．

もう一つが，Stryker Biotech社のOP-1 Putty (rhBMP-7)である．腰椎後側方固定の再手術時に自家骨が用意できない状況では使用が認められており，自家骨移植と臨床成績に差がないことが報告されている．これはウシコラーゲンとrhBMP-7を混合して，パテのようにして使用する．

b. 骨折治癒促進

骨折部位に薬剤を塗布して骨癒合を促進させるという夢も，既に現実のものとなりつつある．Stryker Biotech社のOP-1 Implant(rhBMP-7)は，3.5mgのBMP-7をウシコラーゲンに混合して使用するが，脛骨の偽関節に対して，古典的治療法と差がない成績が得られている．

更に，Medtronic Sofamor Danek社はケージとともに脊椎固定に用いてきたInFUSE Bone Graft(rhBMP-2)を脛骨の新鮮な骨折に応用することを申請し，2004年5月にFDAに認可された．対象は新鮮な脛骨開放性骨折患者で，髄内釘による固定を行うことが条件である．6mgあるいは12mgのrhBMP-2を用いた臨床研究では，12mgのBMP-2を用いた群で，通常の治療法を凌駕する成績が得られている．

おわりに

異所性骨形成を引き起こす物質として発見されたBMPは，発生段階においても重要な働きを有することが示され，複雑なシグナル伝達経路も次第に明らかになってきた．そして，発見当時の夢であった整形外科的利用法が現実のものとなりつつある．しかし，臨床的には使用量があまりに多量であり，BMP活性促進物質などの利用により，BMP量の減量化が図られる必要がある．そのためには，シグナル伝達をはじめとする基礎的研究がますます重要となり，基礎と臨床が互いに刺激し合う環境が必要である．

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The Necessity of Chest Physical Therapy for Thoracoscopic Oesophagectomy

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Radical surgery for thoracic oesophageal cancer is highly invasive and often leads to respiratory complications; thoracoscopic surgery is a less-invasive alternative. We examined the need for chest physical therapy (CPT) after thoracoscopic oesophagectomy. Thirty-six consecutive patients, randomly selected for either thoracotomy or thoracoscopic surgery, were included in a randomized clinical trial and received CPT under the same protocol. During short-term post-operative follow-up, both groups showed a marked reduction in respiratory function and responded to CPT to the same extent, although 2 weeks after surgery some parameters of respiratory function were significantly higher in the thoracoscopy group. Thoracoscopic surgery has been reported to be less invasive than standard thoracotomy, but our results suggest that the procedure is also invasive with respect to respiratory function and that CPT should be performed before and after thoracoscopic surgery.

KEY WORDS: CHEST PHYSICAL THERAPY; THORACOSCOPY; OESOPHAGEAL CANCER; RESPIRATORY FUNCTION

Introduction

Radical oesophagectomy for thoracic oesophageal cancer is an invasive procedure that involves lymphoidectomy in the cervical, thoracic and abdominal regions as well as thoracotomy and laparotomy, and often leads to respiratory failure post-surgery. Recurrent neuroparalysis (which is related to dissection around the recurrent nerve) and reduced coughing ability (caused by dissection around the airway) can also occur; both may cause post-operative complications.

Our previous studies confirmed the efficacy of chest physical therapy (CPT) in preventing a reduction in coughing ability, atelectasis and pneumonia after surgery for oesophageal cancer.[1] We also investigated the effects of this therapy with respect to respiratory function (unpublished data). Several studies have reported that CPT is useful in improving respiratory function after thoracic/abdominal surgery.[2,3] Thoracoscopic surgery is also used in the treatment of oesophageal cancer and has been reported to be less invasive than standard thoracotomy,[4,5] although previous studies have not compared the two procedures with respect to respiratory function. This study aimed to compare thoracoscopic surgery and thoracotomy, with regard to pre- and post-surgical respiratory function and responsiveness to CPT.

Patients and methods

PATIENTS

Patients undergoing oesophagectomy, three-region lymph node dissection and reconstructive surgery for thoracic oesophageal cancer were recruited to the study between May 1994 and May 1998. During this period all patients operated on for oesophageal cancer at Osaka City University Hospital were enrolled. Patients who could not receive CPT because of severe physical conditions were excluded. Randomization was performed using sealed envelopes and the techniques were selected regardless of disease stage. The study was approved by the local research ethics committee and informed consent regarding the procedures was obtained from each patient.

SURGICAL PROCEDURES

Thoracoscopic surgery was performed by inserting a port measuring approximately 1 cm in diameter into four points between the axillary fossa and the seventh intercostal space; neither costal bone resection nor incision of the intercostal muscle was performed. Thoracotomy was performed in the fifth right intercostal space. In all patients, an epigastric median incision was made after total thoracic oesophagectomy; reconstructive surgery of the stomach or jejunum was then performed.

POST-SURGICAL TREATMENT

The tracheal intubation tube was removed the day after surgery and oxygen was administered by mask. Analgesia was provided by continuous epidural block in all patients. An autospirometer AS-505 (Minato, Tokyo, Japan) was used to determine respiratory function, with measurements performed in a supine position. We measured vital capacity, percentage vital capacity, forced expiratory volume in 1 second (FEV_1), percentage FEV_1, and peak expiratory flow as indices of respiratory function on four occasions: before surgery; before and after CPT on day 3 after surgery; and on day 14 after surgery. We also investigated changes in coughing ability before and after CPT on day 3 after surgery.

For pre-operative CPT, respiration training and respiratory muscle training were performed from 1 to 2 weeks prior to surgery until the day before surgery. For post-operative CPT, coughing instruction was performed mainly to improve ventilation and promote expectoration from day 1 to day 3 after surgery. At the same time, patients were instructed to perform deep breathing and abdominal breathing as respiration training. After day 2 post-surgery, training in deep breathing was performed using intensive spirometry to dilate the lungs. Exercise therapy was also used, to achieve early rising. CPT was completed when the patient was able to walk a distance of approximately 100 m without oxygen inhalation or dyspnoea, at about 2 weeks post-surgery.

STATISTICAL ANALYSIS

Values were compared using an unpaired *t*-test. Respiratory function parameters before and after CPT were compared using paired *t*-tests. *P*-values < 0.05 were considered statistically significant.

Results

Thirty-six patients with thoracic oesophageal cancer were recruited to the study: 14 patients were randomized to the Scope group, and underwent thoracoscopy; 22 patients were randomized to the Open group, and underwent thoracotomy. As shown in Table 1, there were no significant differences in age or parameters of

TABLE 1:
Baseline clinical characteristics of patients enrolled in this study of chest physical therapy for oesophagectomy

	Scope group (thoracoscopy) (n = 14)	Open group (thoracotomy) (n = 22)
Age (years)	61.8 ± 8.4	58.9 ± 9.3
Sex (male/female)	14/0	20/2
Vital capacity (l)	3.75 ± 0.94	3.50 ± 0.78
Vital capacity (%)	109.3 ± 21.8	107.8 ± 15.0
FEV_1 (l)	2.88 ± 0.59	2.60 ± 0.64
FEV_1 (%)	81.0 ± 8.1	82.3 ± 12.0
Peak expiratory flow (l/s)	7.95 ± 2.12	7.08 ± 2.30

Values are expressed as mean ± SD. No statistically significant differences were detected between the two groups. FEV_1, forced expiratory volume in 1 s.

pre-operative respiratory function between the two groups.

Intercostal muscle disorder related to right thoracic lateral incision alone was observed as a difference between the two groups. There were no serious post-operative complications, such as suture insufficiency or pneumonia, in either group.

There were no significant differences in respiratory function between the two groups at baseline (Table 1) or before CPT on day 3 (Fig. 1). In both groups, before CPT, respiratory function was reduced on day 3 post-surgery compared with pre-surgical levels, however.

After CPT on day 3 post-surgery, mean vital capacity improved from 1074 ml (before CPT) to 1325 ml in the Scope group and from 1068 ml to 1347 ml in the Open group. Mean FEV_1 improved from 864 ml to 951 ml in the Scope group and from 871 ml to 1028 ml in the Open group. Mean peak expiratory flow improved from 2.30/s to 2.61/s in the Scope group and from 2.10/s to 2.51/s in the Open group. There were significant increases in parameters other than mean FEV_1 and mean peak expiratory flow in the Scope group (Fig. 1). When the improvement ratings for these parameters were compared, values were slightly lower in the Scope group but there were no significant differences between the two groups (Fig. 2).

Coughing ability was classified into three grades (Table 2). On day 3 post-surgery, coughing ability before CPT was evaluated as level III in six patients in the Scope group and in 11 patients in the Open group. In these 17 patients, FEV_1 per kg of body weight before CPT was 10 ml (desired FEV_1 proposed by Pontoppidan *et al.*[6]) or higher. In the remaining patients with a coughing ability of level II or lower, changes in coughing ability were investigated. Table 3 shows that coughing ability improved to level III in six of seven patients with level II coughing ability in the Scope group, and in all 11 patients with level II coughing ability in the Open group. Improvement in coughing ability did not depend on whether values before CPT reached the desired FEV_1 (10 ml/kg).

On day 14 post-surgery there were no significant differences in vital capacity or FEV_1 between the two groups, although peak

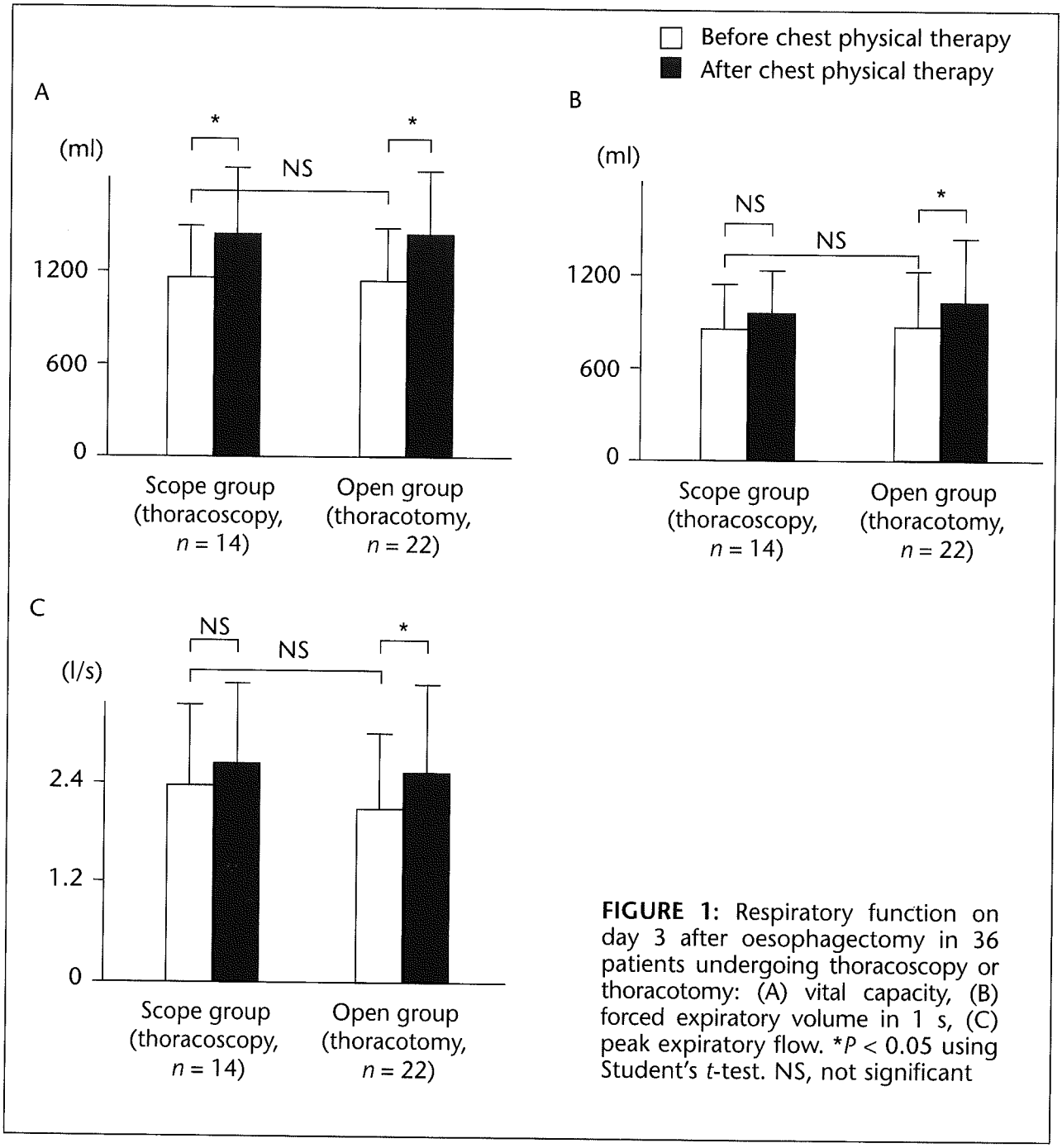

FIGURE 1: Respiratory function on day 3 after oesophagectomy in 36 patients undergoing thoracoscopy or thoracotomy: (A) vital capacity, (B) forced expiratory volume in 1 s, (C) peak expiratory flow. *$P < 0.05$ using Student's *t*-test. NS, not significant

expiratory flow was significantly higher in the Scope group (Fig. 3).

Discussion

Indications are that a reduction in coughing ability is caused by a direct disorder of the respiratory muscle related to thoracotomy/laparotomy, post-operative pain, reduction of pulmonary/thoracic compliance or glottic dysfunction.[3] Peak expiratory flow depends on lung capacity at the zero point of coughing, but a restrictive disorder (inhalation-phase disorder) following thoracic/abdominal surgery may affect the start of coughing at high lung capacity, by not providing sufficient flow.[6]

Of the aetiological factors involved in reduced coughing ability, neither pain nor glottic function are affected by CPT: this therapy is performed to improve the activity of the respiratory muscle and thoracic compliance. Pontoppidan *et al.*[6] reported

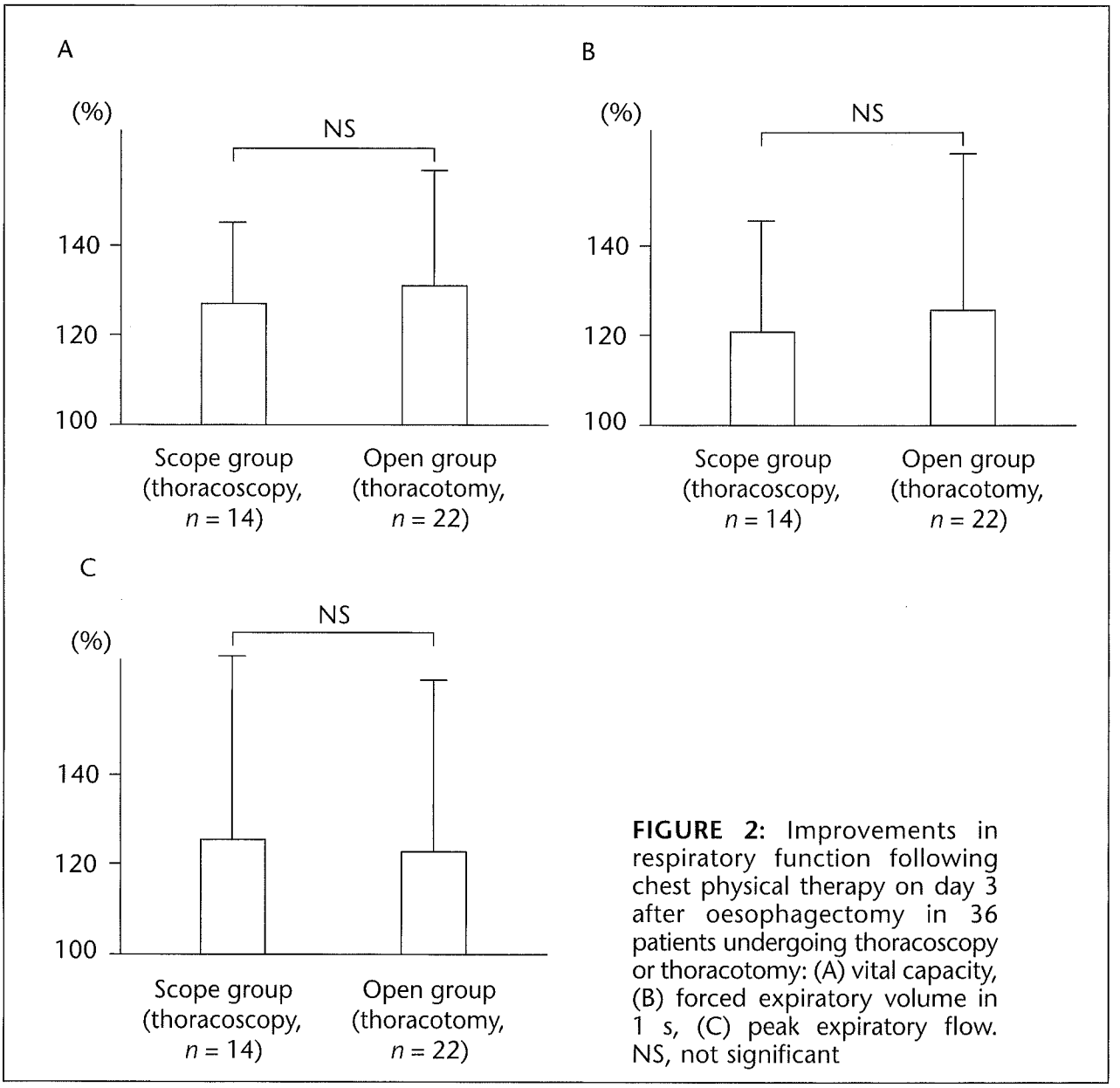

FIGURE 2: Improvements in respiratory function following chest physical therapy on day 3 after oesophagectomy in 36 patients undergoing thoracoscopy or thoracotomy: (A) vital capacity, (B) forced expiratory volume in 1 s, (C) peak expiratory flow. NS, not significant

that, to achieve sputum expectoration without assistance, an FEV_1 of 10 ml/kg of body weight or higher was required for patients with acute respiratory failure. On day 3 post-surgery, however, before CPT only 17 of 32 patients with a FEV_1 of 10 ml/kg body weight had level III coughing ability. In performing radical surgery for oesophageal cancer, the desired FEV_1 for coughing should also be established, considering the marked decreases in vital capacity and FEV_1 that occur immediately after surgery (Table 1 and

TABLE 2:
Ranking of coughing ability in patients undergoing oesophagectomy

I	Loss of cough reflex
II	Existence of cough reflex, but not effective (ineffective expectoration)
III	Existence of cough reflex, and effective (effective expectoration)

TABLE 3:
Improvement in coughing ability by chest physical therapy on day 3 after surgery for thoracic oesophageal cancer

	FEV_1 > 10 ml/kg body weight	FEV_1 < 10 ml/kg body weight
Scope group (thoracoscopy)	II → III (n = 6) II → II (n = 1)	I → I (n = 1)
Open group (thoracotomy)	II → III (n = 8)	II → III (n = 3)

I, Loss of cough reflex; II, existence of cough reflex, but not effective (ineffective expectoration); III, existence of cough reflex, and effective (effective expectoration).

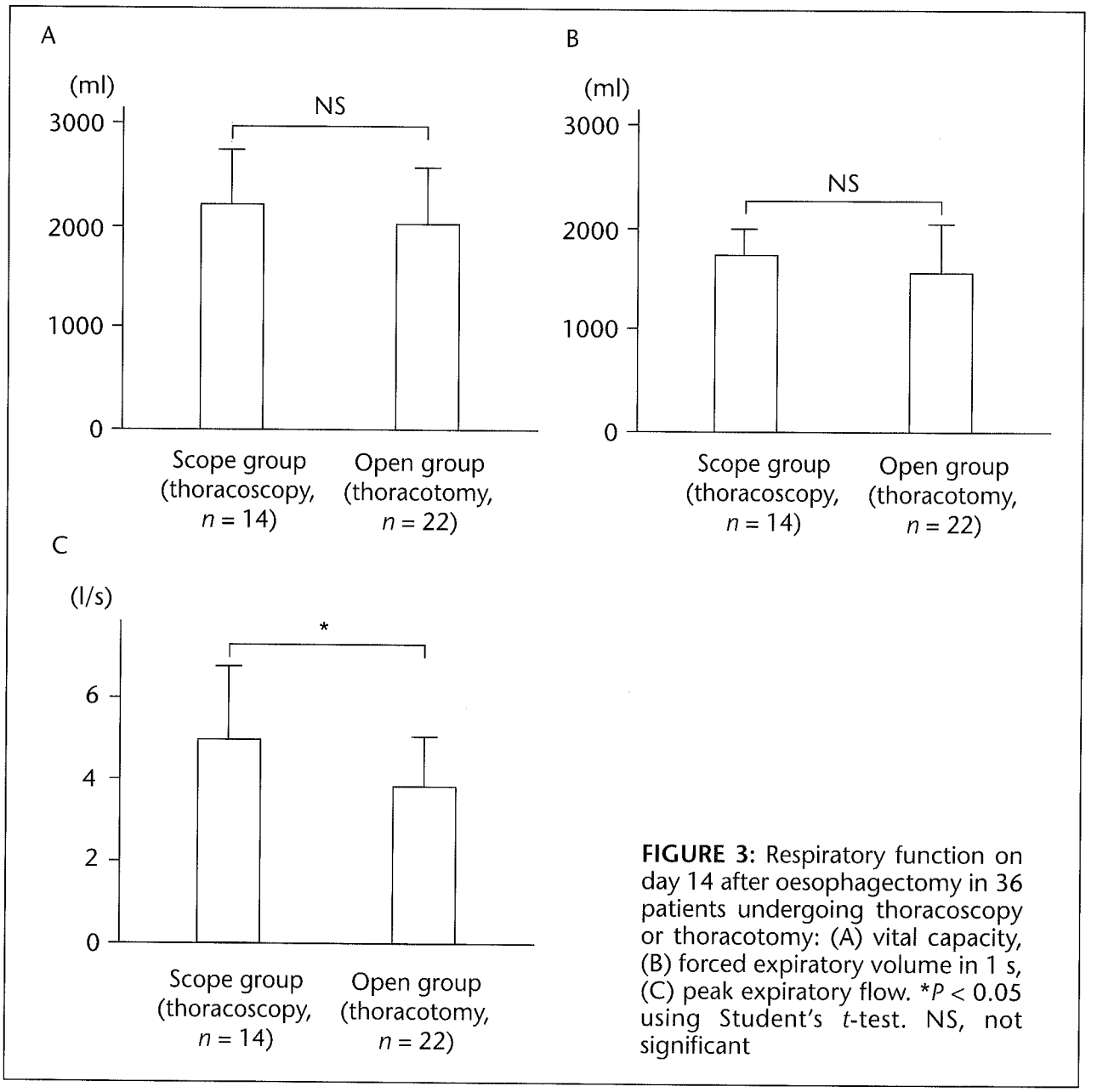

FIGURE 3: Respiratory function on day 14 after oesophagectomy in 36 patients undergoing thoracoscopy or thoracotomy: (A) vital capacity, (B) forced expiratory volume in 1 s, (C) peak expiratory flow. *$P < 0.05$ using Student's t-test. NS, not significant

Fig. 1), although CPT sufficiently improved the coughing level even in patients with low FEV_1 values (Table 3).

To investigate factors influencing the coughing ability of respiratory muscle, patients were divided into a thoracoscopic surgery group and a thoracotomy group. There was no significant difference in respiratory function immediately after surgery between the groups (Fig. 1). Concerning the rate of change after CPT, the thoracotomy group showed higher improvement ratings for two of three items, although there were no significant differences (Fig. 2). Generally, a thoracotomy-related wound is larger than a thoracoscopic surgery-related wound but, with sufficient analgesia, wound size does not negatively affect CPT. In the thoracoscopic surgery group, palpation and chest X-ray showed that the dilatation of the superior lung field involving the site of port insertion was slightly reduced compared with the dilatation of other areas, even when analgesic effects were achieved. This may be because of the long duration of compression associated with hemilateral lung ventilation compared with that in the thoracotomy group. For these reasons, the improvement rating may have been higher in the thoracotomy group.

Incentive spirometry alone does not relieve post-operative respiratory complications.[7,8] CPT relieves various complications, although it does not markedly influence respiratory function,[9,10] and it is regularly performed as standard treatment after thoracic or abdominal surgery.[11] Recently, thoracoscopic surgery has been indicated for an increasing number of patients and there have been considerable changes in surgical procedures.[12] The results of this study suggest, however, that thoracoscopic surgery – which is considered less invasive in the acute post-surgical phase – also requires the respiration training and management that is performed after standard thoracotomy.

The limitation of this study is that patients were randomized to either thoracoscopy or thoracotomy and both groups received CPT: we were examining the effect of surgery rather than physical therapy. In future, CPT should be investigated in a randomized, controlled, study.

Conflicts of interest

No conflicts of interest were declared in relation to this article.

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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

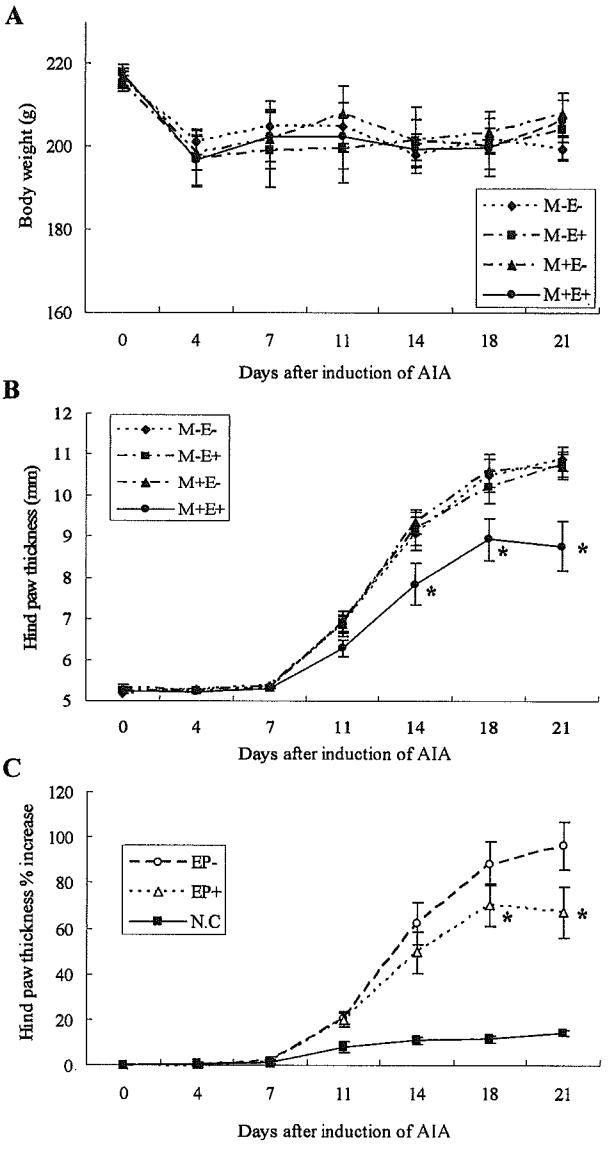

Figure 1. Effects of electrochemotherapy with methotrexate (MTX) on body weight and paw swelling in rats with adjuvant-induced arthritis (AIA). **A,** Weight loss was observed in all groups on day 4. There was no significant weight difference between the 4 groups throughout the entire study period. **B,** 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.

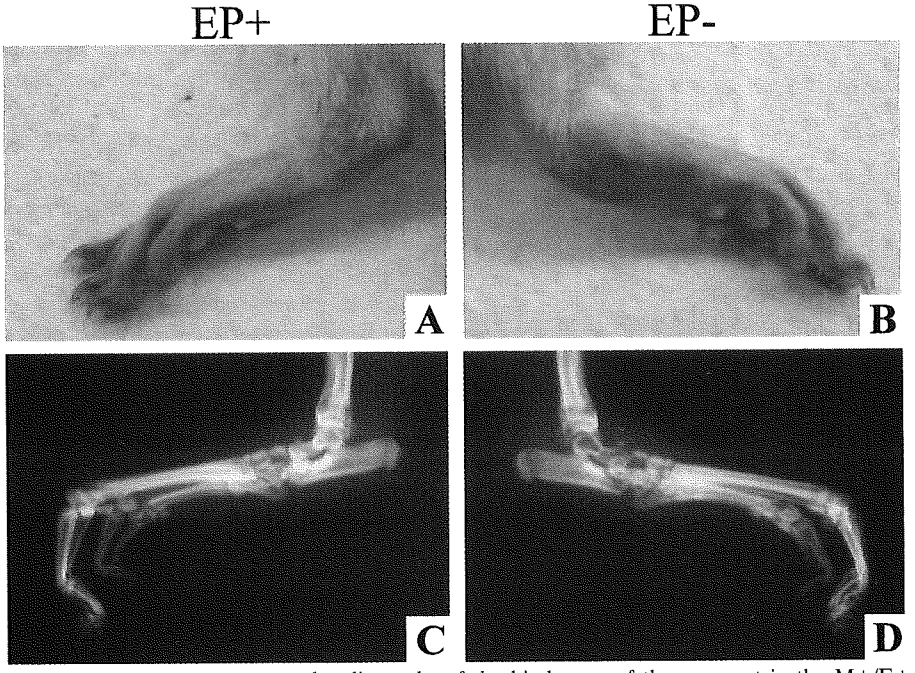

Figure 2. Gross appearance and radiographs of the hind paws of the same rat in the M+/E+ group on day 21. Following administration of MTX (0.125 mg/kg/week), electrical pulses were applied to the left hind paw only (EP+) (**A** and **C**). Note the obvious difference in the degree of swelling and joint 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.