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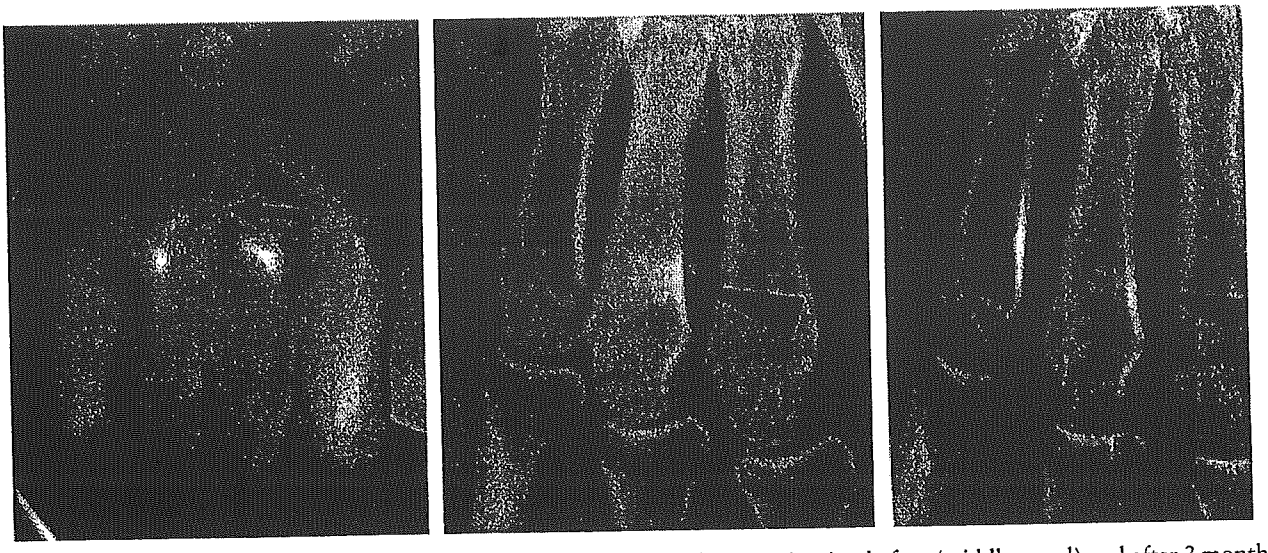

Figure 1. Left panel, subcutaneous nodule with pain (arrow). Radiographic examination before (middle panel) and after 3 months on etidronate (right panel). Arrowheads indicate the positions of nodular calcification.

Our present clinical experience demonstrates a novel therapeutic option for an otherwise incurable complication of Werner syndrome. Moreover, it rediscovers the usefulness of bisphosphonate for ectopic calcification.

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HYPOADIPONECTINEMIA IN BEDRIDDEN FEMALE PATIENTS YOUNGER THAN 75

To the Editor: Older people have several hormonal alterations, but the effect on the endocrine function of adipose tissue in older bedridden patients has not been fully elucidated. Adiponectin is a newly discovered antiinflammatory protein, secreted exclusively by adipocytes, that plays a protective role against atherosclerosis.[1] Hypoadiponectinemia plays a crucial role in atherosclerosis in men, but there have been no studies of plasma adiponectin in bedridden women. The aim of the present study was to estimate plasma adiponectin concentration in bedridden elderly female patients in comparison with age-matched healthy volunteers.

Seventy-four bedridden female patients admitted to geriatric wards and nursing homes in Osaka, Japan, and age-matched volunteers were studied. Clinical diagnoses were defined using detailed physical examination and routine biochemical analyses of blood and urine, as well as clinical tools including computed tomography. Their mean bedridden period ± standard deviation was 49.4 ± 37.4 months. All plasma analyses were performed on samples from fasting subjects. Adiponectin was measured using high-sensitive radioimmunoassay (Linco Research, St. Louis, MO). Bedridden subjects and healthy volunteers were divided into two groups: younger than 75 and aged 75 and older. All statistical analyses were performed using the SPSS (SPSS Inc., Chicago, IL). The statistical differences in the variables were compared using the Mann-Whitney *U* test, and the association between any two parameters was assessed using Spearman correlation.

Table 1. Clinical Characteristics of 74 Bedridden Female Patients and 42 Healthy Age-Matched Volunteers

Characteristic	Healthy Female Volunteers		Bedridden Patients	
	Age 60–74 (n = 22)	Age 75–98 (n = 20)	Age 60–74 (n = 18)	Age 75–98 (n = 56)
Body mass index, mean ± SD	24.1 ± 2.3	23.8 ± 2.7	23.6 ± 2.6	24.9 ± 3.8
Systolic blood pressure, mmHg, mean ± SD	138 ± 22	140 ± 33	154 ± 19*	144 ± 20
Diastolic blood pressure, mmHg, mean ± SD	73 ± 10	77 ± 13	82 ± 14	84 ± 13
Plasma adiponectin, μg/mL, mean ± SD	15.9 ± 7.2	16.2 ± 7.7	11.8 ± 4.8*	14.3 ± 6.9
Serum albumin, g/dL, mean ± SD	4.2 ± 0.3	4.1 ± 0.3	3.6 ± 0.2*	3.5 ± 0.3*
Total cholesterol, mg/dL, mean ± SD	228 ± 24	211 ± 19	183 ± 15*	178 ± 16*
Cerebrovascular accident as cause of bedridden state, %	NA	NA	76	68

*$P < .05$ vs healthy age-matched volunteer.
SD = standard deviation; NA = not assessed.

The clinical characteristics of the subjects are shown in Table 1. Of 74 bedridden patients, the main cause of being bedridden was cerebrovascular accident (CVA, 70%); others were bone fracture, infection, and cardiovascular disease. There was no statistical difference in body mass index between the four groups. Bedridden women aged 60 to 74 were characterized by significantly lower plasma adiponectin (11.8 ± 4.8 μg/mL) concentration than healthy women of the same age (15.9 ± 7.2 μg/mL, $P < .01$). In the entire studied group, a weak, positive, but insignificant, correlation was found between plasma adiponectin concentration and age ($r = 0.18$, $P = .12$). There was no correlation between serum adiponectin and albumin or total cholesterol levels. Additionally, significant correlation was found between plasma adiponectin concentration and the length of time patients had been bedridden when analyzed separately ($r = 0.27$, $P = .04$).

The relationship between aging and plasma adiponectin concentration has recently been described in some manuscripts. Men aged 70 and older have significantly higher plasma adiponectin concentrations than younger men, whereas plasma adiponectin concentration in women does not change significantly with age.[2] The results of the current study in female patients aged 60 and older were consistent with these reports. Sex differences of adiponectin levels are also reported in older diabetic patients.[3]

The main cause of being bedridden was CVA in female patients in this study. The relationship between CVA and lower adiponectin level is controversial. A recent report reported that CVA was associated with hypoadiponectinemia with or without diabetes mellitus,[4] but in a Swedish study, adiponectin levels were not associated with CVA in men.[5] The current results concerning hypoadiponectinemia in younger bedridden female patients mainly caused by cerebrovascular disease would indicate a relationship between hypoadiponectinemia and occurrence of CVA. Hypoadiponectinemia may be due to the severity of cerebrovascular atherosclerosis in bedridden patients.

Precise mechanisms underlying recovery of adiponectin level in long-term bedridden patients are not known. First, being bedridden may affect the decrease in adiposity caused by multiple factors including immobilization, malnutrition, and disease, although several causes could be responsible, including aspiration pneumonia, urinary tract infection, and pressure ulcers. Indeed, the current results showed that albumin and total cholesterol levels in bedridden female patients were significantly lower than in healthy controls. Nutritional restriction due to controlled feeding increases the survival of mammalians by delaying the aging process.[6] Decrease in adiposity due to being bedridden longer may induce higher levels of circulating adiponectin in elderly female patients. A second possibility may be the effects of exercise in bedridden patients. Lack of skeletal muscle exercise is apt to induce adiposity by decreasing the release of cytokine interleukin-6, which stimulates lypolysis.[7] Third, that hypoadiponectinemia was observed mainly in those who had been bedridden for a shorter length of time may reflect greater endothelial dysfunction. A vascular study showed that severity of endothelial dysfunction was closely associated with plasma adiponectin levels in Japanese subjects according to the measurement of forearm blood flow.[8] Elevated adiponectin level would be related to longevity even in bedridden women. Longitudinal studies of serum adiponectin levels and prognosis of bedridden state should be conducted to elucidate the mechanism of lipid metabolism in bedridden patients to help with their future health care.[9]

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SPONTANEOUS FRACTURES OF LONG BONES ASSOCIATED WITH JOINT CONTRACTURES IN BEDRIDDEN ELDERLY INPATIENTS: CLINICAL FEATURES AND OUTCOME

To the Editor: Reports on the occurrence of fractures without any apparent external force in completely bedridden elderly patients under care are limited. One study[1] described six individuals with "spontaneous fractures of long bones" in nursing home patients. Another elderly nonweight-bearing woman with "transfer" and "turning" fracture was also reported.[2] A survey of 11 nursing homes identified 16 subjects with "minimal trauma fractures."[3] Fifty-five "spontaneous long-bone insufficiency fractures" in 53 extremely elderly residents in long-term nursing homes, including 38 bedridden subjects, were recently described.[4]

An observational study was conducted in a hospital and long-term care facility with 1,993 beds for older people (male/female ratio approximately 3/10; mean age 79 for men, 85 for women), from 1998 to 2004 in Japan. Reports of accidents and possible abuse in the hospital were constructed from daily observations of nursing staff. Numbers of bedridden patients and those with joint contracture(s) were approximately 500 and 250, respectively, in the hospital during the study period. Spontaneous fractures were defined as fractures occurring in long bones in bedridden older people, without any apparent external force or abuse, during daily care procedures.

Clinical features of spontaneous fractures, as cited in Table 1, and the outcome up to 1 year after fracture(s) were reviewed from the medical records.

Eighteen bedridden inpatients (one man and 17 women, mean age ± standard deviation 88 ± 9) with spontaneous fractures were identified (Table 1). Their mean period of being bedridden was 7 ± 6 years. Their nutritional state just before they sustained fractures was poor, as evaluated using serum albumin level. Spontaneous fractures affected the femur in 12 cases (8 supracondylar fractures, 2 intertrochanteric fractures, 1 shaft fracture, and 1 neck fracture), the humerus in five cases (2 neck fractures, 2 shaft fractures, and 1 supracondylar fracture), and the proximal phalanx in one case. All spontaneous fractures occurred near joint contractures at proximal or distal sites of extremity bones. Ten patients had previously suffered long-bone fractures during nonbedridden periods, and in six of these 10 cases, spontaneous fractures reoccurred in the same bone. Four of five fractures in hemiplegic patients occurred on the paralytic side. Although one patient died due to worsening of pneumonia 1 month after fracture, 17 of 18 subjects were successfully treated with bandage procedures and showed recovery within approximately 2 months after fracture.

One of the characteristic features of spontaneous fractures in bedridden older people in this study was that one-third of the subjects had had previous fractures of the same bone where the spontaneous fractures occurred. More than half of the patients also had a history of fractures of long bones of traumatic or nontraumatic origin, indicating that elderly subjects with previous long-bone fractures during nonbedridden periods are prone to reoccurrence of long-bone fractures, especially in the healed bone, even after the start of their bedridden status.

As additional evidence of absorbing interest in this investigation, joint contractures adjacent to the fractures were found in all individuals. There were no cases of spontaneous fractures in the population of bedridden elderly without joint contractures during the survey. Joint contractures might be one of the risk factors leading to fractures. Of the 18 bedridden patients with fractures, joint contractures were observed at the proximal site in 17, at the distal site in 16, and at the proximal or distal sites of the fractured bones in all 18 subjects. A marked decrease in bone mass and bone quality due to multiple risk factors, including immobilization, disease, and malnutrition, should also be considered to be a fundamental factor in fracture.[3,4]

It has been reported that bone mineral density (BMD) decreased more rapidly on the paretic side than the nonparetic side,[5] and hemiplegic patients showed more-severe joint contractures on the paretic side than the nonparetic side in this study. The fractures seemed to occur at weakest point of the bone, near the contracted articulation. Joint contractures of an extremity fix the limb to the torso so that the contracted joint acts like a supporting point of leverage and any minimal external force or torque maneuver during passive transfer or lifting on the distal part of a long bone might easily make a bone with low BMD reach its fracture threshold. A subtle external force such as changing a diaper, washing, or putting the patient in an ambulatory or sitting position might produce a deforming force strong enough to make the bone reach its fracture threshold.

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Table 1. Clinical Characteristics of Patients with Spontaneous Fractures of Long Bones

No.	Age	Sex	Bedridden Period (Years)	Main Cause of Bedridden State	Serum Albumin Level (g/L)	Complications	Date of Spontaneous Fracture	Location of Fracture	Episode that Might Have Caused Fracture	History of Fracture of Same Bone	History of Other Long-Bone Fracture	Side of Hemiplegia if Present	Joint Contractures Adjacent to Fractured Bone	Treatment of Fracture	Outcome
1	91	Female	7	Cerebral infarction	29	Aspiration pneumonia, pressure ulcers	27-Oct-98	Shaft of right humerus	Unknown	—	Right hip fracture by falling	Right	Shoulder and elbow	Splint bandage	Died 1 month later from aspiration pneumonia
2	91	Female	2	Multiple cerebral lacunar infarction	33	Pressure ulcers	4-Apr-99	Supracondylar area of left femur	Unknown	—	—	—	Hip and knee	Plaster bandage	Recovered (died 9.5 months later from aspiration pneumonia)
3	91	Female	4	Multiple cerebral lacunar infarction	33	—	25-May-99	Supracondylar area of right femur	"Crack" heard during changing diaper	Left hip fracture by falling	—	—	Hip and knee	Plaster bandage	Recovered
4	100	Female	3	Hip fracture	33	—	31-May-99	Supracondylar area of right femur	Unknown	—	Left hip fracture by falling	—	Hip and knee	Plaster bandage	Recovered (died 3 months later from gastrointestinal bleeding)
5	86	Female	20	Multiple cerebral lacunar infarction	24	Aspiration pneumonia	3-Apr-00	Supracondylar area of left femur	Unknown	—	Left hip fracture by falling	—	Hip and knee	Plaster bandage	Recovered (died 9 months later from aspiration pneumonia)
6	93	Female	3	Senile dementia	27	—	3-Dec-00	Supracondylar area of right femur	Unknown (knee joint found to be swollen and skin reddish when nurse was applying ointment)	Right hip fracture by falling	—	—	Hip and knee	Plaster bandage	Recovered
7	82	Female	5	Intracerebral hemorrhage	31	Aspiration pneumonia	1-Jun-01	1) Shaft of right humerus 2) Shaft of right humerus	Unknown	Fracture of right humerus by falling	—	Right	Shoulder and elbow	Splint bandage	Recovered (died 5 months later from aspiration pneumonia)
8	63	Male	17	Intracerebral hemorrhage	39	—	8-Aug-01	Supracondylar area of left femur	Unknown	—	Left hip fracture by falling	Right	Knee	Plaster bandage	Recovered
9	85	Female	10	Multiple cerebral lacunar infarction	34	Aspiration pneumonia	8-Sep-01	Intertrochanteric area of right femur	Unknown	Fracture of right humerus by falling	—	—	Hip and knee	Splint bandage	Recovered
10	89	Female	5.5	Multiple cerebral lacunar infarction	30	—	15-Nov-01	Neck of left humerus	Unknown	—	—	Left	Shoulder	Splint bandage	Recovered
11	96	Female	3.7	Cerebral infarction	25	—	18-Jan-02	Supracondylar area of left femur	Unknown	Left hip fracture by falling	—	—	Hip and knee	Plaster bandage	Recovered
12	82	Female	22	Multiple cerebral lacunar infarction	35	—	30-Apr-02	Supracondylar area of right humerus	Unknown	—	—	Right	Shoulder and elbow	Adhesive plaster bandage	Recovered
13	94	Female	12	Multiple cerebral lacunar infarction	36	Urinary tract infection	9-May-02	Neck of right humerus	Unknown (rigid contracted joint found to be floppy when changing diaper)	—	—	—	Shoulder and elbow	Splint bandage	Recovered
14	73	Female	2	Parkinson disease	33	Urinary tract infection, pressure ulcers	6-Sep-03	Shaft of right femur	Fracture might have occurred while lifting right leg during treatment of a decubitus ulcer	—	—	—	Hip and knee	Open reduction and internal fixation	Recovered
15	90	Female	4	Senile dementia	37	—	22-Dec-03	Supracondylar area of left femur	Unknown	Left hip fracture by falling	Right hip fracture by falling	—	Hip and knee	Plaster bandage	Recovered
16	89	Female	4	Senile dementia	32	—	30-Dec-03	Proximal phalanx of left second finger	Unknown	—	—	—	Fingers and wrist	Adhesive plaster bandage	Recovered
17	98	Female	2	Multiple cerebral lacunar infarction	32	—	14-Jan-04	Intertrochanteric area of right femur	Unknown	—	—	—	Hip and knee	Traction only	Recovered
18	90	Female	6	Multiple cerebral lacunar infarction	34	—	16-Jul-04	Neck of left femur	Unknown	—	—	—	Hip and knee	Plaster bandage	Recovered

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Electromagnetic Fields Inhibit Endothelin-1 Production Stimulated by Thrombin in Endothelial Cells

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Electromagnetic field (EMF) radiation has been found to induce arteriolar dilatation, but the mechanism of action remains largely unknown. This study investigated the effect of EMF radiation on the production of endothelin-1 (ET-1), a potent vasoconstrictor, by cultured endothelial cells. EMF radiation reduced ET-1 basal levels in human umbilical vein and microvascular endothelial cells, but failed to reduce ET-1 basal levels in bovine and human aortic endothelial cells. EMF radiation significantly inhibited thrombin-stimulated ET-1 production in all four endothelial cell types in a dose-dependent manner. EMF radiation significantly inhibited thrombin-induced endothelin-1 mRNA expression in all four cell types. The inhibitory effect of EMF radiation on ET-1 production was abolished by the nitric oxide synthase inhibitor NG-monomethyl-L-arginine (10^{-3} mol/l). These results demonstrate that EMF radiation modulates ET-1 production in cultured vascular endothelial cells and the inhibitory effect of EMF radiation is, at least partly, mediated through a nitric oxide-related pathway.

KEY WORDS: Electromagnetic fields; Endothelin-1; Nitric oxide; Northern blot hybridization

Introduction

Irradiation by radio frequency (RF) burst-type electromagnetic fields (EMF) has been reported to induce arteriolar dilatation in the foot web of *Xenopus laevis* (frog foot web),[1] promote circulatory arterioles in the rabbit ear,[2] and increase the production of nitric oxide and cyclic guanosine monophosphate (GMP) in the rat cerebellum *in vitro*.[3] These effects have been utilized clinically for alleviating muscular stiffness[4] with a commercially available high-frequency therapeutic device (Matsushita Electric Works Ltd, Osaka, Japan), and the effectiveness of this device for easing lumbar pain has been reported.[5,6] There is also evidence that EMF promotes bone fracture healing,[7,8] affects the immune system,[9] reduces cell differentiation,[10] and stimulates migration of endothelial cells and capillary repair in culture models.[11] High magnetic flux densities of extremely low frequency EMF are also reported to exert acute effects

on leucocyte-endothelium interactions including cell adhesion *in vivo*.[12] Recently, EMF has been reported to augment angiogenesis primarily by stimulating endothelial release of fibroblast growth factor-β-2, inducing paracrine and autocrine changes in the surrounding tissue.[13] Although the clinical effectiveness of EMF therapy has been observed and the mechanism has been shown to involve radiation-induced nitric oxide synthesis,[3] the biological effects of EMF on the vasculature remain largely unknown.

Endothelin-1 (ET-1), a novel endothelium-derived peptide, has been recognized as a locally produced potent vasoconstrictor.[14] The production of ET-1 is up-regulated by thrombin, transforming growth factor-β1, interleukin-1, tumour necrosis factor-α, adrenaline, apolipoprotein A-I, shearing stress and hypoxia.[15 - 17] Its production is down-regulated by nitric oxide, cyclic GMP, prostaglandin E_2, prostacyclin and atrial natriuretic peptide.[16,18,19] ET-1 release from normal human neuronal culture cells is dramatically reduced after exposure (5 min) to a static magnetic field generated by a 0.2 T magnetic resonance tomograph.[20] As a result of these findings, there is considerable interest in whether EMF might improve the local circulation by affecting the production of ET-1.

The present study was performed to investigate the effect of RF burst-type EMF on basal and thrombin-stimulated ET-1 expression and production in cultured endothelial cells from a variety of sources.

Materials and methods

MATERIALS

Thrombin and bovine serum albumin were purchased from Sigma Chemical Co. (St Louis, MO, USA). Dulbecco's modified Eagles' medium (DMEM) was obtained from Nissui Pharmaceutical Co. (Tokyo, Japan). Endothelial cell basal medium-2 (EBM-2) was purchased from Sanko Pure Chemical Co. (Osaka, Japan). Fetal bovine serum was obtained from Flow Laboratories (North Ryde, Australia). All other chemicals used were commercial products of the highest grade available.

CELL CULTURE

Bovine aortic endothelial cells (BAEC) were isolated from bovine aortas according to the previously described method,[21] and grown in DMEM supplemented with 10% fetal bovine serum in 100-mm culture dishes at 37 °C in humidified 5% CO_2. Human umbilical vascular endothelial cells (HUVEC, Lot No. 16197, from a male neonate), human aortic endothelial cells (HAEC, Lot No. 13228, from a female donor aged 58 years old), and human microvascular endothelial cells (HMVEC, Lot No. 14828, from a male neonate) obtained from Sanko Pure Chemical Co. were cultured in EBM-2 medium supplemented with 10% fetal calf serum, 50 µg/ml gentamicin sulphate, 50 µg/ml amphotericin-B, 10 ng/ml epidermal growth factor and 1 mM hydrocortisone under standard conditions[22] at 37 °C in humidified 5% CO_2. Medium was refreshed every 2 days. Cells were passaged at confluence by treatment with 0.05% trypsin/0.02% EDTA in 10 mmol/l phosphate-buffered saline, followed by two washes with medium. BAEC at the fifth to ninth passage and HMVEC and HUVEC at the fourth to sixth passage were used.

ELECTROMAGNETIC FIELD RADIATION SYSTEM

As shown in Fig. 1A, the 96-well culture plate containing endothelial cells was placed on the generator coil. The generator coil of the coil

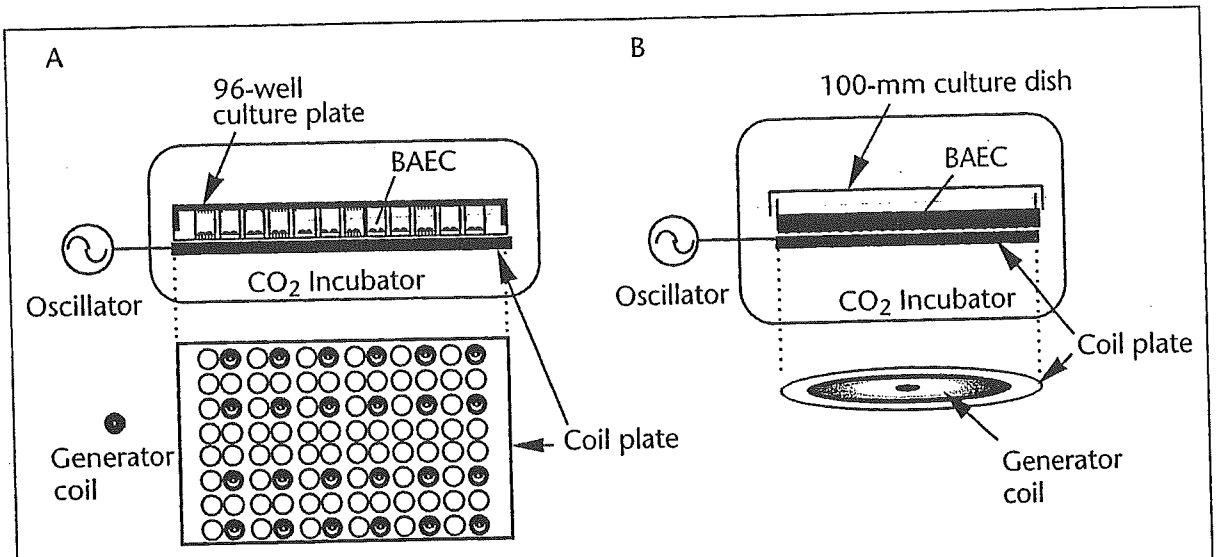

FIGURE 1: Experimental equipment used in the study. A, a 96-well culture plate containing endothelial cells was placed on the coil plate. B, a 100-mm culture dish containing endothelial cells was placed on the coil plate. BAEC, bovine aortic endothelial cells

plate consisted of a concentric nine-turn circular loop coil (i.d. 1.2 mm; o.d. 4.4 mm). The distance between the generator coil and the cells was 2.0 mm. When the amplitude of the oscillator (8116A, 50 MHz Pulse/Function Generator HPIB, Hewlett Packard, Telford, UK) was adjusted to 10 and 16 V peak to peak (Vpp), the intensity of the EMF became 1.25 V/m, 0.98 mW/kg and 1.92 V/m, 2.31 mW/kg, respectively. Fig. 1B shows the 100-mm culture dish containing endothelial cells placed on the coil plate. The generator coil of this coil plate consisted of a concentric ten-turn circular loop coil (i.d. 8 mm; o.d. 44 mm). The distance between the generator coil and the cells was 2.3 mm. When the amplitude of the oscillator was adjusted to 16 Vpp, the intensity of the EMF became 3.59 V/m and 8.06 mW/kg. The oscillator was able to emit various patterns of an oscillating burst-type EMF. As shown in Fig. 2, the frequency was fixed at 10 MHz with a pulse modulation of 10 kHz and 50% burst duty ratio. The EMF applied in the present study was established similarly in type and degree to that in the commercially available device (Matsushita Electric Works Ltd).

DETERMINATION OF ET-1 PRODUCTION

Cells released from confluent stock cultures were seeded into 96-well culture plates at a density of 10^4 cells per well. At confluence, the cells were washed twice with serum-free

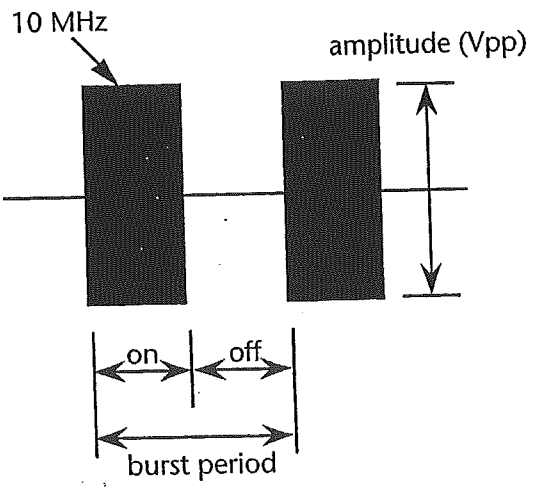

FIGURE 2: The wave-form of the generator coil. The frequency was fixed at 10 MHz with a pulse modulation of 10 kHz and 50% burst duty ratio. Burst duty radio (%) = on duration/burst period

medium and then cultured in medium containing 0.1% bovine serum albumin with or without thrombin. The responses of the cells to thrombin at doses of 0.625 – 10 U/ml were evaluated in our preliminary experiments. We found that thrombin treatment at 10 U/ml was ideal to investigate the effect of EMF. The cells were incubated with or without irradiation of burst-type EMF as described above for 8 h or 24 h. After incubation, the medium of each well was used to determine ET-1 levels using a sensitive sandwich-enzyme immunoassay as previously described.[23] The cells were then washed twice with 10 mmol/l phosphate-buffered saline, followed by the addition of 0.1 ml of 0.1 mol/l NaOH to dissolve the cells, in order to measure cell protein content by the method of Lowry *et al.*[24] using bovine serum albumin as a standard. ET-1 content was expressed in nmol/g of cell protein.

To examine whether nitric oxide may participate in the mediation of ET-1 production by EMF, NG-monomethyl-L-arginine (L-NMMA), a competitive inhibitor of nitric oxide synthase, was used to block the synthesis of nitric oxide in endothelial cell cultures. The EMF effects on ET-1 production in cultured endothelial cells were examined during exposure to L-NMMA (10^{-3} mol/l).

ANALYSIS OF ET-1 MRNA

For ET-1 mRNA analysis, BAEC released from confluent stock cultures were seeded on 100-mm culture dishes at a density of 5×10^4 cells/ml. At confluence, the medium was refreshed with DMEM containing 0.1% bovine serum albumin with or without 10 U/ml thrombin and with or without exposure to EMF radiation (16 Vpp) for 24 h. Total RNA extraction and Northern blot analysis were performed on vascular endothelial cells using 20 µg total RNA/lane, as previously described.[25] The probe used in the studies was a human prepro-ET-1 cDNA (1.17 kb) prepared from the EcoRI site of plasmid pUC18.[26,27] The membranes were rehybridized with a human glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) cDNA probe (1.1 kb) (Clontech Laboratories Inc., Palo Alto, CA, USA). To correct for loading differences, the densitometric signal for each RNA sample hybridized to the ET-1 probe was divided by that hybridized to the GAPDH probe. The size of the ET-1 transcript was estimated from the positions of 28s and 18s ribosomal RNA.

STATISTICAL ANALYSIS

Results are expressed as mean ± SD. Statistical analysis was performed by unpaired Student's *t*-test. A value of $P < 0.05$ was considered statistically significant.

Results

EFFECT OF EMF RADIATION ON ET-1 PRODUCTION IN ENDOTHELIAL CELLS

Basal and thrombin-stimulated ET-1 release from BAEC and human endothelial cells increased in a time-dependent manner (Fig. 3A). Thrombin (10 U/ml) enhanced ET-1 production above the basal levels in BAEC and human endothelial cells (Fig. 3B). EMF radiation had different effects on the basal secretion of ET-1 in the different endothelial cell types: EMF radiation (16 Vpp) reduced ET-1 basal levels in HUVEC and HMVEC, but failed to reduce endothelin-1 basal levels in BAEC; and EMF radiation enhanced the ET-1 basal level in HAEC (Fig. 4). However, EMF radiation significantly inhibited thrombin-stimulated ET-1 production in all bovine and human endothelial cell cultures in a dose-dependent manner (Fig. 4). The inhibitory effects of EMF radiation on ET-1 production in each endothelial cell culture were

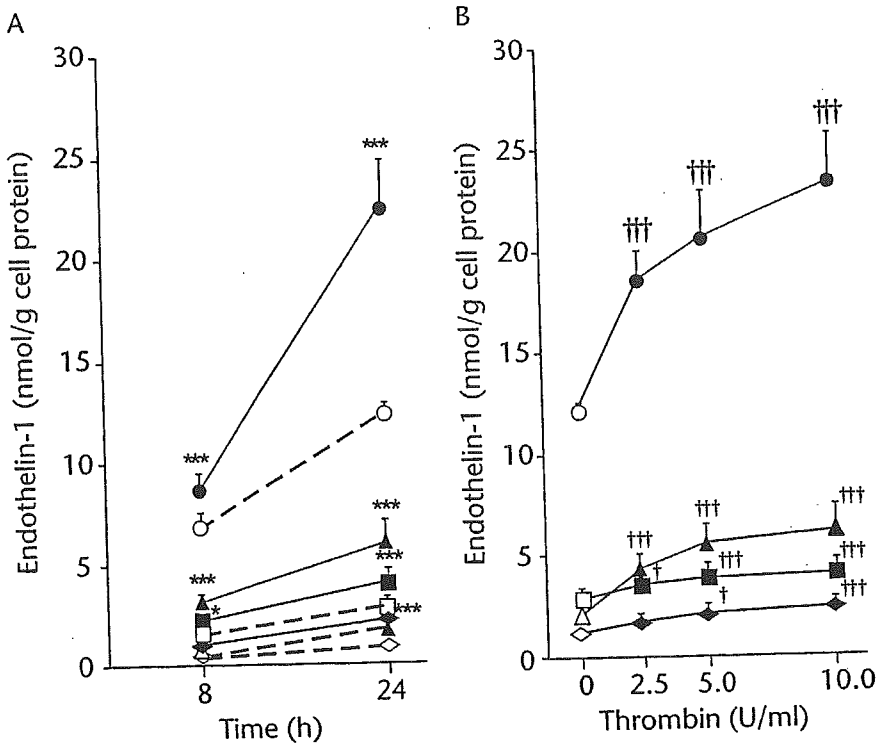

FIGURE 3: Time-dependent (A) and 24-h dose-dependent (B) secretion of endothelin-1 by cultured bovine aortic endothelial cells (BAEC: circles), human umbilical vein endothelial cells (HUVEC: squares), human aortic endothelial cells (HAEC: triangles), and human microvascular endothelial cells (HMVEC: diamonds) treated with (white symbols) or without (black symbols) thrombin (10 U/ml). Values are expressed as mean ± SD, $n = 8$. $^{*}P < 0.05$ and $^{***}P < 0.001$ versus respective basal levels at the same time point (A), and $^{\dagger}P < 0.05$ and $^{\dagger\dagger\dagger}P < 0.001$ versus cultures without thrombin (B)

abolished in the presence of L-NMMA, an inhibitor of nitric oxide synthase (Fig. 4).

EFFECT OF EMF RADIATION ON ET-1 MRNA LEVELS IN ENDOTHELIAL CELLS

Figure 5 shows the time course of endothelin-1 mRNA expression in response to thrombin (10 U/ml) in BAEC, HUVEC, HAEC and HMVEC. Northern blot hybridization showed that the maximal elevations of ET-1 mRNA levels after thrombin treatment were reached after 1 h in all the bovine and human endothelial cells (Fig. 5). EMF radiation (16 Vpp) did not affect basal level of ET-1 mRNA, but significantly inhibited thrombin-stimulated increase of ET-1 mRNA in all bovine and human endothelial cell cultures (Fig. 6).

Discussion

The present study demonstrated that EMF has an inhibitory effect on thrombin-stimulated ET-1 production in cultured BAEC and three types of human vascular endothelial cells – HAEC, HMVEC and HUVEC.

The main vascular effects of ET-1 are transient vasodilatation, and profound and sustained vasoconstriction, as well as proliferation of vascular smooth muscle cells.[17] Most of the ET-1 is released abluminally towards the vascular smooth muscle and less is released luminally, and it functions in an autocrine and/or paracrine manner.[17,28] The synthesis and release of ET-1 is up-regulated by thrombin,[15,17]

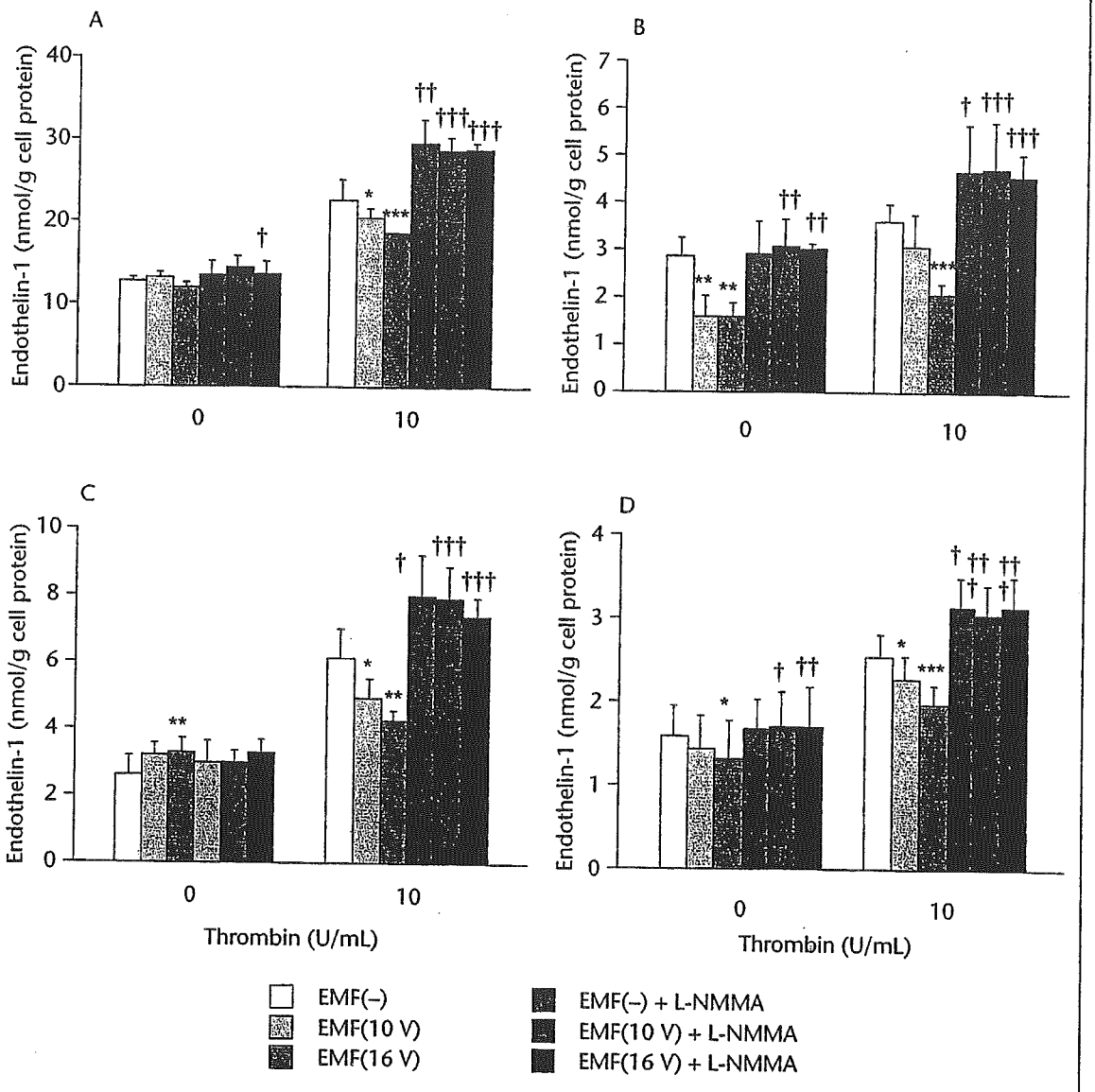

FIGURE 4: The effects of electromagnetic fields (EMF) on basal and thrombin-stimulated endothelin-1 production in cultured bovine aortic endothelial cells (BAEC, A), human umbilical vein endothelial cells (HUVEC, B), human aortic endothelial cells (HAEC, C), and human microvascular endothelial cells (HMVEC, D). Values are expressed as mean ± SD, $n = 8$, for each endothelial cell type. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus treated cultures not exposed to EMF radiation; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ versus treated cultures without addition of NG-monomethyl-L-arginine (L-NMMA)

hypoxia,[16] acidic pH[29] and certain cytokines.[15,17] The imbalance between ET-1 and other vasoactive substances may therefore be responsible for an alteration of the peripheral vascular resistance under physiological or pathological conditions. We propose that the inhibition of local production of ET-1 in the vasculature by EMF may improve local circulation. Miura *et al.*[3] have reported that EMF radiation can increase nitric oxide production in rat cerebellum, suggesting a mechanism for EMF-induced vasodilatation. Nitric oxide has been reported to inhibit ET-1 synthesis.[16,18]

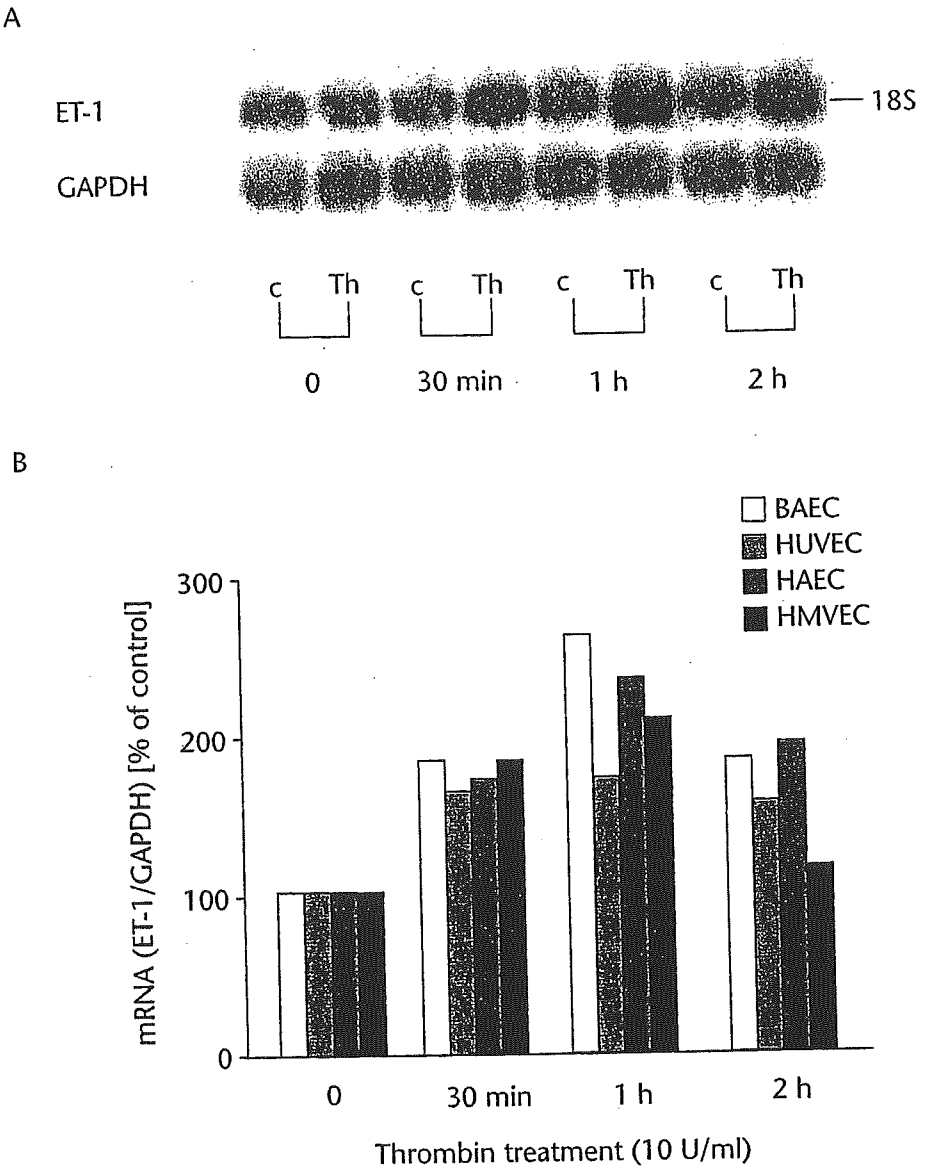

FIGURE 5: The time course of endothelin-1 (ET-1) mRNA expression in response to 10 U/ml thrombin (Th) in cultured bovine aortic endothelial cells (BAEC), human umbilical vein endothelial cells (HUVEC), human aortic endothelial cells (HAEC), and human microvascular endothelial cells (HMVEC). (A), representative blot of Th-treated HUVEC; c, control plates (vehicle only); (B), average multiples of induction of ET-1 mRNA levels in endothelial cells under control and Th-stimulated conditions over time. Data are expressed as the mean of three experiments. GADPH, glyceraldehyde-3-phosphate-dehydrogenase

The present study demonstrated that L-NMMA, an inhibitor of nitric oxide synthase, completely abolished the inhibitory effect of EMF on ET-1 production, suggesting that EMF inhibits ET-1 production through a nitric oxide-related pathway. It is reported that thrombin receptor activation increases the release of nitric oxide,[30] raising the possibility that EMF may decrease thrombin-mediated ET-1 release through augmented release of nitric oxide. This possibility was confirmed by the observation that the EMF dose-dependent suppression of thrombin-stimulated ET-1 release was completely

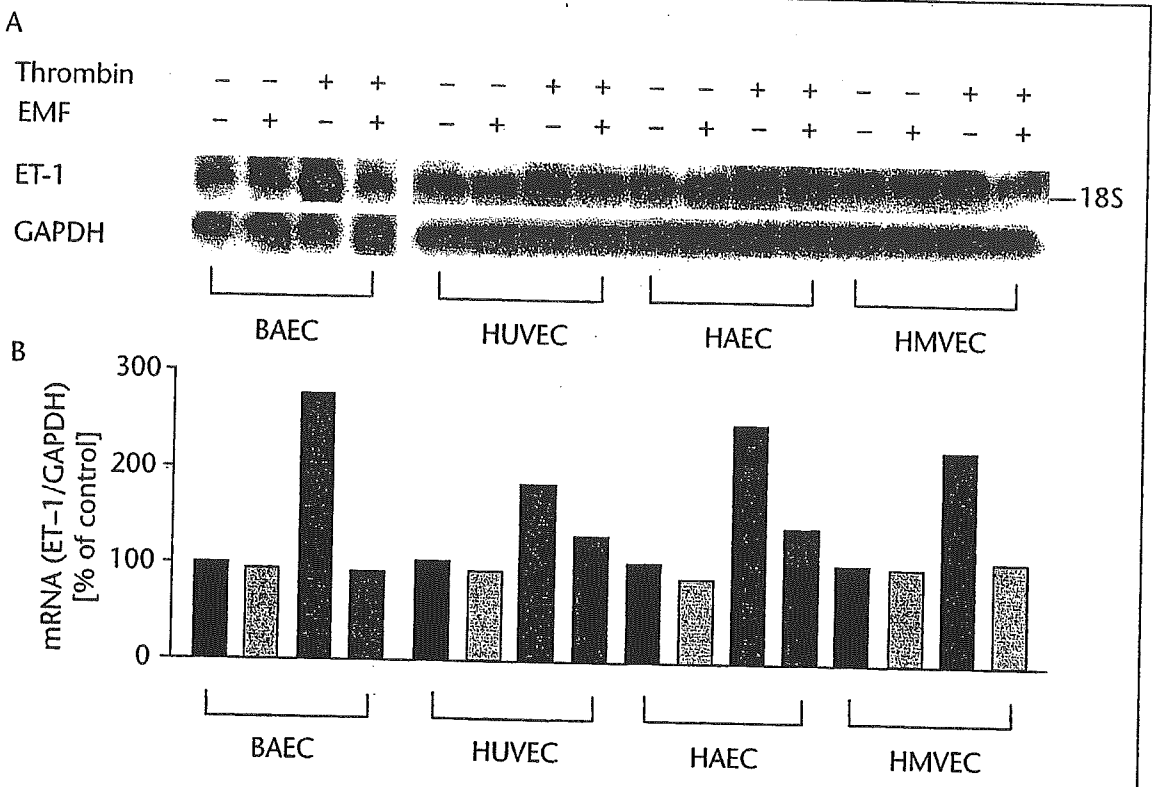

FIGURE 6: Inhibition of thrombin-stimulated endothelin-1 (ET-1) mRNA expression by electromagnetic fields (EMF) in cultured endothelial cells. Northern blot analysis was performed using 20 μg total RNA/lane: lane 1, control (■); lane 2, EMF radiation alone (▩); lane 3, thrombin alone (■); and lane 4, thrombin plus EMF radiation (■), are shown for bovine aortic endothelial cells (BAEC), human umbilical vein endothelial cells (HUVEC), human aortic endothelial cells (HAEC), and human microvascular endothelial cells (HMVEC). (A), representative blot of one of two identical experiments with a similar pattern in each endothelial cell. (B), average multiples of induction of ET-1 mRNA levels in endothelial cells. Data are expressed as the mean of three experiments

restored by the addition of L-NMMA in all the endothelial cell types studied. On the other hand, the basal production of ET-1 was not inhibited by EMF radiation in BAEC and HAEC cultures, but was inhibited in HMVEC and HUVEC. The reason for the different response to EMF radiation among the three human cell types is unclear. The HMVEC and HUVEC we used were from male neonates, while HAEC were from a female donor aged 58 years old. There is the possibility that the response to EMF radiation is age- or sex-related, or dependent upon the vascular source of the endothelial cells.

In a previous study,[15] the basal and thrombin-induced ET-1 production in endothelial cells varied according to the site of origin of the cells (microvascular endothelial cells > arterial endothelial cells > venous endothelial cells). In the present study, the basal and thrombin-stimulated ET-1 was highest in the HAEC, followed by HUVEC and HMVEC. The reason why the basal and thrombin-stimulated ET-1 production in our study was different from that in the previous study[15] remains unclear. Despite the different effects of EMF on the basal production of ET-1 among the four endothelial cell types, EMF similarly suppressed the thrombin-induced increase in

ET-1 production. These observations indicate that EMF might improve the local circulation under the vascular injury by affecting the production of ET-1.

The dose of thrombin used for this study (10 U/ml), which we also used in a previous study,[27] exerted almost maximal responses in ET-1 secretion in all of the bovine and human endothelial cell types. Moreover, the time-course of expression of ET-1 mRNA in the present study was comparable with that of our previous investigation.[27]

An interesting observation was the opposite effects of EMF on the basal secretion of ET-1 in different cultured endothelial cell types. EMF radiation substantially enhanced ET-1 secretion in HAEC, but considerably suppressed it in HUVEC. These effects suggest that EMF might reduce the shift of intravascular effusion into the extravascular space. However, EMF was also shown to inhibit the thrombin-induced increase in ET-1 production among every endothelial cell type studied.

In conclusion, the present study demonstrated that EMF radiation modulates endothelin-1 production in cultured vascular endothelial cells and that the inhibitory effect of EMF radiation is mediated through a nitric oxide-related pathway. The modulation of ET-1 production observed in the present study may represent an additional mechanism for the EMF regulation of vascular tone. Whether this mechanism could partly explain the effectiveness of EMF for clinically alleviating muscular stiffness[4] and lumbar pain,[5,6] however, requires further *in vivo* research.

Acknowledgment

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Conflicts of interest

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

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ORIGINAL ARTICLE

Incidence of adverse drug reactions in geriatric units of university hospitals

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Background: Adverse drug reactions (ADR) in elderly people are often attributed to functional decline and polypharmacy.

Methods: In this study, a multi-institutional retrospective survey was undertaken to investigate the current status of ADR in geriatric units of university hospitals. The inpatient databases from 2000 to 2002 for five university hospitals were studied, and a total of 1289 patients were analyzed.

Results: The incidence of ADR, as determined by attending physicians, was 9.2% on average, but varied from 6.3 to 15.8% among the institutions. Factors significantly related to ADR were the number of diagnoses, the number of geriatric syndromes, the number of prescribed drugs, an increase of two or more drugs during hospitalization, longer hospital stay, emergency admission, depression and apathy.

Conclusion: These results are mostly consistent with previous reports and provide important information on drug treatment in elderly people.

Keywords: adverse drug reaction, elderly, medication error.

Introduction

Adverse drug reactions (ADR) in elderly people are common causes of admission to hospitals and are important causes of morbidity and mortality.[1,2] The risk of ADR has been shown to be related to the number of prescribed drugs and elderly people tend to receive more medications than younger people,[3] which are sometimes inappropriately prescribed.[4] Indeed, the risk of ADR is exponentially rather than linearly related to the number of medications taken.[5] Factors that predispose to pharmacological ADR include the dose, drug formulation, pharmacokinetic or pharmacodynamic abnormalities and drug interactions. Frail elderly patients may be more vulnerable because of impaired homeostatic reserve, multiple medication use, cognitive decline and impaired functional status. Drug therapy taking account of safety as well as effectiveness is still needed in the elderly, although there is accumulating evidence on drug therapy in the elderly with hypertension and hyperlipemia.[6,7]

Although the incidence of ADR for specific drugs can be obtained by large-scale examination and post-marketing surveillance studies by pharmaceutical companies, little data are available on ADR in the elderly as a whole. Previously, we reported the incidence of ADR in inpatients of the geriatric unit of the University of

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Tokyo Hospital, and showed that drug overdose and polypharmacy are important factors in ADR.[8,9] However, it is necessary to confirm whether similar results are obtained in geriatric units of other hospitals. Therefore, in this study, we analyzed the inpatient databases of five university hospitals with geriatric units, and examined the incidence of ADR and factors related to ADR.

Methods

Subjects

We performed a retrospective investigation of the hospital records of five university hospitals with geriatric units: Kyorin University Hospital, University of Tokyo Hospital, Kyoto University Hospital, Kanazawa Medical University Hospital and Tohoku University Hospital. We surveyed the records of inpatients from January 2000 to December 2002 in these hospitals, and a total of 1289 cases were used for analysis.

Investigation and analysis

We studied the incidence of ADR as judged by attending physicians during hospitalization, along with the number of medications taken on admission and on discharge. We also examined the number of final diagnoses on discharge, the length of hospital stay, age, sex and body weight of each patient, and whether or not the admission was emergent. We investigated the number of geriatric syndromes in the cases at Kyorin University Hospital and the University of Tokyo Hospital and performed comprehensive geriatric assessments (CGA). The 30 most significant of 51 geriatric syndromes are listed in Table 1. The CGA included Barthel Index on admission and discharge to evaluate activities of daily living (ADL), Hasegawa's Dementia Scale-Revised (HDS-R) to assess cognitive function, Geriatric Depression Scale 30-items (GDS-30) to assess depressive mood, and Vitality Index to assess energy.[10]

The data were expressed as means ± SD. The unpaired *t*-test was used to compare the data between two groups, and comparison among multiple groups was performed by ANOVA followed by Newman-Keuls' test. The incidences were compared using the χ^2 test. Correlation was analyzed according to Pearson's correlation coefficient. A value of $P < 0.05$ was considered statistically significant.

Results

Frequency of adverse drug reaction

In the analysis of a total of 1289 cases, the incidence of ADR was 9.2%. We analyzed the incidence at each hospital and found that the lowest incidence was 6.6%, while the highest was 15.8% among the five hospitals studied (Fig. 1).

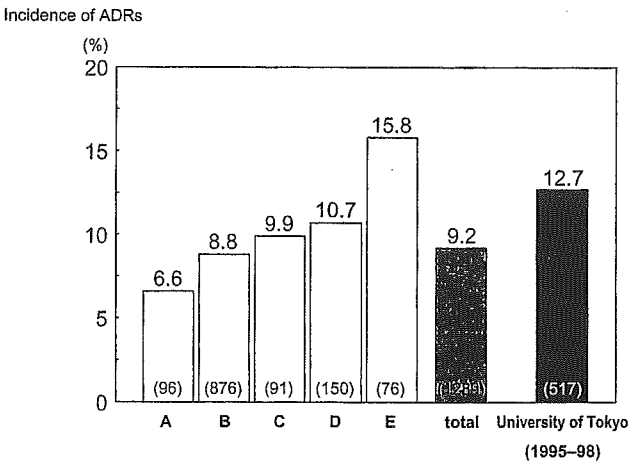

Figure 1 Incidence of ADR in inpatients of geriatric units of five university hospitals. The incidence of ADR in the geriatric unit of University of Tokyo Hospital in 1995–98 is shown as a reference.[9] The numbers of patients surveyed are shown in parentheses.

Table 1 List of major geriatric syndromes

Consciousness disturbance	Chest pain/chest oppression	Edema
Delirium	Palpitation/shortness of breath	Dehydration
Dementia	Arrhythmia	Hearing impairment
Insomnia	Abdominal pain	Motor disturbance
Depression	Constipation	Visual impairment
Dizziness/vertigo	Diarrhea	Back pain
Headache	Body weight loss	Fever
Anemia	Appetite loss	Arthralgia
Pressure ulcers	Nausea/vomiting	Osteoporosis
Falls	Malnutrition	Bleeding tendency
Hemoptysis	Dyspnea	Dysphasia
Urinary incontinence	Pollakisuria	Cough/sputum

Factors related to adverse drug reactions

Background factors related to ADR in cases with or without ADR are summarized in Table 2. There was no significant difference in sex, age or body weight between the two groups. However, patients with ADR had more diagnoses, were taking more drugs on discharge, and stayed longer in hospital than those without ADR ($P < 0.05$). They also showed a tendency to be taking more drugs on admission ($P = 0.08$). When we analyzed the relationship between ADR and the increase in medication during hospitalization, the incidence of ADR in patients with an increase of two or more drugs was 14.4%, which was significantly higher than in those with an increase of one drug (7.9%) and those without an increase (7.8%). Moreover, the incidence of ADR was higher in patients who received emergency admission than in those with scheduled admissions (12.5% vs 7.8%, $P < 0.05$).

The relationship between the factors related to ADR and the variation in ADR among the hospitals was analyzed. In hospital A, where the incidence of ADR was lowest, the number of diagnoses at discharge (2.8 ± 1.1 diseases), number of medications (4.3 ± 1.9 drugs), and the length of hospital stay (28.5 ± 6.8 days) were lowest among the five hospitals. Intriguingly, the mean age of the patients in hospital A was 82 years, while it was 67 years in hospital E, where the incidence of ADR was highest. The mean age of the patients was 71–72 years at other hospitals.

Age was positively correlated with the number of diagnoses ($r = 0.219$, $P < 0.001$) and the number of drugs at discharge ($r = 0.213$, $P < 0.001$), as previously reported.[8,9]

Geriatric syndrome and CGA were analyzed in relation to ADR in the cases at University of Tokyo Hospital and Kyorin University Hospital. The number of geriatric syndromes was significantly higher in patients with ADR than in those without ADR (Table 3). Patients with ADR showed depressed moods and apathy, as assessed by GDS and the Vitality Index, compared to those without ADR, while cognitive function and basic ADL, as assessed by HDS-R and Barthel index, did not differ between the two groups (Table 3).

Table 2 Characteristics of patients with or without adverse drug reactions (ADR)

	ADR (–)	ADR (+)
Number of patients	1170	119
Sex (female, %)	46%	50%
Age (years)	72 ± 14	73 ± 14
Body weight (kg)	56 ± 14	54 ± 14
Number of diagnoses	4.1 ± 2.0	4.9 ± 2.3*
Number of drugs on admission	5.0 ± 3.6	5.7 ± 4.1**
Number of drugs on discharge	5.3 ± 3.3	6.2 ± 3.7*
Length of hospital stay (days)	28 ± 27	38 ± 27*

*$P < 0.01$; **$P = 0.08$ by unpaired t-test.
Data are means ± SD.

Discussion

In this study, we surveyed ADR in the geriatric units of five university hospitals and found that the number of diagnoses, number of geriatric syndromes, number of prescribed drugs, an increase of two or more drugs during hospitalization, longer hospital stay, emergency admission, depression, and apathy were related to the incidence of ADR in elderly inpatients. Our study indicates that the number of diagnoses and drugs would be a better predictor for ADR in the elderly than age.

According to reports on ADR from the USA and Europe, the incidence of ADR in elderly inpatients is 6–15%.[11] The incidence was 1.5–2 fold higher in patients older than 70 years than in patients younger than 60 years. In nursing home residents, the incidence of ADR per year has been reported to be 15–20%.[11] In the outpatient setting, ADR were found in more than 10%

Table 3 Geriatric syndrome and comprehensive geriatric assessment in patients with or without adverse drug reactions (ADR)

	ADR (–)	ADR (+)
Number of geriatric syndromes	4.6 ± 3.8 (866)	6.4 ± 4.7** (85)
Barthel Index on admission	84 ± 28 (854)	80 ± 31 (82)
Barthel Index on discharge	86 ± 27 (840)	85 ± 28 (79)
HDS-R	23.0 ± 8.2 (358)	24.4 ± 6.3 (35)
GDS-30	10.2 ± 6.0 (325)	12.5 ± 6.8* (33)
Vitality index	9.0 ± 2.1 (535)	8.4 ± 2.6* (52)

*$P < 0.05$; **$P < 0.01$ by unpaired t-test. Data are mean ± SD. Numbers in parentheses indicate number of patients studied.
HDS-R, Hasegawa dementia scale-revised; GDS-30, Geriatric depression scale-30 items.

of elderly patients, although the study relied on self-reporting and review of medical records.[11] Only a few studies have been reported in Japan; the incidence was 12.7% in elderly inpatients of the geriatric unit of University of Tokyo Hospital.[9] In the present survey, the average incidence was 9.2%, ranging from 6.6 to 15.8% among facilities, but was similar to that reported previously.[9] Although the incidence varied among hospitals, it is important to note that the incidence of ADR was more than 5% in all hospitals.

Adverse drug reactions were judged by attending physicians in this study, whereas they were determined by objective review of the medical records in addition to judgment by attending physicians in the previous report from the geriatric unit of University of Tokyo Hospital. In the present study, the incidence of ADR in this facility was 8.8%, which was 30% lower than that in our last survey. This difference may be attributable to underestimation by the attending physicians rather than a decrease in ADR over this short period of 3 years. Therefore, if another authorized person judged the ADR strictly, the overall incidence rate might have been slightly higher.

Our results on the incidence of ADR in elderly patients may add important information. However, all the facilities in this survey were geriatric units of university hospitals, where most of the inpatients were older than 65 years and the doctors in those units are careful in prescribing medication to elderly patients. Therefore, our data might not be directly applicable to elderly patients in other hospitals or units. In fact, ADR were found in nearly half of elderly inpatients of the neuropsychiatry unit of University of Tsukuba Hospital (unpubl. obs, Mizukami *et al.*). In addition, our data in university hospitals, which are acute care hospitals, might not be applicable to chronic care facilities such as long-term care facilities. Since the introduction of the fixed payment system, Diagnosis Procedure Combination system, to university hospitals in Japan in 2003, drug treatment in university hospitals might be changing in the future. Therefore, the incidence of ADR in various types of hospitals in Japan needs to be studied.

In this study, depression and apathy were found to be associated with ADR in addition to the accumulation of diseases and geriatric syndromes, polypharmacy, an increase of prescribed drugs during hospitalization, longer hospital stay and emergency admission. This result is consistent with other reports.[9] However, the causal relationship remains unknown. A higher number of diseases or geriatric syndromes can lead to an increase in ADR through polypharmacy[8,9] while ADR themselves may increase diseases or geriatric syndromes. Similarly, longer hospital stays can increase the risk of ADR, while ADR prolong the duration of hospitalization. The latter point is critical to medical economics as well. Age was not associated with ADR in this study, inconsistent with other studies. This might be due to effects of education on pharmacotherapy in elderly patients for several years at university hospitals. Although we did not analyze the types or classes of ADR in this survey, it has been reported that severe ADR such as neuropsychiatric disorders or cardiovascular injury occur in elderly patients.[9]

Recently, evidence has been accumulating on drug therapy in the elderly. However, there are very few data available in people aged 75 years and older or in frail elderly people. Therefore, it is necessary to establish the safety and effectiveness of drug therapy in these patients in the future. Evidence-based medicine in the elderly aims to discontinue unnecessary drugs and to avoid polypharmacy. On the other hand, a fixed payment system such as the long-term care insurance system in Japan forces doctors to reduce prescribed drugs from a business viewpoint. Indeed, it has been reported that 0.6 drugs were on average discontinued within a month after admission to long-term care facilities, although adverse drug withdrawal events were very few.[12] Because minimally prescribed drugs have not increased ADR in patients with dementia and a low capacity for medication management,[13] it is necessary to cut down unnecessary drugs in frail elderly patients based on evidence-based medicine. In the USA, Beers' criteria are available to identify potentially inappropriate medication use, in order to reduce drug-related problems.[14] In Japan, however, we do not have such guidelines for drug treatment in the elderly. Because the drugs and medical situation in Japan are different from those in the USA, we need to establish our own guidelines, which will be published this year. In addition, we need to accumulate clinical evidence to support the guidelines. We also need to utilize pharmacists more efficiently, because they are an underused resource in avoiding medication errors and can provide important safeguards for elderly patients in hospitals and nursing homes.

Elderly patients are exposed to more medications and have an increased risk of ADR, many of which are avoidable. Knowledge of pharmacological principles and age-related effects on pharmacokinetics/pharmacodynamics is essential to promote safe prescribing. Other factors related to ADR such as polypharmacy, long admission and depression should also be evaluated during hospitalization.

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