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Paradoxical Derepression of Collagenase Gene Expression by the Antirheumatic Gold Compound Aurothiomalate

YUICHI MAKINO, HIROTOSHI TANAKA, and ISAO MAKINO
Second Department of Internal Medicine, Asahikawa Medical College, Asahikawa 078, Japan
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SUMMARY

The neutral metalloproteinase collagenase is known to be, among others, one of the key enzymes promoting joint destruction in patients with rheumatoid arthritis. Because inflammatory cytokines, e.g., interleukin-1 and tumor necrosis factor-α, are considered to activate collagenase gene expression through activation of the transcription factor activator protein-1, we examined whether the water-soluble gold compound aurothiomalate (AuTM) influenced collagenase gene expression, using phorbol ester-treated human fibroblasts. However, AuTM did not prevent phorbol ester-mediated activation of activator protein-1 DNA-binding activity and subsequent induction of collagenase gene expression. In contrast, AuTM counteracted the repressive effects of glucocorticoids on collagenase gene expression and restored collagenase mRNA levels. The molecular target of this paradoxical AuTM action was suggested to be the glucocorticoid receptor.

Gold has been advocated for the treatment of a number of human diseases, e.g., RA, bronchial asthma, and pemphigus (1). Among various gold preparations, AuTM is one of the most widely used compounds for RA (1). AuTM is water soluble and contains a sulfur moiety attached to the gold atom (1). The use of gold in the treatment of RA is still empirical and, although a number of mechanisms have been reported, it is not understood exactly how gold works (1).

The MMPs are a family of proteolytic enzymes that can degrade all of the components of the extracellular matrix (2), and the neutral metalloproteinases are considered to play a particularly important role in joint destruction in patients with RA (2). Recent studies showing that mRNA for collagenase (MMP-1, EC 3.4.24.7) is actively expressed in rheumatoid synovium suggest that the expression of collagenase, as well as that of stromelysin (MMP-3), exceeds the expression of inhibitory peptides, i.e., tissue inhibitors of metalloproteinases, *in situ* in patients with active RA (3–8).

Levels of collagenase mRNA are increased by inflammatory cytokines such as interleukin-1 or tumor necrosis factor-α (9), both of which have been shown to be increased in synovial fluids in patients with RA (9). Protein kinase C agonists such as PMA mimic such stimulatory effects of these cytokines (9), and PMA-mediated induction of collagenase synthesis occurs at the level of transcription via the involvement of the transcription factor AP-1 (9, 10), which consists of Fos and Jun proteins joined by a leucine zipper dimerization domain (10). Although the mechanism of interleukin-1- or tumor necrosis factor-α-mediated induction of collagenase gene expression has not yet been demonstrated to be mediated exclusively by protein kinase C and AP-1, such involvement has been strongly indicated (11, 12). Recently, a growing body of evidence has suggested that gold compounds inhibit protein kinase C in several cell types (13–15), indicating that gold might repress subsequent activation of AP-1 and collagenase gene expression.

Glucocorticoid hormone is a potent anti-inflammatory drug (16) and is known to repress collagenase gene expression (17–19). *Trans*-repression of the collagenase promoter by glucocorticoids has been considered to be mediated by suppression of AP-1 activity, presumably via direct protein-protein interaction between GR and AP-1 (17–19). Although we have preliminarily reported that AuTM may interfere with the DNA-binding and *trans*-activation activity of GR (20), the effect of AuTM on this negative modulatory activity of GR has not yet been examined. Given this background, we studied the effect of AuTM on collagenase gene expression, and we also asked whether GR-mediated repression of collagenase gene expression might be affected by AuTM.

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ABBREVIATIONS: RA, rheumatoid arthritis; AuTM, aurothiomalate; GR, glucocorticoid receptor(s); MMP, matrix metalloproteinase; PMA, phorbol myristate acetate; CMV, cytomegalovirus; EMSA, electrophoretic mobility shift assay; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MOPS, 3-(*N*-morpholino)propanesulfonic acid; CAT, chloramphenicol acetyltransferase; GRE, glucocorticoid-responsive element; AP-1, activator protein-1.

Materials and Methods

Cells. Monolayer cultures of normal adult skin fibroblasts (CRL 1454) were initiated from cells obtained from the American Type Culture Collection (Rockville, MD). COS-1 cells were supplied by the Japan Cancer Resource Bank. Ligand binding assays revealed the presence of GR in both cell types, at concentrations of approximately 10^5 and 10^4 sites/cell, respectively.

Reagents and antibodies. Sodium AuTM was obtained from Aldrich (Milwaukee, WI). Sodium thiomalate, malate, and dexamethasone were purchased from Sigma Chemical Co. (St. Louis, MO). Antibodies against c-Fos, c-Jun/AP-1, and NF-κB were commercially obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA).

Plasmids. The collagenase promoter plasmid −73/+63Coll-CAT (21) was a generous gift from Dr. P. Angel (Kernforschungs zentum Karlsruhe, Karlsruhe, Germany). The expression vectors for c-Jun and c-Fos (CMVjun and CMVfos, respectively) (22) were provided by Dr. T. Curran (Roche Institute for Molecular Biology, Nutley, NJ). The GR expression vectors pRShGRα and I550 were gifts from Dr. R. M. Evans (Salk Institute, La Jolla, CA) (23). pGRE-Luc is a glucocorticoid-inducible luciferase reporter plasmid and was constructed as follows. The oligonucleotide containing the GRE tandem repeat (noncoding strand, 5′-CGGATCCTGTACAGGATGTTCTAGCTACGGATCCTG TACAGGATGTTCTAGCTACG-3′, with flanking *Kpn*I and *Eco*RI sites) and a 122-base pair *Eco*RI-*Hind*III fragment of the human immunodeficiency virus long terminal repeat (positions −9193 to −9071 from the transcription start site) were inserted into a pGVB luciferase expression vector (Toyo Ink, Tokyo, Japan) that had been opened with *Kpn*I and *Hind*III. The resultant recombinant plasmid was verified by double-stranded DNA sequencing (TaKaRa, Kyoto, Japan). The β-galactosidase expression plasmid pCH110 (Pharmacia LKB, Uppsala, Sweden) was used as an internal control for transfection efficiency.

Cell culture. Cells were maintained in Dulbecco's modified Eagle's medium (GIBCO Laboratories, Grand Island, NY), pH 7.0, supplemented with 10% heat-inactivated fetal calf serum and antibiotics. Cultures were serum-starved by growth in antibiotic- and 0.2% lactoalbumin hydrolysate-supplemented Dulbecco's modified Eagle's medium for 48 hr before all experiments. Serum steroids were stripped with dextran-coated charcoal, and cells were cultured in a humidified atmosphere of 5% CO_2 at 37°, unless otherwise specified.

RNA isolation. Total RNA was isolated according to conventional methods (24). Briefly, cells were cultured in 10-cm-diameter plastic dishes (IWAKI Glass, Funabashi, Japan), in the absence or presence of ligands, for the indicated time periods. After being washed twice with phosphate-buffered saline, cells were lysed with a solution containing 4 M guanidinium thiocyanate (Fluka Chemical Co., Hauppauge, NY), 25 mM sodium citrate, pH 7.0, 0.5% sodium *N*-lauroylsarcosine, and 0.1 M 2-mercaptoethanol. RNA was further purified by phenol/chloroform extraction and was then precipitated with isopropanol. RNA pellets were solubilized in diethylpyrocarbonate-treated filtered water, and the concentration of RNA was determined spectrophotometrically.

Northern blot hybridization. Twenty micrograms of total RNA were denatured in loading buffer (0.02 M MOPS, pH 7.0, 50% formamide, 2.2 M formaldehyde, 0.01 M sodium acetate, 1 mM EDTA, 2% Ficoll, 0.01% xylene cyanol, 0.01% bromphenol blue) by heating at 70° for 5 min and were separated on a 1% formaldehyde-agarose gel. The fractionated RNA was immobilized onto a polyvinylidene fluoride membrane (Immobilon-N; Nihon Millipore, Japan) using a pressure blot apparatus (Stratagene, La Jolla, CA), and RNA was cross-linked by means of UV irradiation. The cDNA probe for human collagenase was described previously and kindly provided by Dr. Goldberg (Washington University School of Medicine, St. Louis, MO) (25). The cDNA probe for β-actin was from Dr. D. Cleveland (University of California, San Francisco, CA) (26). These cDNA probes (25–50 ng) were labeled with [α-^{32}P]dCTP (3000 Ci/mmol; Amersham) using a random primer labeling kit (BcaBEST; TaKaRa). The membrane was prehybridized with a QuikHyb hybridization solution (Stratagene) for 15 min at 68° and was hybridized to 2×10^6 cpm/ml ^{32}P-labeled probe for 60 min at 68°. After being washed for 30 min in 0.1% sodium dodecyl sulfate/2× standard saline citrate (0.3 M sodium chloride, 0.03 M sodium citrate) at 25° and for an additional 30 min in 0.1% sodium dodecyl sulfate/0.1 × standard saline citrate at 60°, the membrane was exposed to X-ray film (Hyperfilm-MP; Amersham), with intensifying screens, at −70°, usually overnight.

Preparation of nuclear extracts. Nuclear extracts were prepared as described previously (27). In brief, the nuclei were suspended at 0–4° in 15 μl of 20 mM KCl, 20 mM Tris, pH 7.6, 0.2 mM EDTA, 1.5 mM $MgCl_2$, 25% glycerol, 5 mM 2-mercaptoethanol, 0.5 mM phenylmethylsulfonyl fluoride (Sigma). Then, 60 μl of the same buffer containing 600 mM KCl were carefully added, and the nuclei were incubated for 30 min on ice. The nuclear debris was pelleted by centrifugation in an Eppendorf centrifuge for 15 min at 4°, and the resultant supernatant was used as the nuclear extract in the present study. Protein concentrations were determined using the Pierce protein assay kit (Pierce, Rockford, IL), according to the manufacturer's protocol.

EMSA. EMSA was performed as described by Lin *et al.* (28), with minor modification. In brief, the double-stranded AP-1 oligonucleotide probe was end-labeled with [α-^{32}P]dCTP (Amersham) using the Klenow fragment of DNA polymerase I (TaKaRa), and unincorporated nucleotides were chromatographically separated with a Nick column (Amersham). The nuclear extracts (usually 2 μg/reaction) were incubated with 0.5 ng of ^{32}P-end-labeled AP-1 oligonucleotide probe (approximately 20,000 cpm), in a 20-μl reaction mixture containing 10 mM HEPES, pH 7.6, 50 mM KCl, 0.1 mM EDTA, pH 8.0, 5 mM $MgCl_2$, 5 mM dithiothreitol, 10% glycerol, 1 mg/ml bovine serum albumin, and 100 μg/ml poly(dI·dC) (Pharmacia LKB), for 15 min on ice. The unlabeled competitor DNA or various antibodies were included as indicated. The reaction mixture was then loaded onto a 4% nondenaturing polyacrylamide gel containing 0.25× TBE (1× TBE is 89 mM Tris-borate, 89 mM boric acid, and 2 mM EDTA). The gels were subjected to electrophoresis at 350 V for 2 hr and dried. Results were visualized by autoradiography.

The synthetic oligonucleotide AP-1 probe was described by Lin *et al.* (28). The nonspecific competitor DNA was a synthetic oligonucleotide encompassing randomly arrayed nucleotides. The upper strand sequences of the AP-1 probe and the nonspecific competitor are as follows: AP-1 probe, 5′-AGCTTGGTGACTCATCCG-3′; nonspecific competitor, 5′-GGATCCACCCTGTCTCATGAATATGCAAATCAG GTGAG-3′.

Transfection and enzyme assays for CAT and luciferase. Transient transfection was performed as described previously (27). In brief, cells were plated in 10-cm-diameter plastic culture dishes (IWAKI Glass) to 30–50% confluency and washed three times with phosphate-buffered saline, and medium was replaced with Opti-MEM medium (GIBCO Laboratories). Plasmid cocktail was mixed with 30 μl of Lipofectin reagent (GIBCO Laboratories) and added to the culture. After 12 hr of incubation, the medium was replaced with fresh medium supplemented with 10% fetal calf serum, and culture was continued, in the presence or absence of various ligands, for 24 hr. Cell extracts were recovered by solubilization with PGC-51 solution (Toyo Ink) and subsequent centrifugation, and the extracts were suitable for enzyme assays for β-galactosidase, CAT, and luciferase. After normalization of transfection efficiency with respect to β-galactosidase expression, CAT and luciferase enzyme activities were determined according to the manufacturers' recommendations (Promega and Toyo Ink, respectively). For quantitation in the CAT assay, the radioactivity of the spots was counted using a BAS2000 PhosphorImager analyzer (Fuji Film, Minamiashigara, Japan). In the luciferase assay, a Lumat LB 9501 luminometer (Berthold Japan, Tokyo, Japan) was used for semiautomatic and quantitative determination of chemiluminescence.

Results

AuTM did not affect PMA-mediated induction of collagenase mRNA expression. Because primary synovial cells are difficult to obtain in large quantities and often show cellular

heterogeneity, normal human skin fibroblasts were used for the present studies. As shown in Fig. 1, basal levels of collagenase mRNA were slightly detectable. After stimulation with PMA, the steady state collagenase mRNA levels were increased in a dose-dependent fashion (Fig. 1), as was the case when primary synovial fibroblasts were used (5). Pretreatment of fibroblasts with AuTM, however, apparently did not affect the induction of collagenase mRNA by PMA (Fig. 1). Note that AuTM concentrations used in these experiments were clinically attainable (1, 29–31).

AuTM counteracted glucocorticoid-mediated repression of collagenase gene expression. Alternatively, AuTM might cooperate with and/or potentiate other known or as yet unknown negative regulators of the collagenase promoter; therefore, we studied whether AuTM influenced the negative effect of glucocorticoids on collagenase gene expression. Dexamethasone treatment of the fibroblasts reduced PMA-induced steady state levels of collagenase mRNA (Fig. 2), as reported previously (17), indicating the presence of functional GR in these cells. Interestingly, addition of AuTM rather derepressed collagenase gene expression even in the presence of dexamethasone (Fig. 2, *lanes 6-8*). The effect of AuTM was partial but dose dependent (Fig. 2, *lanes 6-8*) and specific for AuTM, because addition of either thiomalate or malate apparently did not mimic this antiglucocorticoid action of AuTM (data not shown).

We then further studied the mechanism of this derepression by AuTM in transient transfection assays using the reporter gene −73/+63Coll-CAT, in which the DNA fragment spanning base pairs −73 to +63 from the transcription start site of the collagenase promoter was fused with the CAT gene to confer AP-1 responsiveness (21). In the absence of PMA, AuTM did not significantly influence CAT expression (Fig. 3, compare *lanes 1* and *2*), and dexamethasone treatment produced marginal suppression of CAT activity (Fig. 3, compare *lanes 1* and *3*). PMA stimulation strongly increased CAT expression (Fig. 3, compare *lanes 1* and *4*), probably via activation of AP-1. Addition of AuTM did not significantly alter PMA-stimulated CAT activity (Fig. 3, compare *lanes 4* and *5*) and, in contrast, dexamethasone efficiently suppressed the induction of CAT

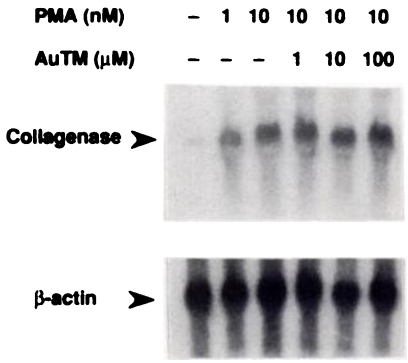

Fig. 1. Northern blot analysis of steady state levels of collagenase mRNA. Human skin fibroblasts were cultured for 24 hr in the absence or presence of AuTM at the indicated concentrations, and PMA or vehicle was added. Twelve hours later, RNA was extracted and equal amounts of total RNA (20 μg) from each sample were separated on a formamide/formaldehyde-agarose gel, as described in Materials and Methods. Blots were probed sequentially with collagenase cDNA and β-actin cDNA. Experiments were repeated three times with almost identical results, and a representative autoradiogram is shown.

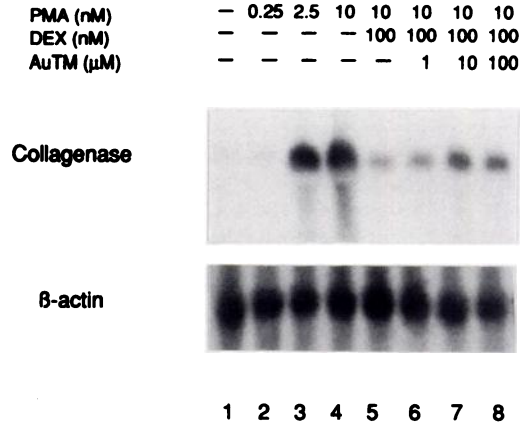

Fig. 2. Differential regulation by dexamethasone (*DEX*) and AuTM of steady state levels of collagenase mRNA. Human skin fibroblasts were cultured in the absence or presence of dexamethasone and/or AuTM for 24 hr, and PMA or vehicle was added as indicated. Twelve hours later, RNA was extracted and equal amounts of total RNA (20 μg) from each sample were separated on a formamide/formaldehyde-agarose gel, as described in Materials and Methods. Blots were probed sequentially with collagenase cDNA and β-actin cDNA. Experiments were repeated at least three times with almost identical results, and a representative autoradiogram is shown.

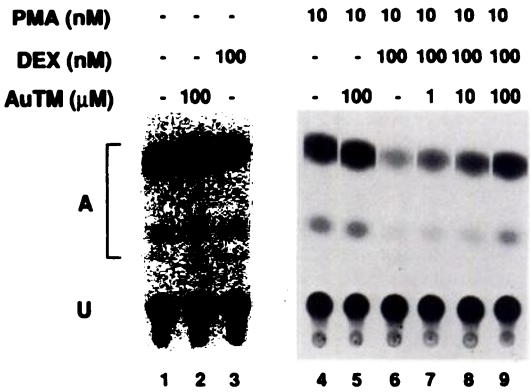

Fig. 3. Effects of dexamethasone (*DEX*) and AuTM on collagenase promoter activity. After serum depletion, human skin fibroblasts were transfected with 5 μg of the collagenase promoter-CAT reporter construct −73/+63Coll-CAT and cultured for 24 hr in the absence or presence of dexamethasone and/or AuTM at the indicated concentrations. Then, 10 nM PMA or vehicle was added, culture was continued for 24 hr, and cellular extracts were prepared for CAT enzyme assays. All procedures are described in Materials and Methods. Experiments were repeated at least three times with almost identical results, and a representative autoradiogram is shown. A and U, acetylated and unacetylated forms, respectively.

activity by PMA (Fig. 3, compare *lanes 4* and *6*), again indicating the presence of functional GR in these fibroblasts. Analogously to the results of mRNA studies (see Fig. 2), AuTM efficiently competed with the repression effects of dexamethasone and restored CAT expression (Fig. 3, *lanes 6-9*). The effect of AuTM occurred in a dose-dependent manner, at concentrations similar to those used in Northern blot experiments (compare Fig. 3, *lanes 6-9*, with Fig. 2), and neither thiomalate nor malate showed significant effects (data not shown). From these results, we suggest that AuTM does not affect either basal levels or PMA-induced activation of the collagenase promoter but counteracts glucocorticoid-mediated repressive effects on PMA-induced collagenase gene expression.

AuTM did not affect nuclear AP-1 DNA-binding activity. To examine the effect of AuTM on nuclear AP-1 DNA-

binding activity in PMA-stimulated fibroblasts, EMSA was performed. In PMA-stimulated fibroblast nuclear extracts, the presence of AP-1-specific DNA-binding activity was confirmed by the following results: 1) the formation of protein-DNA complexes was competitively inhibited not by the addition of a molar excess of randomly assigned oligonucleotide DNA but by the addition of a molar excess of unlabeled AP-1 oligonucleotide DNA (Fig. 4A) and 2) addition of either anti-c-Fos or anti-c-Jun antibodies supershifted specific protein-DNA complexes, whereas those effects were not observed when anti-NF-κB antibodies were used instead (Fig. 4A). As shown in Fig. 4B, PMA strongly induced nuclear AP-1 DNA-binding activity; however, AuTM did not significantly influence the PMA-induced levels of nuclear AP-1 DNA-binding activity, either in the absence or in the presence of dexamethasone. Thus, we considered that enhancement of AP-1 activity by AuTM was not relevant to an explanation for AuTM-mediated restoration of collagenase promoter activity.

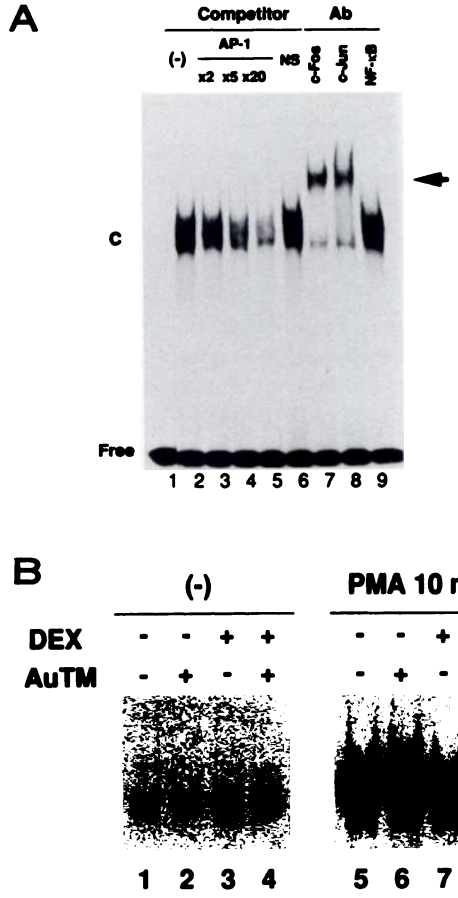

Fig. 4. EMSA for AP-1. A, Demonstration of AP-1-specific DNA-binding activity in the nuclear extracts from PMA-treated (2 hr) human skin fibroblasts. Two micrograms of the nuclear extracts were incubated with ^{32}P-labeled oligonucleotide probe containing the consensus AP-1 binding site, and protein-DNA complexes were fractionated in an EMSA. Where indicated, a 2-, 5-, or 20-fold molar excess of specific (AP-1) competitor DNA (*lanes 3-5*, respectively), a 20-fold molar excess of nonspecific (*NS*) competitor DNA (*lane 6*), or various antibodies (*Ab*) that were raised against c-Fos, c-Jun, or NF-κB (*lanes 7, 8,* and *9*, respectively) were included in the reaction mixture. *Lane 1*, without protein. *Arrow*, position of supershifted protein-DNA complexes. *C*, protein-DNA complexes formed. *Free*, free probe. B, Demonstration that AuTM and dexamethasone (*DEX*) do not affect AP-1 DNA-binding activity. Human skin fibroblasts were cultured for 24 hr in the absence or presence of 100 μM AuTM and/or 100 nM DEX and were further cultured for 12 hr with or without stimulation with 10 nM PMA, and then AP-1 DNA-binding activity was monitored by EMSA.

AuTM attenuated glucocorticoid-mediated repression of collagenase gene expression through extinction of GR function. We then examined the alternative possibility that AuTM might functionally antagonize the repressive effect of glucocorticoids. To determine whether the presence of GR is necessary to elicit such effects of AuTM, we used COS-1 cells (because of low levels of endogenous GR in these cells), and the wild-type GR expression vector pRShGRα was co-transfected with the expression vectors for c-Fos and c-Jun, as well as the reporter plasmid −73/+63Coll-CAT, into COS-1 cells as indicated (Fig. 5). Transfection of c-Fos/c-Jun expression vectors was involved instead of PMA treatment, and the vectors efficiently stimulated collagenase promoter activation (Fig. 5, *columns 1* and *4*). Without the transfection of pRShGRα, dexamethasone slightly decreased CAT activity (Fig. 5, *columns 4* and *6*) and the derepression effect of AuTM was not evident (Fig. 5, *columns 6* and *7*), suggesting that the amount of GR was insufficient to elicit effects of either AuTM or dexamethasone. When pRShGRα was co-transfected, both the repressive effects of dexamethasone and functional antagonism by AuTM became evident; dexamethasone strongly repressed CAT activity, which was partially but significantly rescued by the presence of AuTM (Fig. 5, *columns 8-11*). Together, these data indicate that the presence of considerable amounts of GR appears to be essential for both dexamethasone-mediated repression and AuTM-mediated derepression of the collagenase

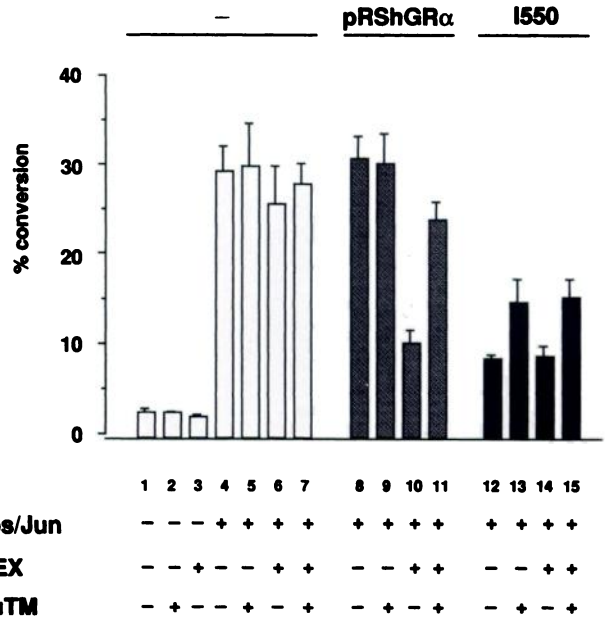

Fig. 5. Regulation of collagenase promoter activity by AP-1, GR, and AuTM. COS-1 cells were co-transfected with 1 μg each of Fos/Jun expression vectors (CMVFos and CMVJun) (24), in the absence (−; columns 1–7) or presence of 1 μg of the GR expression vectors (pRShGRα; columns 8–11, I550; columns 12–15) 5 μg of the collagenase promoter-CAT reporter construct −73/+63Coll-CAT. Total amounts of plasmids in the transfection cocktail were adjusted to 10 μg by addition of the pGEM3Z vector. After transfection, the cells were cultured in the presence or absence of 100 nM dexamethasone (*DEX*) and/or 100 μM AuTM, as indicated, for 24 hr and were assayed for CAT enzyme activity. Experiments were repeated four times and results are expressed as percentage conversion of chloramphenicol into its acetylated forms (mean + standard error).

promoter activity. We then asked whether AuTM could directly interfere with GR function. To simply address this issue, we co-transfected −73/+63Coll-CAT and the expression plasmid for the mutant GR I550, which lacks the ligand binding domain and is known to be a constitutively active receptor even in the absence of ligands (23). As shown in Fig. 5, I550 *trans*-repressed collagenase promoter activity in the absence of ligands (Fig. 5, *columns 12* and *14*); moreover, AuTM efficiently quenched the negative modulatory effect of I550 (Fig. 5, *columns 13* and *15*), strongly suggesting that AuTM did not affect ligand-GR interaction but suppressed GR function directly. If that is the case, it is of particular interest whether *trans*-activation function of GR is also interfered with by AuTM. For that purpose, the glucocorticoid-inducible reporter plasmid pGRE-Luc and the GR expression vectors were co-transfected into COS-1 cells and the cells were treated with dexamethasone and AuTM as indicated in Fig. 6. In the presence of wild-type GR, cellular luciferase activity was strongly induced in a hormone-dependent manner (Fig. 6, *columns 4* and *6*); however, the additional presence of AuTM reduced the hormone induction response of the reporter gene (Fig. 6, *columns 6* and *7*). In cells that expressed truncated, constitutively active receptor (Fig. 6, *column 8*), the apparent antiglucocorticoid effect of AuTM was also seen, without the requirement for ligands (Fig. 6, *columns 8* and *9*). When these data are taken together, we conclude that AuTM affects GR function and abolishes both *trans*-activation and *trans*-repression functions of the receptor.

Discussion

Gold compounds have been documented in numerous clinical trials to produce a remissive effect (see Ref. 1 for review).

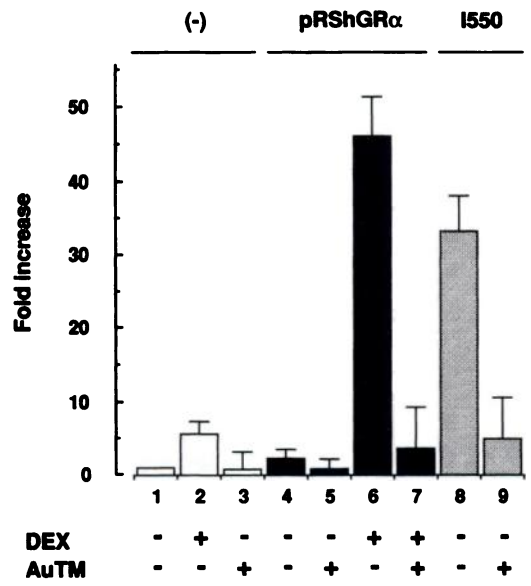

Fig. 6. AuTM suppression of the *trans*-activation function of GR. COS-1 cells were co-transfected with 5 μg of the glucocorticoid-inducible reporter construct pGRE-Luc without (−; columns 1–3) or with 1 μg of the GR expression vectors (pRShGRα; columns 4–7, I550; columns 8 and 9). Total amounts of plasmids in the transfection cocktail were adjusted to 10 μg by addition of the pGEM3Z vector. After transfection, the cells were cultured in the presence or absence of 100 nM dexamethasone (*DEX*) and/or 100 μM AuTM, as indicated, for 24 hr and were assayed for luciferase activity. Experiments were repeated four times and results are expressed as the fold increase, from basal levels, of luciferase expression (mean + standard error).

Although a body of evidence indicates that the antirheumatic activity of gold compounds is related to effects on various mediators of inflammation upstream of collagenase activation (1), the antierosive effects of these compounds have not yet been proven, at least mechanistically. It therefore appears to be particularly important to study the effects of gold compounds on collagenase activity and/or production. Thus, in this communication we have focused on the effect of AuTM on collagenase gene expression and have found that AuTM apparently does not affect PMA-dependent induction of collagenase gene expression but, rather, weakens the negative modulatory effect of glucocorticoids and paradoxically restores collagenase promoter activity. This antiglucocorticoid action is suggested to be due to the interaction between AuTM, or Au(I), and GR.

Recent studies have suggested that antirheumatic gold compounds may have direct inhibitory actions on protein kinase C in several types of cells (13–15). Because AP-1 is considered to be one of the nuclear signals for protein kinase C (10), one may speculate that AuTM suppresses collagenase gene expression at the level of transcription through reductions in AP-1 activity. In our study, however, we showed that AuTM did not alter the basal or PMA-induced levels of collagenase gene expression. Consistently, we demonstrated that nuclear AP-1 DNA-binding activity was not significantly affected by the treatment of the cells with AuTM (Fig. 4). In addition, AuTM apparently did not affect the *trans*-activation function of transiently expressed c-Fos/c-Jun proteins (Fig. 5). Therefore, our data may indicate that AuTM-sensitive protein kinase C might be different from that which is involved in AP-1 activation. In fact, protein kinase C exists as a family of at least eight different isoforms, and it is also documented that some of the isoforms differ from one another with respect to their relative abundance in different tissues or cells, the dependence of their enzyme activity on calcium or lipids, and their susceptibility to treatment with PMA (30–32). These negative results, however, do not directly contradict the clinical effects of AuTM on collagenase production and/or activity, because inhibition of collagenase expression could be secondary to reduced levels of cytokines and/or reduced responsiveness to cytokines (1). Follow-up experiments, therefore, would be necessary to clarify the effects of AuTM on cytokine expression and cytokine-induced collagenase activation.

In the present study, an unexpected but striking finding is that AuTM at least partially liberated collagenase gene expression from glucocorticoid-dependent repression. Glucocorticoids down-modulate both endogenously induced (PMA treatment) and transiently expressed (co-transfection of expression vectors for c-Fos and c-Jun) AP-1 activity, the mechanism for which is suggested to be mutual interference between AP-1 and GR (17–19). Concerning the restoration of collagenase gene expression by AuTM, AuTM did not appear to affect basal transcription machinery. Moreover, we showed that AuTM did not alter the PMA-induced level of collagenase promoter activity, which was further supported in EMSA by the fact that AP-1-specific DNA-binding activity in fibroblast nuclear extracts was not influenced by treatment with AuTM. Together, these data indicate that, as a possible explanation for liberation of collagenase gene expression from glucocorticoid-mediated repression, AuTM might, directly or indirectly, suppress GR function itself. Evidence for this hypothesis was provided by the fact that transient expression of wild-type GR increased not only

glucocorticoid-mediated repression but also AuTM-mediated derepression of collagenase gene expression. Moreover, we also demonstrated that ligands were not necessary for AuTM-mediated repression of GR function. Thus, AuTM may affect functional coupling between GR and AP-1, resulting in facilitation of the AP-1 effect on collagenase promoter activation, at least in part, in a ligand-independent manner. Additional studies using cloned or purified factors would clearly facilitate understanding of these AuTM effects at the molecular level.

We have recently shown that AuTM suppresses the DNA-binding activity of GR (20). Together with results presented in this paper, this indicates that AuTM, or Au(I), may more or less universally work as an antiglucocorticoid wherever GR regulates gene expression, either positively or negatively. To confirm this hypothesis and enable pharmacological modulation of glucocorticoid action, the molecular interplay between Au(I) and GR is now under vigorous investigation in our laboratory. In any case, additional studies are clearly needed to elucidate the molecular nature of gold action and the versatility of chrysotherapy in patients with RA.

Acknowledgments

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Send reprint requests to: Hirotoshi Tanaka, Second Department of Internal Medicine, Asahikawa Medical College, Nishikagura-4-5-3, Asahikawa 078, Japan.