Induction of Cellular Antioxidative Stress Genes through Heterodimeric Transcription Factor Nrf2/Small Maf by Antirheumatic Gold(I) Compounds*

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Gold(I)-containing compounds have long been used in the treatment of rheumatoid arthritis (RA), but the molecular mechanism of their action has remained largely unknown. In this paper we have demonstrated that gold(I) drugs selectively activate the DNA binding of a heterodimer consisting of the basic-leucine zipper transcription factors Nrf2 and small Maf. Once bound to its recognition DNA sequence termed antioxidant-responsive element or Maf-recognition element, Nrf2/small Maf induces a set of antioxidative stress genes, including heme oxygenase-1 and γ-glutamylcysteine synthetase, whose products have been demonstrated to contribute to the scavenging of reactive oxygen species and to exhibit anti-inflammatory effects. Our findings suggest that stimulation of antioxidative stress response through activation of Nrf2/small Maf may be a pharmacologically important part of the actions of gold(I) drugs for the treatment of rheumatoid arthritis. Alternatively, activation of Nrf2/small Maf may be a protective response of cells against toxic effects of the drugs.

Gold(I)-containing compounds are a part of the group of disease-modifying antirheumatic drugs and have been used for the treatment of rheumatoid arthritis (RA) for many decades. However, their exact mechanism of action is not known. Among three available gold(I) drugs, aurothioglucose (AuTG) and aurothiomalate (AuTM) are injected intramuscularly, whereas auranofin (AUR) is orally administrated. Both AuTG and AuTM are water-soluble, whereas AUR is water-insoluble, lipid-soluble, and plasma membrane-permeable. AUR is considered to be converted to a water-soluble form after cellular uptake (1).

RA is a chronic inflammatory disease characterized by the migration of activated phagocytes and leukocytes into synovial tissue, which causes progressive destruction of cartilage and bone, and joint swelling. Therefore, anti-inflammatory and immunomodulatory effects of gold(I)-containing drugs are well documented. For example, it has been reported that gold(I) drugs down-regulate the expression of a number of genes involved in promoting inflammation. The genes for collagenases, interleukin-2 (IL-2) receptor, cytokines (IL-1, IL-2, IL-6, IL-8, and tumor necrosis factor-α), and producers of chemical mediators (cyclooxygenase-2 and inducible nitric-oxide synthase) are all down-regulated by gold(I) drugs (2–4). It has been suggested that transcriptional down-regulation of these proinflammatory genes is mediated by the inhibition of AP-1 (Jun/Fos) and NF-κB transcription factors. In fact, the gold(I) drugs have been shown to selectively inhibit the DNA binding activities of AP-1 and NF-κB in vitro (2, 5), and inhibit IκB kinase activity in vivo (6).

In addition to proinflammatory gene products, reactive oxygen species (ROS) produced by activated macrophages play a pivotal role in the destruction of joint tissue in RA. Recently, ROS have been recognized to act as intracellular second messengers of cytokines and growth factors, and it has been reported that administration of superoxide dismutase, a ROS scavenging enzyme, can induce remission of RA (7). Although it has also been demonstrated that gold(I)-containing compounds inhibit the production of ROS from activated macrophages (8, 9), the molecular basis of their inhibitory action is largely unknown.

When mammalian cells are exposed to insults that induce oxidative stress, including ROS, ionizing radiation, and various chemicals, a set of antioxidative stress proteins and enzymes are induced as a part of defense system (10, 11). Some of these proteins and enzymes are well characterized and include γ-glutamylcysteine synthetase (GCS) (12–14), glutathione S-transferases (GSTs) (11, 15), NADPH:quinone oxidoreductase (NQO1) (11, 16), and heme oxygenase-1 (HO-1) (17). GCS catalyzes the rate-limiting step of synthesis of glutathione, which is an efficient ROS scavenger, and the GSTs conjugate glutathione to ROS and hydrophobic electrophiles. NQO1 catalyzes the two-electron reduction of xenotoxic quinone and its derivatives, which are then conjugated to glutathione by GSTs. HO-1 is the rate-limiting enzyme in oxidative degradation of heme to biliverdin, carbon monoxide, and iron. The importance of HO-1 in cellular defense against oxidative damage has been recently recognized (18, 19). For example, its catabolite biliverdin is subsequently converted to bilirubin, which is an efficient scavenger of ROS (20). It has also been shown that carbon monoxide and iron exhibit protective activity against oxidative stress as well as having anti-inflammatory, anti-apoptotic, and immunomodulatory activities (21, 22).

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The abbreviations used are: RA, rheumatoid arthritis; AuTG, aurothioglucose; AuTM, aurothiomalate; AUR, auranofin; IL, interleukin; ROS, reactive oxygen species; GCS, γ-glutamylcysteine synthetase; GST, glutathione S-transferase; NQO1, NADPH:quinone oxidoareductase; HO-1, heme oxygenase-1; ARE, antioxidant-responsive element; MARE, Maf-recognition element; bZIP, basic-leucine zipper; CNC, Cap’n’Collar; TPA, phorbol 12-O-tetradecanoate-13-acetate; TRE, TPA-responsive element; GFF, green fluorescent protein; RBGP, rabbit β-globin minimal promoter.
The coordinated induction of these antioxidative stress genes is mediated through cis-regulatory DNA sequences termed antioxidant-responsive elements (ARE: GTGACnnmGCA) located in the gene promoter or enhancer region (11, 23). The ARE is closely related to the Maf-recognition element (MARE: TGAGCTCAAGCTGAGACATGGCG-3', a common reverse primer). Amplified 4.0-kilobase fragments were digested with MluI and XhoI, and were inserted into pGL2-Basic plasmid (Promega, Madison, WI) to construct pHO-1-luc and pHO-1(ARE)-luc, respectively.

To construct expression plasmids for sense and antisense Nrf2 (pEF/Nrf2 and pEF/nrf2-AS), the entire open reading frame of the human nrf2 cDNA was amplified by polymerase chain reaction using the primers 5'-GAGCCGCCTGGCAGGACATGG-3' (forward primer for pHO-1-luc), 5'-GGAGCTCAAAGCGCGACGCACTCATGTA-3' (reverse primer for pHO-1(ARE)-luc), and 5'-GAGCCGCCTGGCAGGACGAGCTGTTG-3' (common reverse primer). Amplified 4.0-kilobase fragments were digested with MluI and XhoI, and were inserted into pGL2-Basic plasmid (Promega, Madison, WI) to construct pHO-1-luc and pHO-1(ARE)-luc, respectively.

DNA fragments containing the human HO-1 promoter region (32) were polymerase chain reaction-amplified from genomic DNA isolated from human embryonic fibroblast cells using specific primers, 5'-GAGCCGCCTGGCAGGACATGG-3' (forward primer for pHO-1-luc), 5'-GGAGCTCAAAGCGCGACGCACTCATGTA-3' (reverse primer for pHO-1(ARE)-luc), and 5'-GAGCCGCCTGGCAGGACGAGCTGTTG-3' (common reverse primer). Amplified 4.0-kilobase fragments were digested with MluI and XhoI, and were inserted into pGL2-Basic plasmid (Promega, Madison, WI) to construct pHO-1-luc and pHO-1(ARE)-luc, respectively.

To construct an expression plasmid containing a fusion of green fluorescent protein (GFP) and full-length Nrf2, a BsrGl restriction site was introduced just upstream of the initiator methionine codon of Nrf2 using polymerase chain reaction, and then the entire Nrf2 fragment was ligated into the BsrGl site of pEGFP-1 (CLONTECH, Palo Alto, CA). An EGFp-Nrf2 fragment from this plasmid was then inserted into the expression vector pHygEFP to create the plasmid pHygEFP/EGFP-Nrf2. The plasmid pHygEFP/EGFP-Nrf2AT-A vector (31) that lacks 400 amino-terminal amino acids of Nrf2 was constructed by deleting a 6.6-kilobase fragment of pHygEFP/EGFP-Nrf2.
IL-1, IL-2, IL-6, IL-8, tumor necrosis factor-α, cyclooxygenase-2, and inducible nitric-oxide synthase probably by inhibiting transcriptional activities of NF-κB and AP-1 (Jun/Fos) transcription factors, both of which play critical roles in the induction of these genes (2–6). To confirm this, we transfected a reporter plasmid containing multiple copies of the b2-site (5xB/luc) or TRE (3xTRE/luc), to which members of NF-κB and Jun/Fos (AP-1) families bind, respectively, into the U937 (myelomonocytic leukemia) cell line. Luciferase activities in the cells transfected with these reporter plasmids were induced by TPA treatment. Similarly to previous reports (5, 6), these activities were partially suppressed by pretreatment of the cells with AUR (Fig. 1). Thus, coordinated repression of the transcriptional activity through these cis-regulatory elements may account for the down-regulation of proinflammatory genes by gold(I) drugs.

We also assayed for the cis-regulatory element MARE/ARE, to which various homo- and heterodimers of the bZip family members, Jun, Fos, Maf, and CNC, have been shown to bind (16, 24–28). Surprisingly, luciferase activity in U937 cells transfected with reporter plasmid 3xMARE/luc (constructed using three copies of MARE-containing oligonucleotide number 7, designed to exclude the binding of AP-1 (Jun/Fos) dimers, see Table I) was significantly induced by treatment with AUR but not with TPA alone.

Activation of MARE by AUR was also observed in HepG2 (hepatocellular carcinoma) (Fig. 2, left panel) and HeLa (cervical carcinoma) cells (see below), and was dose-dependent. The other antirheumatic gold(I) drugs, AuTG and AuTM, also stimulated the luciferase activity, although the response was weaker than that observed for AUR (Fig. 2, right panel). Higher but clinically achievable concentration of AuTG and AuTM were required, probably because they are lipid-insoluble and diffuse across the plasma membrane less efficiently than lipid-soluble AUR. Addition of thioglucose tetra-acetate, thioglucose, or thiomalate, which are structurally related to AUR, AuTG, and AuTM, respectively, but lack gold and have no antirheumatic activities, did not stimulate transcription through MARE. Another antirheumatic drug, d(-)-penicillamine, which contains the thiol group but not gold, was inert to activate transcription. Thus, activation of MARE is specific to gold(I)-containing antirheumatic compounds.

Identification of Nrf2/Small Maf as the AUR-responsive Factor—MARE/ARE is related to the TRE and binds many combinations of homo- and heterodimers of members of Jun, Fos, Maf, and the CNC families (24–28). To identify factor(s) mediating transcriptional stimulation by gold(I) compounds through MARE/ARE, we utilized a set of oligonucleotides that we have previously designed to bind preferentially to certain dimers (Table I) (24, 25). Three copies of each of the oligonucleotides were introduced into a luciferase reporter plasmid that was then transfected into HepG2 cells. The cells were then tested for luciferase induction following AUR treatment. As shown in Table I, 3x#1/luc and 3x#11/luc, to which all of these bZip dimers bind efficiently, were responsive to AUR treatment. Luciferase activity of cells transfected with 3x#29/luc, which is preferentially bound by the Maf/Jun, Maf/Fos, and Maf/CNC heterodimers, was also stimulated by AUR. In contrast, 3xTRE/#23/luc did not respond to AUR, whereas 3xMARE/#7/luc, to which Jun/Jun and Jun/Fos cannot bind, did. These results suggest that Maf family members, but not the Jun and Fos family members, are involved in the induction of transcription by AUR. A reporter plasmid containing the ARE of the mouse GST-Ya gene was also induced by AUR. In contrast, the unrelated serum responsive element (SRE) of the human c-fos gene was not induced by AUR, again indicating the specific activation of MARE/ARE and its related cis-elements by AUR.

We next prepared nuclear extracts from untreated or AUR-treated HepG2 cells and subjected them to gel mobility shift analysis (Fig. 3A). A DNA-protein complex, whose intensity was induced in the nuclear extract from AUR-treated cells, was detected using oligonucleotide probes 7 and 29. The binding of this factor to probe 29 was specifically inhibited by the addition of unlabeled oligonucleotide 29 itself and other AUR-responsive oligonucleotides 1 and 11. Binding was not inhibited by unlabeled probes 17 (negative control) and 23 (TRE), clearly demonstrating that the DNA-binding specificity of this factor is very similar to the specificity of the factor responsible for AUR-
responsiveness in HepG2 cells. The AUR-inducible DNA-protein complex was also detected in nuclear extracts of HeLa, Jurkat (T-cell leukemia), and U937 cells (Fig. 3 B).

To identify this factor, we added specific antisera into the DNA-binding reaction mixture. We found that antiserum against small Maf proteins (MafK, MafF, and MafG) abolished the formation of the DNA-protein complex (Fig. 3 C), whereas antiserum against large Maf proteins (v-/c-Maf and MafB) did not (data not shown). We further tested the effects of antisera raised against candidate heterodimeric partners for the small Maf proteins and found that an antiserum against Nrf2, a member of the CNC family proteins, abolished the formation of the DNA-protein complex. The complex was supershifted by the addition of an anti-NF-E2 p45 serum that also cross-reacts with Nrf2. In contrast, antisera raised against c-Fos or other members of the CNC family, Nrf1 and Bach1/Bach2, had no

### Table I

| Oligonucleotide | Binding preference | Transactivation |
|-----------------|--------------------|-----------------|
|                 | Maf/Maf | Maf/Jun | Maf/Fos | Maf/CNC | Jun/Jun | Jun/Fos | AUR | Nrf2 |
| No. 17          | TGAGGACTCTCATCA   | +      |       |       |       |       | 1.6 | 0.8 |
| No. 7 (MARE)    | TGCTACTTACAGCA    | +      | +    | +    |       |       | 156 | 862 |
| No. 11          | TGATGACTCATCA     | ++     | ++   | ++   |       |       | 53  | 114 |
| No. 23 (TRE)    | TTGACACTCATGG     | +      | +/-  | +    |       |       | 31  | 49  |
| No. 29          | TGGCGACTCTATTG    | +/-   | +    | +    |       |       | 113 | 162 |
| ARE (GST-Ya)    | TGTCGACAGCA       | +/-   | +    | +    |       |       | 74  | 128 |
| SRE(c-fos)      | CCATATTAG        | ND    | ND   | ND   |       |       | 3.7 | ND  |

*a The same nucleotides as consensus MARE (oligonucleotide number 1) are underlined.

*b Relative binding affinities of homo- and heterodimers of Maf, Jun, Fos, and the CNC family members to the oligonucleotides.

*c Fold activation of luciferase activity of reporter plasmid containing each oligonucleotide by AUR treatment (2.5 μM) or co-transfection of Nrf2 expression vector.

*d ND, not determined.

**Fig. 3. Activation of DNA binding of Nrf2/small Maf by AUR.** A, nuclear extract of AUR-treated or untreated HepG2 cells were analyzed by gel mobility shift assay using probes 7 or 29. A 10-fold molar excess of unlabeled oligonucleotides was added to act as competitor. The AUR-induced DNA-protein complex is indicated by an arrow. An asterisk indicates homodimers of the small Maf proteins (see text). B, detection of an AUR-inducible DNA-binding factor in various cell lines. C, gel mobility shift analysis of nuclear extract from HepG2 cells in the absence or presence of antisera indicated at the top.
These results clearly indicate that the DNA-binding complex contained Nrf2 and either one or all of the small Maf members. The faster migrating complex on the gel detected by oligonucleotide probe 7 is probably composed of homodimers of small Maf proteins based on its mobility and reactivity to antisera (data not shown).

We next tested the involvement of the Nrf2 and the small Maf proteins in transcriptional stimulation of MARE/ARE by AUR. Transfection of an expression plasmid for Nrf2 together with 3xMARE-luc into HepG2 cells resulted in a large increase in luciferase activity without AUR treatment of the cells, whereas co-transfection of an expression plasmid for Nrf1 resulted in only weak induction (Fig. 4A). We also tested the effect of Nrf2 co-expression on a set of reporter plasmids and found that sequence specificity for the activation by Nrf2 was similar to that for the AUR responsiveness (Table I). Further-

**Fig. 4.** Transcriptional activation of MARE by AUR through Nrf2/small Maf. A, HepG2 cells were co-transfected with 3xMARE-luc and Nrf1 or Nrf2 expression plasmid or empty vector, and were treated with or without AUR (5 μM). The luciferase activity in untreated, empty vector-transfected cells is set at 1. B, repression of AUR-induced transactivation of MARE by an antisense expression plasmid for nrf2 but not for c-fos. The luciferase activity in AUR-treated, empty vector-transfected cells is set at 100 (%). C, suppressive effects of dominant negative mutants of the small Maf members on AUR-induced transcription.

**Fig. 5.** Transcriptional activation of HO-1 promoter by AUR. Reporter plasmids containing human HO-1 promoter fragment were schematically shown. These plasmids were co-transfected into HeLa cells with Nrf2 expression vector or empty vector, and the cells were treated with or without AUR (2.5 μM). The luciferase activity of pHO-1/luc in untreated cells was set at 1.
more, transfection of a plasmid expressing antisense nrf2 resulted in a significant reduction of stimulation by AUR, whereas an antisense c-fos expressing plasmid did not (Fig. 4B). We have previously constructed dominant negative mutants of the small Maf proteins (MafK-R22E, MafF-R22E, and MafG-R22E), that contain mutations in their DNA-binding domains and cannot bind to DNA, although they can form dimers through their intact leucine zipper domains (24, 25, 35). Transfection of these mutant expression plasmids efficiently abolished the induction of transcription through MARE by AUR (Fig. 4C), whereas a similar v-Maf mutant (v-Maf-MD26.22) (25) had little effect. Taken together, these results clearly demonstrated that Nrf2 and the small Maf proteins mediate the induction of transcription through MARE by AUR, probably by forming heterodimers.

Induction of Antioxidative Stress Genes by Gold(I) Containing Drugs through Nrf2—Nrf2/small Maf have recently been recognized as key transcriptional regulators for basal and induced expression of a set of oxidative stress-responsive genes and phase II xenobiotic detoxifying enzyme genes through MARE/ARE located in the transcriptional regulatory region of these genes (10, 11). From this information, we suspected that such genes are also induced by gold(I) drugs. To test this possibility, we first examined if AUR could stimulate the human HO-1 promoter activity. The HO-1 expression is known to be up-regulated by various stimuli including heat shock, UV irradiation, oxidative stress, heme, cytokines, antioxidants, electrophiles, and heavy metals. Recently, induction of the HO-1 gene by heme, antioxidants, and heavy metals is revealed to be mediated by binding of Nrf2 to the ARE (or the stress response element), StRE located in its enhancer region (17). We thus constructed a luciferase reporter plasmid containing the human HO-1 gene promoter (about 4.0 kilobase), and transfected it into HeLa cells. We found that the luciferase activity was increased not only by Nrf2 co-transfection but also by AUR treatment of the cells (Fig. 5). This stimulation was ARE-dependent, because deletion of the ARE from the promoter resulted in reduction of its basal and AUR and Nrf2 induced activity (Fig. 5).

We next examined mRNA expression of oxidative stress-responsive genes by Northern blot analysis. As far as we have tested, expression of mRNAs for HO-1, heavy subunit of GCS (GCSh), and NQO1 were induced in a dose-dependent manner by AUR, AuTG, and AuTM in HepG2 cells (Fig. 6A). Induction of these genes by AUR was also observed in the other cell lines, HeLa, U937, and Jurkat (Fig. 6B).

We next examined if HO-1 induction by AUR is mediated by Nrf2. Immunofluorescent staining with an anti-HO-1 antiserum revealed that the HO-1 protein was readily detected in HepG2 cells treated with AUR but not in untreated cells (Fig. 7). When transfected with an expression vector for the GFP-Nrf2 fusion protein, cells positive for GFP fluorescence were also positive for HO-1 staining without AUR treatment (Fig. 7). These data indicate that generation of HO-1 protein is induced by Nrf2. To further substantiate this finding, we constructed

**FIG. 6.** Induction of antioxidative stress genes by gold(I) drugs. A, Northern blot analysis of total RNA isolated from HepG2 cells treated with increasing concentrations of AUR (2.5, 5, and 10 µM), AuTG (100, 250, and 500 µM), or AuTM (25, 50, and 100 µM) for 16 h using 32P-labeled HO-1, GCSh, and NQO1 cDNAs as probes. B, mRNA expression of HO-1, GCSh, and NQO1 in AUR-treated (2.5 µM) or untreated HeLa, U937, and Jurkat cells.

**FIG. 7.** Induction of HO-1 by AUR through Nrf2. Untransfected or transfected HepG2 cells with expression vector for GFP fusion proteins were treated or untreated with AUR, and were stained with anti-HO-1 antiserum. Left panels indicate nuclear staining with 4,6-diamidino-2-phenylindole. Right panels indicate fluorescence of GFP fusion proteins (green) and anti-HO-1 staining (red).
an expression vector for fusion protein of GFP and amineterminal truncated Nrf2 (GFP-Nrf2TA). This truncated Nrf2 lacks the transactivator domain and is expected to act in a dominant-negative manner. We transfected this expression plasmid into HepG2 cells, and the cells were treated with AUR and then stained with HO-1 antiserum. Cells positive for GFP-Nrf2TA fluorescence were negative for staining with anti-HO-1 (Fig. 7). These results demonstrate that HO-1 induction by AUR was mediated by the Nrf2 protein.

**DISCUSSION**

We have demonstrated that gold(I)-containing antirheumatic compounds specifically activate the heterodimeric transcriptional activator Nrf2/small Maf, which then activates a set of antioxidative stress genes by binding to the cis-regulatory DNA sequence MARE/ARE present in their promoter or enhancer regions.

We have shown that HO-1 expression is stimulated by gold(I) drugs through the activation of Nrf2/small Maf. HO-1 induction by these drugs has been reported previously when using mouse peritoneal macrophages (39). Thus, HO-1 is specifically induced by antirheumatic gold(I)-containing drugs in a wide variety of cells. Anti-inflammatory and immunomodulatory activities of gold(I) drugs may be elicited in part by inducing HO-1 protein, because its catalytic biliverdin is subsequently converted to bilirubin, an efficient scavenger of ROS (20). The other two catabolites, carbon monoxide and iron, exhibit protective activity against oxidative stress as well as anti-inflammatory, anti-apoptotic, and immunomodulatory activities (21, 22). The functional importance of HO-1 in protection against oxidative damage has been consistently reported in the literature (18, 19).

We have also shown that other known downstream target genes for Nrf2/small Maf, namely GCS (12, 14) and NQO1 (11), were induced by gold(I) drugs. Accumulating evidence suggests that Nrf2 is involved in the induction of a set of oxidative stress response proteins and phase II xenobiotic detoxifying enzymes such as GSTs (11, 15), the heavy and light subunit of GCS (GCSH and GCSI) (12, 13), ferritin heavy (H) and light (L) chains (40), and A170 stress protein (10). Therefore, these genes may also be targets for gold(I) drugs. Peroxiredoxin/MS23 may also be a downstream target of Nrf2/small Maf because it has been reported to be induced by gold(I) drugs (39). The above mentioned proteins, including HO-1, have been shown to contribute to the homeostatic control of the cellular redox state, and to the protection of cells from oxidative damage. For example, GCS (GCSH and GCSI) is the rate-limiting enzyme in the synthesis of glutathione, which is an efficient intracellular ROS scavenger. Also, peroxiredoxin/MS23 is a thioroxygen peroxidase with antioxidant activity. We speculate that the orchestrated induction of these genes by the antirheumatic gold(I) drugs may protect synovial cells and joint tissues from ROS produced during inflammatory response. This is supported by recent findings which showed that cells from Nrf2 knockout mice are highly sensitive to oxidative stress-induced cell death as a result of a deficiency in the induction of the antioxidative stress genes (10). At present, however, it is unclear that induction of antioxidative stress response by the gold(I) drugs is required for their antirheumatic activities. We cannot exclude a possibility that the induction of these genes is caused by toxic effects of the drugs. Evaluation of biological activities of the drugs against experimental arthritis using Nrf2 or HO-1 knockout mice may help to elucidate the significance of this pathway.

Nrf2 has been shown to be required for both basal and phenolic antioxidant-induced expression of phase II xenobiotic detoxifying enzyme genes, GSTs (Ya and Pi class) and NQO1, by acting through the MARE/ARE located in their promoter regions (11, 15, 16). MARE/ARE has also been found in the regulatory regions of the genes for HO-1, GCSH, GCSI, and ferritin H and L chains, and has been shown to play an important role in the induction of these genes through various stimuli, including UV irradiation, hyperoxia, heavy metals, electrophilic agents, and antioxidants (12–14, 17, 40). All of these stimuli, including phenolic antioxidants, induce cellular oxidative stress. It is now understood that the transcriptional activity of MARE/ARE is regulated by oxidative stress-sensitive nuclear translocation of Nrf2. Normally, Nrf2 is anchored to the cytoplasm through its interaction with Keap1 protein. When mammalian cells are exposed to insults that induce oxidative stress, including ROS, ionizing radiation, and various chemicals, Nrf2 is translocated to the nucleus where it forms heterodimers with small Maf proteins that then bind to MARE/ARE (11, 29). As Au(I) ion has affinity for thiol, gold(I) drugs may act as oxidative stress inducers within cells. However, it seemed not plausible that they are nonspecific stress inducers because they could activate transcription through MARE more efficiently and at lower concentrations than other heavy metals such as cadmium, mercury, and cobalt ions. We are now investigating the mechanism of activation of DNA binding activity of Nrf2/small Maf by the drugs.

We have also demonstrated that antirheumatic gold(I)-containing compounds repressed the transcriptional activities of AP-1 and NF-kB transcription factors. These transcription factors play a central role in the inducible expression of proinflammatory genes such as IL-1, IL-6, tumor necrosis factor-a, and cyclooxygenase-2. Thus, the down-regulation of these genes by the gold(I) drugs may be the result of the negative effect of the drugs on these transcription factors. The antirheumatic effect of gold(I)-containing compounds is likely to be elicited through its action on multiple transcription factors, i.e., by its capacity to inhibit the transcriptional activities of NF-kB and AP-1, and through its capacity to activate Nrf2/small Maf, which leads to the transcriptional down-regulation of proinflammatory genes and the up-regulation of antioxidative stress genes, respectively. In addition to NF-kB and AP-1, this paper presents evidence to suggest that Nrf2/small Maf is a new target molecule for these antirheumatic gold(I) drugs. The elucidation of the exact pathway and mechanism of action of gold(I) drugs on Nrf2/small Maf may reveal new targets for future development and discovery of antirheumatic and antiinflammatory drugs.

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