Current Topics

Drug Discovery: Recent Progress and the Future

Review

Potential Anticancer Activity of Auranofin

Takefumi Onodera, Isao Momose,* and Manabu Kawada

Institute of Microbial Chemistry (BIKAKEN), Numazu, Microbial Chemistry Research Foundation; 18–24 Miyamoto, Numazu, Shizuoka 410–0301, Japan.

Received October 1, 2018

Gold compounds have a long history of use in medicine. Auranofin was developed more than 30 years ago as an oral therapy for rheumatoid arthritis. Now, however, auranofin is rarely used in clinical practice despite its efficacy for treating rheumatoid arthritis because more novel antirheumatic medications are available. Although its use in clinical practice has decreased, studies on auranofin have continued and it shows promise for the treatment of several different diseases, including cancer and bacterial and parasitic infections. Several potential novel applications of auranofin for treating human disease have been proposed. Auranofin inhibits the activity of thioredoxin reductase (TrxR), an enzyme of the thioredoxin (Trx) system that is important for maintaining the intracellular redox state. Particularly in cancers, TrxR inhibition leads to an increase in cellular oxidative stress and induces apoptosis. TrxR overexpression is associated with aggressive tumor progression and poor survival in patients with breast, ovarian, and lung cancers. The Trx system may represent an attractive target for the development of new cancer treatments. Therefore, the TrxR inhibitor auranofin may be a potent anticancer agent. This review summarizes the current understanding of auranofin for cancer therapy.

Key words auranofin; anticancer drug; thioredoxin reductase; thioredoxin system; drug repurposing

1. Introduction

Gold elements and compounds have long been used to treat various pathologies. In the first half of the 20th century, parenteral gold compounds, such as sodium aurothiomalate and aurothioglucone, were reported to be effective therapeutic agents for rheumatoid arthritis.1–3) Gold compounds subsequently became the main treatment for rheumatoid arthritis.4,5) In 1976, a novel oral gold-containing drug for rheumatoid arthritis, auranofin (1-thio-β-D-glucopyranosatetriethylphosphine gold-2,3,4,6-tetraacetate), was developed by Smith Kline and French laboratories6) (Fig. 1). Auranofin is an Au(I) complex containing an Au–S bond stabilized by a triethyl phosphate group. Auranofin was approved by the U.S. Food and Drug Administration (FDA) for the treatment of rheumatoid arthritis in 1985.7) Although auranofin was established for clinical use, its mechanism of action as an antiarthritic gold drug remained controversial. Recently, auranofin has been investigated as a potential therapeutic agent for a number of human diseases, including cancer, neurodegenerative disorders, acquired immunodeficiency syndrome, and parasitic and bacterial infections.8–11) Here, we focus mainly on the repurposing of auranofin as an anticancer drug.

Auranofin exhibits powerful antitumor activity in various in vitro and in vivo tumor models12,13) and has dose-dependent inhibitory activity against DNA, RNA, and protein synthesis at cytoxic concentrations. The inhibition of DNA replication by auranofin is not an important factor for inducing cytotoxicity,14) and its direct interaction with DNA also does not contribute to its cytotoxicity.15) Auranofin induces apoptosis in several cancer cell lines by increasing the level of reactive oxygen species (ROS) and changing the cellular redox state.16) Interestingly, in tumor cells, auranofin inhibits the deubiquitinating enzyme associated with the 19S regulatory subunits of proteasomes, consequently inducing apoptosis.17,18) Auranofin may interact with several intracellular proteins causing cell proliferation and inhibition of the signaling pathways involved in cancer progression.19) Furthermore, auranofin is a strong inhibitor of mammalian thioredoxin reductases (TrxRs) in the cytosol and mitochondria.19,20) TrxR inhibition is closely related to important alterations in the intracellular redox state and induces severe oxidative stress and cytotoxic effects in vitro. The inhibitory activity of auranofin against TrxR may lead to further pharmacologic effects and account for its efficacy against rheumatoid arthritis. Cancer progression is closely related to the intracellular redox state. This review summarizes the evidence supporting the development of drugs targeting TrxR for the treatment of cancer.

2. Characteristic Features of Thioredoxin and TrxR

Mammalian TrxRs are selenium-containing flavoenzymes first isolated and purified from rat liver as dimers with two identical subunits having a molecular mass of 58 kDa.21) The TrxR family comprises three members, TrxR1, TrxR2, and TrxR3,22) and each isozyme is encoded by TXNRD1, TXNRD2, and TXNRD3, respectively, on separate genes. Although their overall structures are similar, the expression levels and localization of these proteins differ. TrxR1 mainly localizes in the cytosol, TrxR2 localizes in the mitochondria, and TrxR3...
neurodegenerative disorders such as Alzheimer’s and Parkinson’s diseases. The cellular redox balance is also maintained by the glutathione (GSH) system. The GSH system utilizes NADPH as an electron donor like the Trx system. GSH is a tripeptide comprising three amino acids (cysteine, glutamic acid, and glycine) and is involved in many biologic processes as the major free thiol group in most viable cells. GSH exists in both a reduced form (GSH) and an oxidized form (GSSG) in cells. Under normal physiologic conditions of cells and tissue, more than 90% of the entire GSH pool is present in the reduced form and less than 10% exists in the oxidized form. GSH also acts as a cofactor of glutathione peroxidases, the principal defense mechanism against hydrogen peroxide and other peroxides. The balance between GSH and GSSG is regulated by glutathione-disulfide reductase (GSR), which converts the oxidized form (GSSG) to the reduced form (GSH) using NADPH as an electron donor. The three-dimensional structure of GSR is similar to that of TrxR (Fig. 2). GSR is a critical molecule for resisting oxidative stress and maintaining the reducing environment of the cell and is activated in response to oxidative stress. The ratio of the GSSG and GSH in cells is often regarded as an indicator of cytotoxicity. Additionally, glutaredoxin utilizes the reducing power of GSH to catalyze disulfide reductions in the presence of NADPH and GSR. Glutaredoxin is involved in regulating various cellular functions, including electron transport and protein folding. Thus, both the GSH and the Trx systems cooperatively play a role in preventing damage to important cellular components caused by ROS, such as free radicals, peroxydases, and lipid peroxides.

3. Role of the Trx System in Cancer
Trx and TrxR are overexpressed in various cancer cells and are potentially related to cancer cell proliferation and tumor growth. High expression levels of these proteins correlate directly with poor prognosis in a variety of cancers, including lung, ovarian, and breast cancers (Fig. 4). Trx1 has an important role in transmitting oxidative signals as a molecular switch in kinase signaling pathways. The reduced form of Trx binds to apoptosis signal-regulated kinase 1 (ASK1), and the Trx-ASK1 complex inhibits apoptosis; conversely, the dissociation of the Trx-ASK1 complex activates ASK1 and induces apoptosis in cancer cells. In -

Fig. 1. Structure of Auranofin

![Fig. 1. Structure of Auranofin](image)

Fig. 2. Domain Structures of Human TrxR1, TrxR2, and GSR

The different domains are presented in each schematic diagram. The hTrxR structure contains an FAD and NADPH binding domain as well as an interface domain between the two monomer subunits. TrxR2 also has a mitochondrial localization sequence in the N-terminal. The domain structures of hTrxR and GSR are similar, but hGSR does not have a selenocysteine residue at the C-terminal active site. The active site sequences are noted in the upper part of the motifs. Selenocysteine (U) is present at only the penultimate C-terminal amino acid in hTrxR1 and hTrxR2. (Color figure can be accessed in the online version.)

is expressed at very low levels in various tissues. These proteins have a conserved -Cys-Val-Asn-Val-Gly-Cys- catalytic site and a C-terminal -Gly-Cys-SeCys-Gly- sequence that communicates with the catalytic site, which are essential for their redox activity. TrxR activity is regulated by nicotinamide adenine dinucleotide phosphate (NADPH), which is produced by glucose-6-phosphate dehydrogenase, the rate-limiting enzyme of the pentose phosphate pathway. Mammalian thioredoxin (Trx) comprises homodimers and undergoes reversible oxidation or reduction states. Trx is a 12-kDa protein that reduces the two-cysteine groups in the conserved -Cys-Gly-Pro-Cys- catalytic site. The reduced form of Trx [Trx-(SH)2] reduces oxidized protein substrates that typically contain a disulfide group. The oxidized form of Trx [Trx-[SS]] is reduced by TrxR in an NADPH-dependent manner. Humans have two types of Trx, Trx1 (encoded by the TXN gene) and Trx2 (encoded by the TXN2 gene). Trx1 is found in the cytoplasm and is also detected in the nucleus of normal endometrial stromal cells, tumor cells, and primary solid tumors. In contrast, Trx2 contains a mitochondrial translocation sequence at the N-terminal region of the protein and is important for controlling the homeostasis of mitochondrial ROS. The Trx system, comprising Trx and TrxR, participates in extensive biologic processes that are involved in redox homeostasis, antioxidant defense, transcription factor regulation, and cell proliferation and replication. In addition, the Trx system has important roles in various physiologic and pathologic conditions in cancer, parasitic infection, and neurodegenerative disorders such as Alzheimer’s and Parkinson’s diseases. The cellular redox balance is also maintained by the glutathione (GSH) system. The GSH system utilizes NADPH as an electron donor like the Trx system. GSH is a tripeptide comprising three amino acids (cysteine, glutamic acid, and glycine) and is involved in many biologic processes as the major free thiol group in most viable cells. GSH exists in both a reduced form (GSH) and an oxidized form (GSSG) in cells. Under normal physiologic conditions of cells and tissue, more than 90% of the entire GSH pool is present in the reduced form and less than 10% exists in the oxidized form. GSH also acts as a cofactor of glutathione peroxidases, the principal defense mechanism against hydrogen peroxide and other peroxides. The balance between GSH and GSSG is regulated by glutathione-disulfide reductase (GSR), which converts the oxidized form (GSSG) to the reduced form (GSH) using NADPH as an electron donor. The three-dimensional structure of GSR is similar to that of TrxR (Fig. 2). GSR is a critical molecule for resisting oxidative stress and maintaining the reducing environment of the cell and is activated in response to oxidative stress. The ratio of the GSSG and GSH in cells is often regarded as an indicator of cytotoxicity. Additionally, glutaredoxin utilizes the reducing power of GSH to catalyze disulfide reductions in the presence of NADPH and GSR. Glutaredoxin is involved in regulating various cellular functions, including electron transport and protein folding. Thus, both the GSH and the Trx systems cooperatively play a role in preventing damage to important cellular components caused by ROS, such as free radicals, peroxydases, and lipid peroxides.

3. Role of the Trx System in Cancer
Trx and TrxR are overexpressed in various cancer cells and are potentially related to cancer cell proliferation and tumor growth. High expression levels of these proteins correlate directly with poor prognosis in a variety of cancers, including lung, ovarian, and breast cancers (Fig. 4). Trx1 has an important role in transmitting oxidative signals as a molecular switch in kinase signaling pathways. The reduced form of Trx binds to apoptosis signal-regulated kinase 1 (ASK1), and the Trx-ASK1 complex inhibits apoptosis; conversely, the dissociation of the Trx-ASK1 complex activates ASK1 and induces apoptosis in cancer cells. Trx1 is also reported to have an active functional role in cancer metastasis and progression. In mice inoculated with human breast cancer MCF-7 cells and transfected with a redox domain mutant of Trx1, tumor formation is almost completely inhibited and metastasis to other organs is not observed. Furthermore, human breast cancer MDA-MB-231 cells transfected with redox domain-inactivated Trx1 protein exhibit decreased expression of matrix metalloproteinase-9 and reduced invasion. These findings suggest that Trx1 has a key role in promoting cancer cell proliferation and metastasis. Inhibiting TrxR disrupts redox homeostasis by elevating oxidative stress and inducing apoptosis and necrosis. Yoo et al. reported that knocking down TrxR1 suppresses tumor formation in a mouse xenograft model, demonstrating the functional importance of TrxR1 in tumor progression. TrxR1 knockdown also inhibits cancer cell proliferation and DNA replication. In addition, tumor cell TrxR1 expression levels are related to the sensitivity of the cells to cytotoxic drugs in a drug-dependent manner. For example, the cytotoxicity of cisplatin is increased in cells expressing high
Mitochondria have recently been effectively targeted for anticancer therapy. One role of mitochondria is the synthesis of ATP by oxidative phosphorylation, which is indispensable for the survival of eukaryotic cells. Tumor cells require large amounts of ATP to synthesize the cellular components, such as lipids, proteins, and nucleotides, needed for rapid cell growth. Most ATP in tumor cells is generated via the oxidative phosphorylation pathway, similar to normal cells. Because the majority of ROS are produced as a by-product of oxidative phosphorylation, high levels of ROS are detected in almost all cancers. Therefore, suppressing the antioxidant system dramatically increases oxidative stress in tumor cells and inhibits tumor growth and apoptosis. These findings suggest that the Trx system should be a promising target for anticancer drugs. In addition, the GSH system is one of the defense mechanisms involved in adapting to oxidative stress. GSH is highly expressed in tumor tissues. Enhancing both Trx and GSH metabolism in tumor tissue confers drug resistance to chemotherapy in cancer cells. Tumors lacking levels of TrxR1 compared with cells expressing low levels.

Fig. 3. Trx System
The Trx system is one of the main antioxidant pathways in the body and is involved in the maintenance of redox status in mammalian cells. TrxR uses NADPH to catalyze the conversion of the oxidized form of Trx to the reduced form of Trx. NADPH is supplied by the pentose phosphate pathway.

Fig. 4. Kaplan–Meier Plots
Trx and TrxR1 expression is correlated with overall survival rate in cancer patients. High expression levels of Trx and TrxR1 are associated with a short overall survival in breast cancer (A, D), ovarian cancer (B, E), and lung cancer (C, F) patients based on Kaplan–Meier analysis. The expression data are from Kaplan–Meier Plotter (http://kmplot.com/analysis/).
TrxR1 are highly sensitive to pharmacologic GSH deficiency because the survival and proliferation of TrxR1-deficient tumors strictly depends on a functional GSH system in vitro and in vivo.44) Simultaneously targeting both the Trx and GSH systems may have synergistic effects in cancer treatment.57) The Trx and GSH systems are the major antioxidant systems required for cell survival. Concomitant inhibition of these two systems is highly effective in killing tumors and is expected to be a promising strategy for anticancer drug therapy.

4. Auranofin as an Anticancer Agent

Auranofin inhibits mammalian TrxR1 and TrxR2.19,20) Auranofin-induced inhibition of TrxR decreases the reduced form of Trx and increases the oxidized form of Trx in cells (Fig. 5). Normally, the reduced form of Trx supplies reducing equivalents to peroxiredoxin, which converts H₂O₂ to H₂O, and to ribonucleotide reductase, which reduces ribonucleotide to deoxyribonucleotide for DNA synthesis. Elevated levels of reduced Trx increase cell proliferation, activate transcription factors that regulated gene expression, and suppress programmed cell death, resulting in cell survival.58) Conversely, increases in oxidized Trx in cells lead to an increase in ROS, as well as the accumulation of oxidized proteins, DNA damage, and apoptosis.36,59) Thus, inhibiting the antioxidant mechanism can kill a variety of cancer cells, making these compounds an attractive target for anticancer drugs. The anticancer activity of auranofin has been investigated in various cancers, including non-small cell lung cancer (NSCLC), gastrointestinal stromal tumor (GIST), and osteosarcoma (OS). In 2016, Li et al.50) observed that auranofin inhibits the growth in some NSCLC cell lines with an IC₅₀ value of less than 1.0 µM. In addition, they reported that the overexpression of TrxR in auranofin-sensitive Calu3 cells induces partial resistance to auranofin, and high expression of TrxR is one cause of auranofin tolerance. Further, auranofin inhibits the expression of several key proteins in the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway. Li et al.50) also observed that the intraperitoneal administration of auranofin inhibits tumor growth by 67% compared to controls in a study using an NSCLC xenograft tumor model. Pessetto et al.61) showed that auranofin markedly inhibits GIST cell growth and viability. Auranofin stimulates caspase-3/7 activity and induces apoptosis in GIST cells by inhibiting TrxR and the subsequent elevation of ROS. Parrales et al.62) found that combining auranofin with vorinostat or rapamycin synergistically induces apoptosis in OS cells. Auranofin suppresses the cell growth of OS and tumor growth in an OS xenograft model. Thus, combination therapy with auranofin and other drugs having different mechanisms of action is meaningful for expanding treatment options.

The pharmacokinetics of auranofin were evaluated in humans because it was approved by the FDA for treating rheumatoid arthritis. After oral dosing with auranofin, almost all is absorbed in the alimentary tract within the first 20 min.8) Approximately 25% of the dosage is detected in the plasma and it predominantly binds to albumin.7,63,64) The plasma concentration of auranofin reaches 60 to 90 µg/L within 1 to 2 h.65–67) The terminal plasma half-life of auranofin ranges from 17 to
25d and it is eliminated from the body after an average of 55 to 80d.\textsuperscript{27} A total of 85% of auranofin is excreted via the feces, and the remaining 15% via the kidneys.\textsuperscript{26,28} Based on its pharmacokinetics, auranofin was recently evaluated in clinical studies for treating cancer (see www.clinicaltrials.gov trial numbers NCT01419691, NCT01737502, NCT01747798, and NCT03456700).

5. Conclusion and Perspectives
Auranofin, a gold-containing antirheumatic drug, has been studied for its potential repurposing for the treatment of many other diseases in addition to rheumatism. Auranofin inhibits TrxR activity and increases intracellular ROS levels. The active sites of TrxR, -Cys-Val-Asn-Val-Gly-Cys- on the N-terminal side and -Gly-Cys-SeCys-Gly on the C-terminus, are essential for its enzyme activity. Because auranofin has high affinity for protein thiol and selenol groups,\textsuperscript{20-28} it is thought that it binds the active sites of TrxR and inhibits its activity. Auranofin-induced inhibition of TrxR causes the accumulation of ROS in cells and induces apoptosis and cell death. The importance of redox regulation in cancer cell proliferation and resistance to chemotherapy is widely accepted. The findings from many studies indicate the potential of auranofin for cancer therapy based on manipulating the redox state in cells. We found that auranofin-induced inhibition of TrxR showed preferential cytotoxicity to human pancreatic cancer cells under nutrient-deprived conditions that mimicked the tumor microenvironment (unpublished data). Therefore, antioxidant machinery such as the Trx system may be an attractive target for cancer therapy.

The GSH system is also an antioxidant pathway that guards against oxidative stress. Concomitantly targeting both the Trx and GSH systems can have synergistic effects for treating cancer.\textsuperscript{57} In addition, auranofin has been evaluated as an adjuvant therapy for sensitizing cancer cells to cytotoxic agents by altering their redox state.\textsuperscript{60} Therefore, combination therapy with auranofin and other drugs possessing different mechanisms of action may be useful for cancer therapy. Simultaneous inhibition of Trx and GSH, however, also induces death in mice.\textsuperscript{59}

The risks of inhibiting both systems must therefore be carefully considered because both systems are also important in normal cells and tissues. A better understanding of the redox-regulatory mechanisms of TrxR and other related molecules will provide important information for the development of new cancer therapies.

Finally, novel uses for clinically established drugs \textit{i.e.}, drug repurposing, is a very promising and effective strategy in the modern drug discovery process. Drug repurposing helps to reduce much of the effort involved in evaluating the safety and pharmacokinetics of drugs. Moreover, drug repurposing can markedly reduce the time and cost of drug development. It is expected that auranofin will be a successful example of drug repurposing.

Acknowledgment  We sincerely appreciate all of the members of the Institute of Microbial Chemistry for their useful advice and extensive discussions.

Conflict of Interest  The authors declare no conflict of interest.

References