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Novel use of old drug: Anti-rheumatic agent auranofin overcomes imatinib-resistance of chronic myeloid leukemia cells

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Abstract

Patients with chronic myeloid leukemia (CML) are commonly treated with a specific inhibitor of BCR-ABL tyrosine kinase, imatinib mesylate (IM). Unfortunately, CML patients develop IM-resistance, which has emerged as a significant clinical problem. Somatic mutations, especially T315I mutation, in BCR-ABL kinase domain represent the most common mechanism underlying drug resistance to tyrosine kinase inhibitors (TKI), including imatinib. Thus, it is urgent to develop novel therapeutic strategies to overcome TKI-resistance. The anti-rheumatic gold (I) compound Auranofin (AF), was recently approved by US Food and Drug Administration for Phase II clinical trials to treat leukemia. In a recent study, we discovered that AF can selectively inhibit 19S proteasome-associated deubiquitinases (UCHL5 and USP14), which mediates its anticancer effects. More recently studies we have shown that AF inhibits the growth of both Bcr-Abl wild-type cells and IM-resistant Bcr-Abl-T315I mutation cells *in vitro* and *in vivo*. AF-induced Bcr-Abl down regulation is associated with diminished mRNA expression and caspase-dependent Bcr-Abl cleavage. More importantly, we unraveled that AF cytotoxicity is mediated by proteasome inhibition rather than previously suspected reactive oxygen species (ROS) generation. These findings support that AF overcomes IM-resistance through Bcr/Abl-dependent and -independent mechanisms, identifying a potentially new strategy for cancer treatment.

Keywords

auranofin; CML; Bcr-Abl; imatinib resistance; proteasomal deubiquitinases; reactive oxygen species

Introduction

The Bcr-Abl fusion protein produced by the Philadelphia chromosome is present in more than 95% of chronic myelogenous leukemia (CML) [1, 2]. The deregulated tyrosine kinase

activity of the Bcr-Abl oncoprotein, is critical for malignant transformation by activating several signaling cascades, including the PI3K/AKT, JAK2/STAT5, and MAPK/ERK pathways [3]. Imatinib mesylate (IM), a selective Bcr-Abl kinase inhibitor by blocking the ATP-binding site of the kinase domain, has achieved a remarkable success in treating CML. Although IM treatment shows great efficacy in patients with newly diagnosed CML at the chronic phase, and to a less extent, at the accelerated and blastic-phase, imatinib-treated CML patients nearly all acquire IM-resistance and clinical relapse develops over time [4]. Point mutations in Bcr-Abl kinase domains, which prevent the binding of IM with Bcr-Abl, are believed to be the main cause of imatinib resistance. Among the known mutations, the gatekeeper T315I point mutation is the most stubborn mutation and poses a major challenge in the treatment of CML [5]. To overcome the acquired IM-resistance, new tyrosine kinase inhibitors (TKI) such as nilotinib (AMN107), dasatinib (BMS-354825), and bafetinib (INNO-406), were developed and have proven to be effective against almost all mutations except the T315I mutation [6–8]. Therefore, novel methods to overcome IM resistance are desperately needed. Other studies show that LAQ824 and triptolide have significant activity against imatinib-resistant CML cells by decreasing Bcr-Abl mRNA and protein levels, proposing a new approach to overcoming imatinib resistance [9–11].

The ubiquitin proteasome system is the principal mechanism for protein degradation in the cell, pivotal role to both protein quality control and the regulatory degradation in a wide variety of cellular processes including cell survival and proliferation. Accordingly, proteasome inhibitors have become potentially attractive anticancer drugs [12]. The 26S proteasome is assembled from a 20S core particle (CP) that is capped by the 19S regulatory particle (RP) at one or both ends. Deubiquitinating enzymes or deubiquitinases (DUBs) are proteases that are capable of removing the ubiquitin from ubiquitinated proteins and thereby alter the conformation and functions of a wide range of cellular proteins and by doing so regulate a variety of cellular processes and functions. In mammals, there are three DUBs associated with the 19S RP: RPN11, USP14, and UCH-L5. The physiological roles of the 19S-associated DUBs are not completely known. It has been suggested that RPN11 performs ubiquitin chain amputation by cleaving the entire ubiquitin chain from the substrate in a process coupled to degradation [13]. In contrast, the action of USP14 and UCH37 is thought to trim ubiquitin chains from the distal end, thereby shortening the ubiquitin chain and antagonizing proteasomal degradation [14,15]. Several DUBs have been found to be involved in cancer progression; therefore, they are emerging targets for cancer therapy [16]. We and others have reported that metal (Cu, Zn)-containing compounds can induce cytotoxicity in human cancer cells via targeting the 20S proteasome and 19S proteasome DUBs [17,18]. These findings have prompted us to search for clinically used compounds that can selectively inhibit 19S proteasome-associated DUBs rather than the 20S proteasome.

Auranofin (AF), a gold (I) -containing agent, has been approved by the FDA in 1985 for the treatment of rheumatoid arthritis. It has also been reported that AF has anti-cancer effects against various human cancers, including breast, ovarian, lung, as well as lymphoma and leukemia [19–21]. AF is currently in phase II clinical trials for the treatment of leukemia such as chronic lymphocytic leukemia (<http://clinicaltrials.gov/ct2/show/NCT01419691>). After we carefully analyzed the cytotoxic effect of AF and its reported mechanisms, it became

apparent to us that some of the characteristics induced by AF are very consistent with the changes induced by proteasome inhibition; thus we propose that like copper compounds [18] AF may target the proteasome. Interestingly, we found that different from bortezomib, AF inhibits 19S proteasome-associated DUBs UCHL5 and USP14 but not the 20S proteasome activity. Notably, in cancer cells from acute myeloid leukemia patients, therapeutic doses of AF even more strongly inhibited proteasome function than bortezomib [22]. Importantly, we discovered that selective inhibition of tumor growth by AF *in vitro* and *in vivo* depends on its proteasome inhibiting action. However, most of the previous reports believe that AF induces apoptosis *via* the inhibition of thioredoxin reductase activity and the production of intracellular reactive oxygen species (ROS) levels [19]. To clarify this seemingly discrepancy, we used two kinds of antioxidants to scavenge ROS and found that thiol-containing antioxidant NAC could rescue AF-mediated proteasome inhibition and cell apoptosis, while non-thiol-containing antioxidant TBHQ could not. This is because that NAC could bind with and inactivate AF as detected by HPLC assay and reported previously [23] but TBHQ could not bind with AF. These results further confirm that AF-mediated apoptosis is associated with proteasome inhibition rather than ROS generation.

The 20S proteasome inhibitor bortezomib has been reported to be effective in overcoming IM-resistance in CML cells. Bortezomib treatment induces cytotoxicity in CML cells through inhibition of Bcr/Abl, inactivation of NF- κ B, and induction of cell cycle arrest and apoptosis [24, 25]. Unfortunately, bortezomib had minimal efficacy and considerable toxicity in patients with IM-refractory CML [26]. Moreover, our previous study demonstrated that proteasome inhibitor gambogic acid downregulates Bcr-Abl by caspase-dependent Bcr-Abl cleavage and overcomes IM-resistance in CML cells [27]. Hence, it is necessary to explore the potential of 19S proteasome-associated DUB inhibition as a treatment of BCR-ABL-positive, in particular IM-resistant leukemia. Our recent studies showed that AF induces apoptosis in CML cells bearing wild-type Bcr-Abl and T315I mutation Bcr-Abl and inhibits the growth of Bcr-Abl-T315I xenografts *in vivo*; we also found that AF down-regulated the levels of total and phosphorylated Bcr-Abl proteins in KBM5 and KBM5-T315I cell lines. Furthermore, we found that AF treatment reduced Bcr-Abl mRNA level to the some extent in both cell lines. The degree of Bcr-Abl mRNA reduction is less dramatic than that of Bcr-Abl protein reduction, suggesting that AF-induced suppression of Bcr-Abl transcription may not account entirely for, but is likely partially responsible for, the decreased Bcr-Abl protein levels. This proposition is further supported by comparing the effects of AF and bortezomib on Bcr-Abl expression. Both DUB inhibitor AF and proteasome inhibitor bortezomib caused the accumulation of ubiquitinated proteins, caspase activation, and Bcr-Abl down-regulation, but AF seemed to be more potent than bortezomib in downregulating total and phosphorylated Bcr-Abl proteins, consistent with the notion that AF inhibits Bcr-Abl gene expression [28].

Similar to what we observed earlier in other cancer cell lines [22] we found that AF induced accumulation of ubiquitinated proteins and proteasome-specific substrate proteins in KBM5 and KBM5-T315I cell lines; only thiol-containing antioxidants but not non-thiol-containing antioxidants could completely block AF-induced proteasome inhibition, Bcr-Abl

downregulation and cell apoptosis. These results provide compelling evidence that proteasome inhibition rather than ROS generation plays an essential role in AF-mediated caspase activation and Bcr-Abl down-regulation in CML cells. We and others have reported that Bcr-Abl can be cleaved by caspase activation [27,29]. We observed that broad-spectral inhibition of caspases could reverse AF-mediated cell death, Bcr-Abl decreases and, to certain extent, Bcr-Abl downstream event proteins but did not attenuate accumulation of ubiquitinated proteins. These results suggest that caspase activation induced by AF plays an essential role in downregulation of Bcr-Abl and its downstream events.

Collectively, we have unraveled that AF induces cell apoptosis and overcomes IM-resistance in CML cells through both Bcr/Abl-dependent and -independent mechanisms. On one hand, AF inhibits the expression of Bcr-Abl mRNA and induces Bcr-Abl protein cleavage by caspase activation, thereby inhibiting cell proliferation; on the other hand, as an inhibitor of 19S proteasome-associated DUBs, AF induces proteasome inhibition-dependent apoptosis by activating both the intrinsic and the extrinsic caspase pathways (Figure 1) [22,28]. These findings yield important new insights into the pharmacology of a clinical anti-rheumatic drug AF, identifying AF as a specific inhibitor of 19S proteasome-associated DUBs. Since proteasome inhibition is known to be anti-inflammatory and immunosuppressing it is tempting to speculate that AF's proteasomal DUB inhibition action might have been the primary mechanism for its anti-rheumatic efficacy. Our studies have also illustrated a brand new therapeutic strategy to overcome imatinib resistance in CML cells, a highly significant clinical problem to be solved, further underscoring their potential clinical impacts. In the studies we reported so far, for *in vivo* testing, we used a solid-tumor model which may be not as good as a non-solid leukemia model. It will be important to test the *in vivo* effect of AF on a non-solid leukemia model in our future research [30]. In addition, we have designed a series of AF analogues to find a common mechanism of gold (I) compounds and improve the effects of AF in CML cells that are resistant to imatinib.

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Figure 1. A proposed mechanism by which Auranofin induces cytotoxicity in CML cancer cells
 Auranofin induces cell proliferation inhibition and apoptosis *via* downregulating Bcr-Abl mRNA expression and activating Bcr-Abl cleavage.