

Metal-Based Compounds as Proteasome-Inhibitory Anti-Cancer Drugs

Sara Schmitt and Q. Ping Dou*

Barbara Ann Karmanos Cancer Institute, Departments of Oncology, Pharmacology and Pathology, School of Medicine, Wayne State University, Detroit, MI 48201-2013, USA

Since the war on cancer began forty years ago, significant advances have been made in the treatment of the disease. However, cancer remains the second-leading cause of death in the United States, and resistance and toxicity to current treatments hinder progress in fighting the disease. Thus, the search for new treatment strategies with more specificity and less toxicity continues. The ubiquitin-proteasome pathway (UPP) has emerged as a promising target, and metal-based proteasome inhibitory compounds have been investigated as potential treatment strategies.

The UPP is a tightly regulated process responsible for maintenance of protein homeostasis in cells [1,2]. The UPP selectively degrades proteins involved in several biological processes, including development, differentiation, proliferation, signal transduction, and apoptosis [3], many of which are deregulated in cancer and contribute to tumorigenesis. Because unbalanced protein homeostasis has been implicated in cancer development, progression and "survival, targeting..." the major pathway responsible for protein turnover has emerged as a promising strategy for cancer treatment [4,5]. Increased proteasome activity has been reported in several different cancers, such as colon [6], prostate [7] and leukaemia [8], suggesting that cancer cells may rely more heavily on the UPP than non-cancer cells and that the UPP may be a viable target for cancer treatment. Indeed, targeting this pathway was validated as a strategy by the FDA approval of bortezomib for the treatment of relapsed multiple myeloma and mantle cell lymphoma in 2003. Unfortunately, while many patients have benefited from bortezomib treatment, it has proven ineffective against solid tumours. Additionally, severe side effects and resistance have limited its clinical use. Furthermore, bortezomib efficacy is also affected by its interactions with some natural compounds such as green tea polyphenols [9,10]. Therefore, new proteasome inhibitory compounds must be developed to overcome the adverse effects and resistance associated with bortezomib.

The importance of metals like copper and zinc to normal cellular function has led to the investigation into compounds that bind these biological metals as potential proteasome inhibitory anti-cancer treatments. Studies have shown that copper levels are altered in tumour-bearing mice and humans [11,12], which led to an extensive investigation into the role of copper in carcinogenesis. Various cancers such as breast [13,14], colon [15], prostate [15,16], lung [17] and brain [18] have since been reported to display increased serum and tissue levels of copper. A critical role for copper in angiogenesis was discovered in 1980 [19], and it was later shown that copper induces transcription of VEGF mRNA and protein expression [20,21], contributing to the growth, invasion and metastasis of tumour cells [22,23]. Because of copper's essential role in the growth and development of cancer, copper has emerged as a putative anti-cancer target and as the metal centre in some anti-cancer drugs, especially after the early 1960s discovery of the anti-cancer properties of cisplatin. The mechanism by which cisplatin triggers apoptosis is well-understood: cisplatin interacts with DNA and forms adducts which interfere with replication and transcription [24]. While cisplatin has contributed to the cure or treatment of many cancers since its inception, toxicities and resistance associated with cisplatin treatment have hindered its clinical use and spurred the search

for new, less toxic metal-based treatments [25,26], using biologically active metals like copper, as well as other metals like gold which have been used successfully in the treatment of other ailments, with different mechanisms of action, such as inhibition of the UPP.

Several copper-chelating compounds have been investigated for their ability to bind copper and inhibit the cellular proteasome to cause apoptosis. Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline, CQ), is a lipophilic compound that is currently used to treat Alzheimer's and Huntington's diseases [27] and is able to form stable complexes with copper(II) [28]. CQ-Cu (1:1 ratio) inhibits purified 20S proteasome ($IC_{50}=2.5 \mu M$), as well as intact cellular proteasome in C4-2B and LNCaP prostate cancer cells. In addition to inhibition of proteasomal activity, CQ-Cu suppressed androgen receptor expression, inhibited cellular proliferation and induced apoptosis in these cells [28]. In C4-2B xenograft-bearing mice, tumour growth was significantly suppressed (66%) following CQ treatment, and proteasome inhibition was also evident [28]. Another copper-binding compound, disulfiram (tetraethylthiuram disulfide, DSF) was also tested for its potential as a proteasome inhibitor [29]. DSF is an irreversible aldehyde dehydrogenase inhibitor that is one of two drugs approved for the treatment of alcoholism [30,31]. DSF-Cu (1:1 ratio) also inhibits both purified ($IC_{50}=7.5 \mu M$) and intact breast cancer MDA-MB-231 cellular proteasomes [29]. Cellular proliferation was inhibited and proteasome-inhibition-associated accumulation of ubiquitinated proteins and apoptotic events were also observed. In *in vivo* experiments of breast cancer xenograft-bearing mice, tumour growth was inhibited by DSF treatment [29]. Importantly, treatment with both CQ and DSF resulted in little to no changes in body weight in *in vivo* studies, suggesting little toxicity for these compounds. In fact, several phase I and II clinical trials are underway investigating the tolerability and efficacy of DSF in human subjects. Thus, the use of copper-chelating compounds may provide a promising clinical strategy for the treatment of human cancers.

Gold has also been investigated as a potential inhibitor of the proteasome because gold (III) is isoelectric with Pt(II) and tetracoordinate gold complexes possess the same square planar geometry as cisplatin [32]. Importantly, gold has also been used for decades to reduce inflammation associated with rheumatoid arthritis [33]. A gold (III) dithiocarbamate derivative, $[Au(DMDT)Br]_2$,

*Corresponding author: Q. Ping Dou, Barbara Ann Karmanos Cancer Institute, Departments of Oncology, Pharmacology and Pathology, School of Medicine, Wayne State University, Detroit, MI 48201-2013, USA, Tel: 313-576-8301; Fax: 313-576-8307; E-mail: doup@karmanos.org

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inhibited purified 20S proteasome ($IC_{50}=7.4 \mu M$) as well as intact proteasome in MDA-MB-231 breast cancer cells [34]. Accumulation of ubiquitinated proteins and p27 and induction of apoptosis, associated with proteasome inhibition, were also observed. $Au(DMDT)Br_2$ also inhibited tumour growth *in vivo* [34]. Recently, the therapeutic effects of a gold (I) complex, AUL15, were compared against the activity of a gold (III) complex, AUL12. Both complexes inhibited the growth of MDA-MB-231 cells, with AUL12 being much more potent ($IC_{50}=4.5 \mu M$ vs. $13.5 \mu M$) and both compounds also inhibited purified proteasome, again with AUL12 being more potent ($IC_{50}=1.3 \mu M$ vs. $17.7 \mu M$) [35]. Interestingly, the production of reactive oxygen species was observed following treatment with AUL12, but not AUL15 [35], indicating that oxidative stress induction may play a role in the cytotoxicity of the gold (III) complex.

In addition to copper and gold, gallium has also been investigated for its anticancer properties, because although it does not have a long history of clinical use or any reported physiologic activity, it is able to interact with proteins and play a role in some cellular processes. Gallium is used primarily in diagnostics, but reports have indicated that it may have potential in a therapeutic capacity [36]. A series of gallium complexes with two pyridine phenolate ligands with asymmetric NN'O donor groups were tested for their ability to inhibit proteasome activity and induce apoptosis in C4-2B prostate cancer cells. Chloro-, bromo- and iodo- substituted species [37] were able to inhibit purified proteasome ($IC_{50}=46, 27$ and $16 \mu M$, respectively) and cellular proteasome ($IC_{50}=48, 28$ and $17 \mu M$, respectively) activities [38]. Ubiquitinated protein and p27 accumulation, AR suppression and apoptosis induction, all associated with proteasome inhibition, were observed following treatment with these complexes. Mice-bearing prostate cancer xenografts treated with the bromo-substituted complex exhibited inhibition of tumour growth, associated with the appearance of apoptotic indices [38]. Thus, gallium may serve as a viable option for the specific treatment of tumour cells.

Adverse effects and resistance to treatments remain large problems in the war on cancer. Therefore, it is important to discover better targets and treatments that will be more specific and less toxic. As a major player in the maintenance of cellular homeostasis, the UPP has emerged as a promising target for cancer therapy. The dependence of cancer cells on the UPP, compared with healthy cells, suggests that targeting this pathway may be more specific than other treatment strategies. Recently, several metals, including copper, gold and gallium, among others, have been investigated for their potential as proteasome inhibitors in cancer therapy and the copper-chelating compound DSF is currently being investigated in clinical trials. Thus, the UPP is a viable target for the treatment of human cancers and metal-based compounds are promising proteasome-inhibitory drug candidates.

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