Published in final edited form as:
Biol Trace Elem Res. 2012 October ; 149(1): 5–9. doi:10.1007/s12011-012-9391-z.

Association of metals and proteasome activity in erythrocytes of prostate cancer patients and controls

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Keywords

Prostate cancer; metal; arsenic; cadmium; copper; lead; zinc; proteasome activity; erythrocyte

Introduction

Little is known about the effects that environmental metal exposure may have on proteasome activity within the human body. The proteasome is a multi-protein complex of both permanent and transient interacting proteins, some of which require essential metals such as zinc to function [1;2]. The proteasome is responsible for the degradation of proteins involved in the regulation of cell differentiation, proliferation, signal transduction, and apoptosis, as well as the destruction of misfolded and damaged proteins. Dysfunction of the proteasome and the ubiquitin-proteasome pathway has been implicated in several diseases including cancer [3-6]. Inhibition of proteasome activity is associated with tumor cell death [7]. Several proteasome inhibitors currently under development as chemotherapeutic agents contain metals or metal complexes [8-10]. Both biologically essential metals for example copper [7;11;12] and non-essential metals including cadmium [13] have shown some efficacy as proteasome inhibitors in cell culture.

This study aims to determine whether erythrocyte arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), or zinc (Zn) levels are associated with chymotrypsin–like (CT-like) proteasome activity in prostate cancer patients and control subjects. CT-like activity is considered to be the primary measure of the degradation potential of the proteasome, although trypsin- and caspase-like activities have been shown to be important in protein degradation as well [14]. We hypothesize that there will be differences in the associations of metals and CT-like proteasome activity in men diagnosed with prostate cancer when compared to men without prostate cancer.

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Materials and Methods

Blood samples

Erythrocytes were separated from peripheral blood samples of African American and white prostate cancer cases and controls as part of the Gene-Environment Interaction in Prostate Cancer Study (GECAP)[15] and were stored at −20 °C. A subset of GECAP cases (n=61) and controls (n=61) were selected for study here, and were matched on race, age (± 2 years) and date of blood draw (± 1 year).

Metals

Metal levels were measured by inductively coupled plasma mass spectrometry (ICP-MS). 100 μL of erythrocyte was mixed with 1 mL of concentrated nitric acid (Optima Grade from Fisher) in acid-washed and dried polyethylene tubes, and incubated overnight at room temperature. The following day samples were heated for 15 minutes at 70 °C. For As, Cd, Cu and Pb analysis, 0.3 ml of the digested sample was diluted to 3 mL. For Zn analysis, 0.07 mL of the digested sample was diluted to 3 mL. Internal standards used were Y and In. Calibration standard stock solutions were purchased from VWR or Perkin Elmer. HPLC grade water was used for all the measurements. For samples and standards, the final nitric acid concentration was kept below 20%. Samples were analyzed in a Perkin Elmer Sciex Elan 9000 ICP-MS with a cross flow nebulizer and Scott type spray chamber. The RF power was 1000 and the argon flow was set at 0.92 L/min. The detection limits for the different metals under these experimental conditions were: 0.004 ppb for As, 0.005 ppb for Cd, 0.01 ppb for Cu, 0.01 ppb for Pb and 0.5 ppb for Zn. The reported ICP-MS data are the results of two to three replicate measurements of at least two separately digested samples from each erythrocyte sample.

Proteasome Activity

Proteasome activity was determined by the method previously described [11]. Briefly, proteins were extracted from the erythrocytes in a lysis buffer (50 mM Tris-HCl/pH 8.0, 5mM EDTA, 150mM NaCl, 0.5% NP-40, and 0.1% of protease inhibitor cocktail) for 30 min at 4 °C. Afterwards, the lysates were centrifuged at 14,000 g for 20 min, and the supernatants were collected and protein concentration was measured. Fifteen μg of total protein from each erythrocyte sample was incubated with 40 μM of fluorogenic peptide substrates Suc-Leu-Leu-Val-Tyr-AMC (for CT-like activity) in 100 μl assay buffer (20mM Tris-HCl, ph 7.5) for 2 h at 37 °C. After incubation, production of hydrolyzed fluorescent AMC groups was measured using Wallac Victor^3 multilabel counter with an excitation filter of 365nm and an emission filter of 460nm. Matched case and control pairs were run together in one of five separate batches.

Statistical Analysis

T-tests were used to determine differences between groups and Pearson correlation coefficients were used to assess associations between individual metals and CT-activity. Partial correlations took into account race and CT-batch. Multivariate linear regression models were run separately for cases and controls and included CT-like activity as the dependent variable and all metals and CT-batch as the independent variables. Since cases and controls were matched on race and age and these two variables did not appear to modify results, they were not included in final regression models. Statistical significance was set at p-value <0.05.

Results

Cases and controls ranged in age from 42 to 75 years of age and did not differ in race or age by design. There were no significant differences in mean metal levels or CT-like activity between cases and controls, although cases had slightly lower zinc (12,132 ppb vs.12,496 ppb, p= 0.48), higher copper (892 ppb vs. 756 ppb, p= 0.33), and higher CT-like activity (6,103 RFU vs. 5,601 RFU, p=0.38) as expected [16-18].

As Table 1 shows, we first evaluated correlations between individual metals, as metals may work in concert or be antagonistic to one another with regard to their effects on the proteasome. Among cases there were significant correlations between metals, while in controls there were none. In cases, arsenic was significantly associated with cadmium ($r = 0.49$, $p<.001$), lead ($r = 0.26$, $p=.04$), and zinc ($r = 0.35$, $p=.006$) and cadmium was positively correlated with lead ($r = 0.53$, $p<.001$). Further, Table 1 shows associations between metals and CT-like proteasome activity. There were no significant associations between individual metals and CT-like activity in cases. In healthy controls, however, higher copper was associated with lower CT-like activity ($r = -0.38$, $p=.003$), while higher lead, a non-essential metal and toxicant, was associated with higher CT-like activity ($r = 0.29$, $p=.026$).

In multivariate linear regression models which included all metals and controlled for batch (Table 2), significant associations remained between copper and lead and proteasome activity in controls. There were no significant associations between metals and CT-activity in cases.

Discussion

In this study of erythrocyte metal levels and proteasome activity, we observed significant correlations between the non-essential toxic metals, arsenic, cadmium and lead, in prostate cancer cases but not in controls. Further, we did not observe significant associations between metals and CT-like activity in cases but did observe such associations in controls. We observed a significant inverse association between copper, an essential metal, and proteasome activity, and a significant positive relationship between lead, a non-essential metal, and proteasome activity in controls. These findings suggest that in healthy subjects both essential and non-essential metals may affect proteasome activity. In addition, metal-proteasome associations appear to differ between prostate cancer cases and controls.

The correlation of metals within cases but not controls is of interest. As noted by Schrauzer [19] several metals including As, Cd, and Pb can act as selenium-antagonists and can result in the inactivation of selenium (Se), a metal commonly studied for its prostate cancer protective effects. Studies by the same author with others [20;21], using prostate tissue collected during autopsy, showed that the ratio of Cd to Se was higher in older men compared to younger men, suggesting that changes in metal ratios over the lifespan may play a role in age related tumors such as prostate cancer. In another study [22], lower iron and Zn levels were associated with biochemical recurrence of prostate cancer, while, Cd and Se levels did not differ between those with and without recurrence. Together these findings suggest that multiple metals may be involved in prostate cancer etiology and may play different roles throughout the course of the disease.

The ability of copper to inhibit proteasome activity has been reported [7] and copper complexes have been shown to be effective proteasome inhibitors [11;23]. Our results suggest that, in healthy individuals, copper may be a natural inhibitor of the proteasome, but in prostate cancer cases the ability of copper to inhibit proteasome activity may be lost as part of the disease process. However, high copper levels are known to occur in cancer

patients [17] and our lack of association between copper and CT-activity in cases may be due to non-proteasome related cancer processes that cause and/or require increases in copper.

In terms of our finding of a positive association between lead and proteasome activity in controls, Grunblat-Etkovitz et al. [24] have shown that lead increases proteasome activity in neuroblastoma cell lines and work by Gou et al. [25] and Bardag-Gorce et al. [2] suggests that delta-aminolevulinic acid dehydratase (ALAD) may interact with the proteasome. ALAD is responsible for the second step of heme synthesis. Lead has long been known to inhibit ALAD and ALAD inhibition has been used as a biomarker of lead exposure [26]. It has also been suggested that ALAD is a natural inhibitor of proteasome activity [25]. Lead may inhibit ALAD or interfere with other proteins that make-up or interact with the proteasome, causing increases in proteasome activity. A lack of association between lead and CT-like activity in prostate cancer cases again suggests that the proteasome may change or function differently in men who develop prostate cancer than in men who are disease free.

More work is needed to understand how essential metals and non-essential metals affect the normal proteasome and how differences or changes in these metal-proteasome interactions may play a role in prostate cancer.

Acknowledgments

Sources of funding: Wayne State University President's Research Enhancement Program, NIEHS 5R01 ES011126, CDMRP W81XWH-07-1-0252

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Table 1

Partial correlations* between erythrocyte metals and proteasomal CT-like activity in prostate cancer cases and controls

	Cases n=61					Controls n=61				
r p-value	**As**	**Cd**	**Cu**	**Pb**	**Zn**	**As**	**Cd**	**Cu**	**Pb**	**Zn**
As	1.0	.486	−.061	.264	.350	1.0	.013	.220	.031	.125
		<.001	.65	.04	.006		.92	.10	.81	.34
Cd			−.104	.53	.029			−.044	.130	−.057
			.43	<.001	.83			.74	.32	.66
Cu				−.19	.027				−.008	.122
				.16	.84				.95	.36
Pb					−.012					−.139
					.93					.29
Zn					1.0					1.0
CT-like	−.016	−.10	.20	−.026	−.05	−.082	−.182	−.38	.29	−.018
activity	.90	.44	.13	.84	.68	.54	.17	.003	.026	.89

Abbreviations: Arsenic, As; Cadmium, Cd; Copper, Cu; Lead, Pb; Zinc, Zn; Chymotrypsin-like, CT-like.

Significance level $p < 0.05$.

*Metal-metal Pearson correlations controlled for race. Metal-proteasome activity correlations controlled for race and CT-batch.

Table 2

Associations between metals and proteasomal CT-like activity in cases and controls

	Cases n=61		Controls n=61	
Model	**standardized beta ± SE**	**p-value**	**standardized beta ± SE**	**p-value**
As	0.046 ± 0.121	0.72	0.007 ± 0.098	0.94
Cd	−0.12 ± 0.134	0.37	−0.157 ± 0.087	0.08
Cu	0.133 ± 0.100	0.19	−0.280 ± 0.089	0.002
Pb	0.032 ± 0.120	0.79	0.230 ± 0.088	0.011
Zn	−0.050 ± 0.107	0.64	0.045 ± 0.091	0.62
Batch	0.650 ± 0.105	< 0.001	0.680 ± 0.096	< 0.001

Abbreviations: Arsenic, As; Cadmium, Cd; Copper, Cu; Lead, Pb; Zinc, Zn; Chymotrypsin (CT)-like batch, batch. Significance level $p < 0.05$.