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Original Article

The effect of short and long-term doxorubicin treatment on K562 cells and Prdx6 expression

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ABSTRACT

Peroxiredoxin (Prdx) proteins are a family of antioxidants that protect cells from oxidative stress. The expression of these proteins has been shown to be elevated in many cancers, and recent studies have found peroxiredoxin overexpression in cancer cells lines that are resistant to chemotherapies, suggesting a role for peroxiredoxins in cancer cell progression and/or survival. This study investigated the effect of the chemotherapy drug doxorubicin on K562 leukemia cells growth, viability and Prdx6 expression. We show that treatment of K562 cells with 50 nM doxorubicin for a 72-hour and 4-week period causes a decrease in cell density and viability to approximately 50%, indicating doxorubicin-resistance. We also show that Prdx6 levels are significantly elevated at the end of both treatments compared to non-treated K562 cells. These findings demonstrate doxorubicin-resistance in K562 cells and support a role for peroxiredoxins in the cellular response to doxorubicin in leukemia cells, and possibly other cancer cells.

KEYWORDS: K562, doxorubicin, peroxiredoxin 6.

INTRODUCTION

Chemotherapy resistance is a common occurrence in cancer treatment, varying with the type of cancer. As the problem is so prevalent, many researchers are looking to understand how resistance occurs in individual cancer types, as well as possible therapies to prevent it. Doxorubicin, otherwise known as Adriamycin, is a chemotherapy commonly used to treat several cancers including breast cancer and leukemia [1]. It initiates apoptosis in cancer cells through various mechanisms, depending on the type of cancer. Some of the known mechanisms include disruption of adjacent GC pairs in DNA through hydrogen bonding [2, 3], topoisomerase-II poisoning resulting in double strand breaks [4, 5], and histone eviction in some leukemia [6]. In addition to directly interacting with the DNA, doxorubicin creates a superoxide and hydrogen peroxide when oxidized [7] and generates highly reactive hydroxyl radicals forming a doxorubicin-iron complex [8]. Although doxorubicin is known as a front line drug for several cancers, resistance is still an issue for many patients. The mechanisms of how resistance to doxorubicin occurs are still unknown, but it is believed to involve several proteins and pathways [4].

Doxorubicin is used to treat several leukemia including acute myelogenous leukemia (AML) and acute lymphocytic leukemia (ALL), and has been found useful in treating some chronic myelogenous leukemia (CML) that are resistant to Imatinib [9]. The human K562 cell line is derived from a chronic myeloid leukemia and has been used extensively in research. Some research has been done in doxorubicin-resistant K562 leukemia cells to investigate the mechanisms of resistance in leukemia. Studies range from exploring specific gene mutations in doxorubicin-resistant K562 cells [10] to investigating the effects of inhibiting specific proteins, such as SIRT1, in reducing K562 resistance to doxorubicin [11].

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The Peroxiredoxin proteins constitute a family of thiol-specific antioxidants whose main role is to reduce cellular peroxides and protect cells from oxidative stress [12]. There are six members of the family in mammalian cells, each with a distinct cell and tissue distribution. Prdx1 is localized in the cytosol, close to the plasma membrane, Prdx2 and Prdx6 are generally localized in the cytosol, Prdx3 is found in the mitochondria, Prdx4 locates to the endoplasmic reticulum, and Prdx5 has been found in several cellular compartments [12]. Studies have shown that peroxiredoxins are upregulated in many cancers and down regulated in others [13]. The changes in expression of Prdx in cancers indicate that these proteins may have an important role in the growth and maintaining of the cancer.

While there is growing research examining the role of peroxiredoxins in cancer biology, very little research has been conducted on the role of these proteins in leukemia and leukemic cells. Some studies have shown that targeting Prdx1 increases differentiation in K562 cells, leading to apoptosis [14, 15], suggesting that it may have an important role in maintaining the cancer state. However, there are no studies to date examining the role of peroxiredoxins in doxorubicin-resistant K562 cells. As Prdx6 is localized to the cytosol, where doxorubicin is oxidized [4], we decided to look further into its potential role in doxorubicin resistance in K562 leukemia cells. In addition to its location, Prdx6 is also the only Prdx that contains a single cysteine residue (as the other five are 2-cys) and hence it is known to catalyze reduction reactions in a different way [16]. Knowing the important antioxidant role of Prdx6 and that doxorubicin induces oxidative stress, we hypothesized that Prdx6 expression may be regulated in response to short and long-term doxorubicin treatment, and that it may play a role in resistance to doxorubicin in K562 leukemia cells.

METHODS

Cell culture and treatment

K562 leukemia cells were purchased from ATCC and were cultured in IMDM media supplemented with 10% fetal bovine serum, and maintained at 37 °C with 5% CO_2 in a humidified chamber. For the 72-hour experiments, cells were treated in triplicate in 6-well plates using doxorubicin at a final concentration of 50 nM (or untreated control cells). For the 4-week experiments, cells were treated at a final concentration of 50 nM of doxorubicin in a ventilated T25 flask, and media and doxorubicin were replenished weekly by gently centrifuging the cells, and replenishing with new media and doxorubicin over a four-week period.

Viability and growth measurements

Viability and growth were measured using the trypan blue exclusion method. Cells were diluted 1:1 with 0.4% trypan blue; then cell counts and viability measurements were made using a hemocytometer. A two-tailed t-test was conducted to determine statistical significance in density and viability between control and treated cells.

Protein quantification and western blot

Protein quantification was performed using the Coomasie Plus reagent, and 30 ug of protein from each sample were electrophoresed on 12% gels (Biorad) using SDS-PAGE. Protein was transferred onto a PVDF membrane (Biorad). Mouse anti-actin (Sigma, A5441) and mouse anti-Prdx6 monoclonal (Abcam, ab16947) antibodies were used as primary antibodies, followed by appropriate alkaline-phosphatase conjugated secondary antibodies, and bands were detected using the CDP-star reagent. Blot images were captured using the G-Box and band analysis was done using Syngene GeneTools software.

RESULTS

K562 cells were treated with doxorubicin for a 72-hour period. The cell density and viability were calculated every 24 hours. As shown in Figure 1, the growth of cells was significantly inhibited as early as 24 hours after treatment, and inhibition continued throughout the three days. By 72 hours, cell density in the control cultures was about three times higher than in the treated cultures. In addition to the inhibition in cell density, there was a significant reduction in cell viability in doxorubicin-treated cells, showing approximately 50% viability at 72 hours (Figure 2).

To examine the long-term effect of doxorubicin on K562 cells, cells were treated with 50 nM doxorubicin over a 4-week period. To maintain treatment, cells were spun, washed and retreated

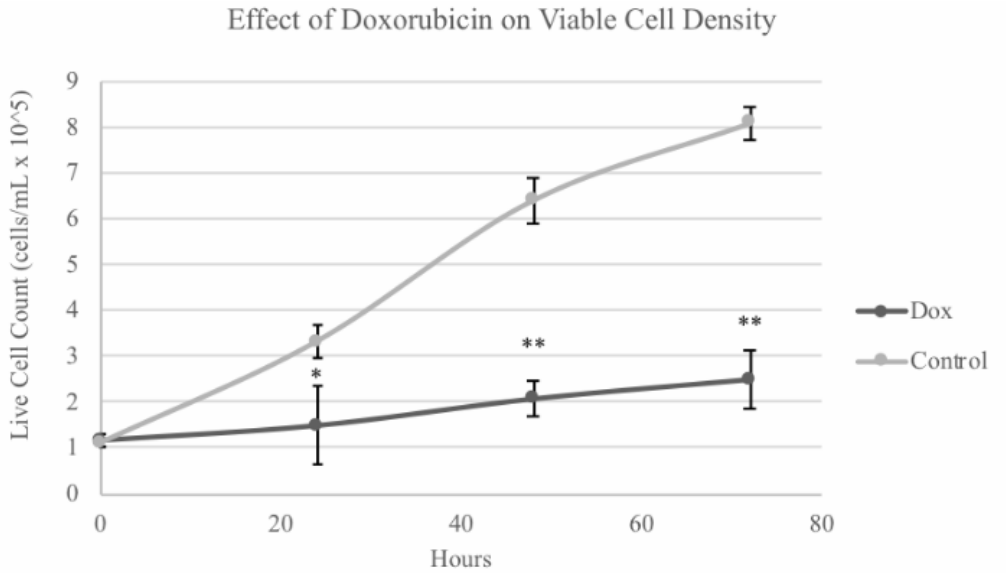

Figure 1. K562 cell density over a 72-hour treatment with doxorubicin. Cells were treated with or without 50 nM doxorubicin and viable cell density was measured daily using trypan blue exclusion. Averages at each time point are shown (+/- stdev). (* $p < 0.05$; **$p < 0.01$).

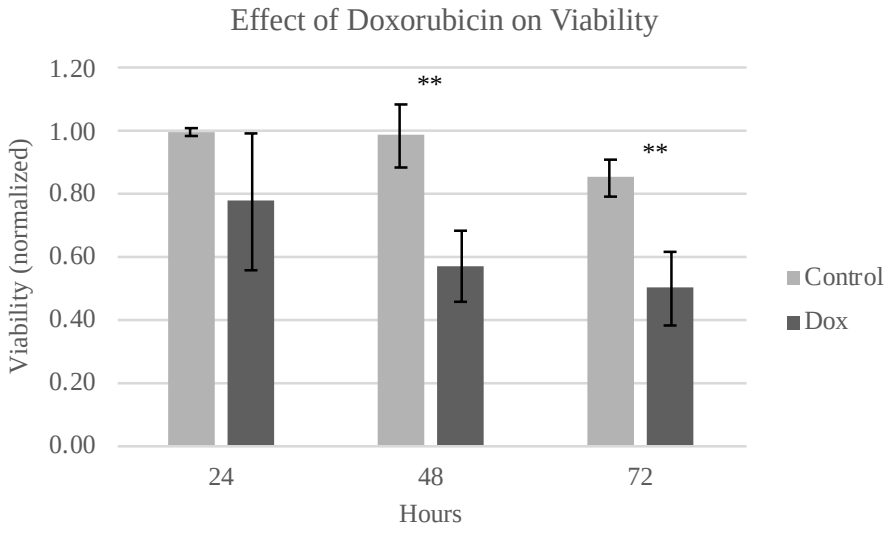

Figure 2. K562 cell viability over a 72-hour treatment with doxorubicin. Cells were treated with or without 50 nM doxorubicin and viability was measured daily using trypan blue exclusion. Normalized averages at each time point are shown (+/- stdev). (**$p < 0.01$).

weekly over the four-week period. The cell density and viability were measured once per week. As shown in Figure 3, while many cells were lost through the treatment, viability of the final culture remained at approximately 50%. This suggested that remaining cells in the doxorubicin-treated cultures included doxorubicin-resistance cells.

We sought to examine the morphology of the treated cells throughout the four-week period. The cultures were photographed at 100X magnification at various time points, as shown in Figure 4. As is evident, treated cells demonstrated a range of cell morphologies as early as three days post treatment. As compared to untreated cells, some cells were

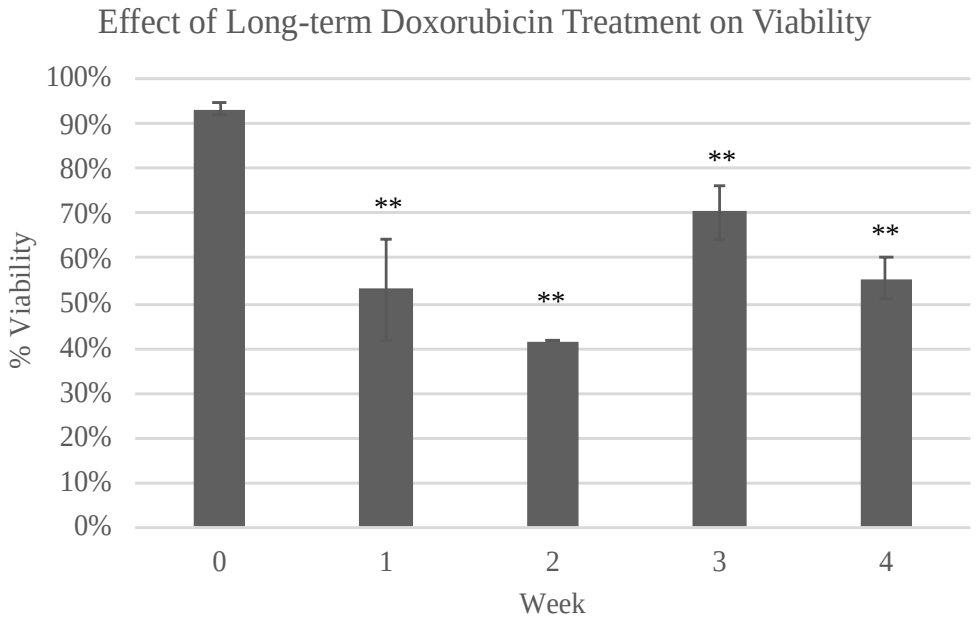

Figure 3. K562 cell viability over a four-week treatment with doxorubicin. Cells were treated with or without 50 nM doxorubicin for four weeks, with weekly replenishment of media and treatment. Viability was measured weekly using trypan blue exclusion. Averages at each time point are shown (+/- stdev). (**p < 0.01).

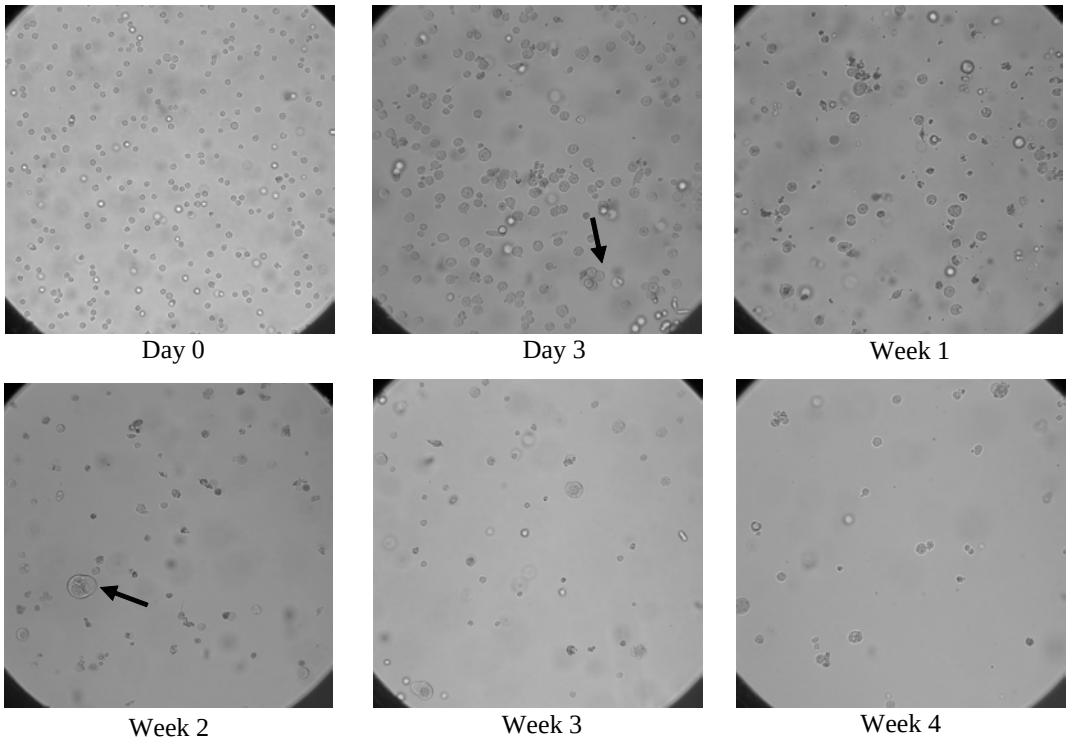

Figure 4. Morphology of K562 cells during doxorubicin treatment. Cells were treated with 50 nM doxorubicin over a four-week time period. Cells were imaged at the indicated days (at 100X). Arrows indicate morphological changes induced by doxorubicin.

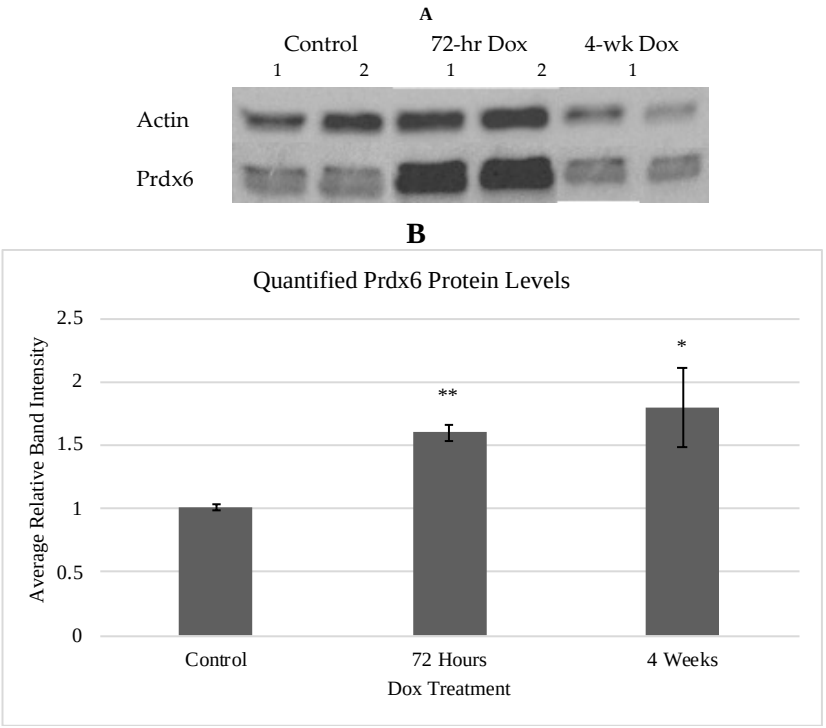

Figure 5. Western blots for expression of control actin and Prdx6. (A) Representative western blot. (B) Band quantification using Syngene GeneTools. Levels were normalized to actin. (*p < 0.05; **p < 0.01).

larger and more granular and asymmetrical in shape. By three and four weeks, vacuoles were visible in the cells, a feature in doxorubicin-resistant K562 cells [17], further supporting that cells were developing resistance to the treatment.

After 3-day and 4-week treatments with doxorubicin, protein extractions were conducted and used to perform western blots to examine peroxiredoxin expression. As shown in Figure 5A, we found that there was a significant increase in Prdx6 after 72-hour treatment as well as 4-week treatment, as compared to control cells. Quantification of Prdx6 levels from replicate samples demonstrate an approximately 50% increase in Prdx6 expression in these time periods (Figure 5B).

DISCUSSION

In this study, our goal was to examine the effect of doxorubicin on K562 cells and explore a possible effect on peroxiredoxin levels in doxorubicin resistance. We found a significant loss of cell density and viability in doxorubicin-treated cells, but viability remained around 50% throughout the duration of the experiment. In addition, there was a visible increase in vacuoles, a sign of resistance in doxorubicin-resistant K562 [17]. K562 cells surviving doxorubicin treatment after 72 hours also showed an increase in Prdx6 expression, which was retained over the longer 4-week time period.

The significant increase in Prdx6 expression after doxorubicin treatment indicates that it may play a role in resistance to doxorubicin in K562 leukemia cells. Prdx6 has been found to be upregulated in other chemotherapy-resistant cancers, such as cisplatin resistant K562 leukemia, MCF-7 breast carcinoma, and SKOV-3 ovarian carcinoma cells [18, 19]. The increase in Prdx6 in all of these chemotherapy-resistant cells lines suggest that the protein is important in protecting the cancer from the chemotherapy mechanisms. Previous studies from our laboratory have shown that inhibiting specific peroxiredoxins in MCF-7 cells increases susceptibility to doxorubicin-induced cell death, consistent with the notion that peroxiredoxins may help facilitate cancer cell survival [20].

In order to investigate the role of Prdx6 in doxorubicin-resistant K562 cells further, future

experiments should be done in which the protein is inhibited using siRNA and cells are treated with doxorubicin to observe if resistance occurs. If inhibiting Prdx6 reduces resistance, possible supplementary drugs can be used with chemotherapy to help prevent resistance. With Prdx6 being the only Prdx that is 1-Cys, compounds like mercaptosuccinate interact specifically with Prdx6 and prevent peroxidase activity [21, 22]. Further experiments can be performed to investigate the effects of using mercaptosuccinate as a supplementary drug with doxorubicin treatment. In addition, these studies should be done using patient samples, or *in vivo*, as using a single cell line may not be as representative. Overall, the results have indicated that Prdx6 may play an important role in resistance, and may be useful, after future experimenting, in decreasing clinical instances of resistance.

CONCLUSION

In conclusion, our study demonstrates that K562 cells are partially susceptible to doxorubicin-induced toxicity, but long-term exposure can select for a resistant subpopulation. Interestingly, cells surviving K562 cell exposure exhibit increased levels of Prdx1, suggesting a possible role for this antioxidant in K562 survival to doxorubicin.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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