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Effect of the antioxidants on Vinblastin and Vincristine treated Human erythrocytes in-vitro

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Abstract

Vinca alkaloids are one of the earliest microtubule-targeting plant based anticancer drugs approved clinically. The Food and Drug Administration (FDA) approved vinblastin and vincristine are used for the of different tumor types like leukemia, Hodgkin's lymphoma, lung cancer, breast cancer in 1961 and 1963, respectively. Although these drugs are commonly used as anticancer drug but still constitute a substantial drawbacks. The side-effects of vinca alkaloids consist of toxicity to blood cells, nausea, vomiting, and many more. In present the haemolytic effect of vinblastin and vincristine was investigated on normal erythrocytes and the protective effect of ascorbic acid, β Carotene, quercetin and tocopherol in presence of these drugs. To study the effect of selected antioxidants and vinca alkaloids osmotic fragility assay was performed. The results suggest that β Caroteneexhibits a protective effect against vinblastin-induced haematological toxicity.

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Introduction

More than 50% of all cancer patients encounter anemia, regardless of the treatment received, and approximately 20% of all patients undergoing chemotherapy will require red blood cell transfusion when hemoglobin concentrations declined below 10 g/dL (Jiahui Liu and Zhichong Wang, 2015). Repeated cycles of chemotherapy may impair erythropoiesis cumulatively. Mechanisms of drug-induced anemia in patients with cancer include stem cell death, blockage or delay of haematopoietic factors, oxidative damage to mature haematopoietic cells, long-term myelodysplasia, immune-mediated haematopoietic cell destruction, and microangiopathy and plasma volume expansion with dilutional anemia and osmotic imbalance of erythrocytes **(**Kwame Asare, 2008).Vinca alkaloids are a subset of drugs extracted from *Catharanthusroseus plants*. The vinca alkaloids have significant anticancer properties. Till date the major vinca alkaloids clinically used are vinblastine, vinorelbine, vincristine and vindesine (Moudi, *et al*, 2013). Among them vinblastine, vinorelbine (Budman, 1997) and vincristine have been approved by FDA for use in the United States only vinflunine is another vinca alkaloid which has been synthesized in the laboratory and has been approved by European countries to treat the second-line transitional cell carcinoma of the urothelium is being developed for other malignancies. After taxol the vinca alkaloids are the second-most-used class of cancer drugs used for the treatment of several types of cancer (Himes *et al*, 1976).

The vinca alkaloids have been generally used in the combination with the other therapies as well. These drugs do not have cross-resistance with drugs that alkylate deoxyribonucleic acid (DNA) and have a different mechanism of action. Vinblastin has been used as an integral part of medicinal treatment regimens for testicular carcinoma and both Hodgkin and non-Hodgkin lymphomas (Jordan *et al*, 1991). It is also used in breast cancer and germ cell tumors. Although the anticancer drugs including vinca alkaloids, are used to treat many types of cancer but they have many side-effects of consist of toxicity to white blood cells, nausea, vomiting, constipation, dyspnea, chest or tumor pain, wheezing and fever. It is also rarely associated with antidiuretic hormone secretion (Ruhlmann and Herrstedt, 2011). Neutropenia is the main dose-limiting toxicity of vinblastin and vincristine. Thrombocytopenia and anemia usually have been seen less. In addition, vincristine is related with hematologic toxicity rarely, severe myelosuppression has been monitored in situations resulting in profoundly increased drug exposure and hepatic deficiency and mechanical red cell trauma (Kufe *et al*., 2003) (Anitha, 2016).

Antioxidants are known to be effective in scavenging free radicals from the blood and other cells so they may reduce the side effect of chemotherapy on the erythrocytes by protecting them from oxidative damage (Birben *et al*, 2012). This oxidative damage caused by the drug may be ameliorated by antioxidants (Miki et.al., 1987). The present study was conducted to investigate the effect of widely used anticancer vinca alkaloids like vinblastine and vincristine on human blood cells and the protective role of antioxidants (ascorbic acid, β Carotene, quercetin and tocopherol) on Vinblastine/Vincristine treated red blood cells *in vitro*.

Materials and methods

Pure vinblastin, vincristine, ascorbic acid, β Carotene, quercetin and tocopherol were purchased from Sigma Aldrich, USA. Dimethyl sulphoxide (DMSO) ordered from Sisco Research Laboratories Pvt Ltd. NaCl, Na_2PO4, and NaH_2PO4 were brought from Panreac Quimica. The dilution of different vinblastin, vincristine, ascorbic acid, β Carotene, quercetin and tocopherol was prepared by dissolving them in required amount of agents in DMSO. The phosphate-buffer saline was prepared by using NaCl, Na_2HPO_4 and NaH_2PO_4 in autoclaved double distilled water.

Blood sampling

5ml of venous blood were collected from healthy volunteers with informed consent. All the experiments were run in duplicate, and the mean value of them was used.

Treatment conditions

To study the osmotic fragility of erythrocytes in the presence of anticancer drugs vinblastin and vincristine antioxidants (ascorbic acid, β Carotene, quercetin and tocopherol). Three different conditions i.e. pre-treatment condition (blood was treated with antioxidants for 30 min prior to the treatment of anticancer drugs). Co-treatment (Antioxidants and anticancer drugs were added together) and post-treatment (anticancer drugs were added before antioxidants) were used in present study.

Osmotic fragility assay

The osmotic fragility assay was conducted to study the vinblastine and vincristine related osmotic hemolysis of erythrocytes. The protocol used is described briefly here, the heparinized blood was incubated with 1 mg/ml concentration of both the drugs separately at 37°C for 30 min. Aliquots of saline solutions, with concentrations ranging from 10 to 1 g/L were prepared. The treated erythrocytes were then transferred to the tubes containing decreasing concentrations of saline solutions. After mixing carefully, the cell suspensions were left to equilibrate for 30 min and then centrifuged at 3000 r/min for 5 min. The absorbance of supernatants was recorded at 540 nm, standardized against a blank (10 g/L saline supernatant corresponds to 0% haemolysis). The recorded optical density (OD) of the supernatant indicates the degree of haemolysis of the erythrocytes. The lysis percentage was calculated by dividing the OD of the supernatant obtained from a particular saline concentration by the OD of the standard (1 g/L) representing 100% haemolysis. Osmotic fragility curves were constructed by plotting the lysis percentage against the **c**oncentration of saline solutions The MEF25, MEF50 and MEF75 (mean erythrocyte fragility) values, which are the saline concentrations at which 25%, 50% and 75%, respectively, red blood cells hemolysis (at standard pH and temperature) were obtained from the curve.

Statistical analysis

The results were reported in mean values plus/minus standard error of the mean. Statistical analysis was performed with Student's t-test and multiple regression analysis $p < 005$ was considered statistically significant.

Results and discussion

The osmotic fragility of the red blood cells in the presence of vinblastine/ vincristine alone or in combination with ascorbic acid, β Carotene, quercetin and tocopherol was measured by scoring hemolysis in saline solution.

Table 1. Pre-treatment of Ascorbic acid, β Carotene, Quercetin, and tocopherol (1 ml/ml) with vinblastin and vincristine (1 ml/ml), resulting in mean erythrocyte fragility (MEF).

SNo	Pre-treatment condition	Mean erythrocyte fragility (MEF)		
		MEF25	MEF50	MEF75
1	Control	0.5825±0.002395	0.4425±0.00125	0.2975±0.002394
2	Vinblastin	0.735±0.002041	0.565±0.003536	0.33±0.002394
3	Vinblastin+Ascorbic acid	0.66±0.01405	0.5675±0.024537	0.4625±0.010873
4	Vinblastin+β Carotene	0.6225±0.001443	0.535±0.0025	0.455±0.002887
5	Vinblastin+Quercitin	0.685±0	0.585±0	0.5025±0.004787
6	Vinblastin + Tocopherol	0.64±0.01375	0.555±0.015326	0.4825±0.020954
7	Vincristine	0.6625±0.01375	0.5475±0.015326	0.4425±0.020954
8	Vincristine+ Ascorbic acid	0.7375±0.00125	0.6225±0.002394	0.435±0.005951
9	Vincristine+β Carotene	0.705±0.01405	0.6±0.024537	0.505±0.010873
10	Vincristine+ Quercitin	0.78±0.001443	0.7125±0	0.62±0.002041
11	Vincristine+ Tocopherol	0.74±0.002041	0.63±0.004732	0.5±0.012479

The concentrations of saline solution causing 25%, 50% and 75% lysis of red blood cells have been evaluated and designated as MEF25, MEF50 and MEF75, respectively. The control values were 0.5825±0.002395, 0.4425±0.00125 and 0.2975±0.002394 respectively.

MEF25, MEF50 and MEF75 for the pre-treatment condition and co-treatment conditions are mentioned in Table 1 and 2 respectively. The results indicating that the pre-treatment of β-carotene shows protective effect but the other antioxidants have only moderate protective effect on erythrocytes, but there was no significant effect of on vincristine (Table 1).In case of co-treatment the treatment of β-carotene along with vinblastin have some protective effect and vincristine doesn't show any positive response(Table 2).

In post-treatment (antioxidants were added to the erythrocytes after exposure of anticancer drugs for 30 min), there was no change on the damage caused by anticancer drugs (Data not shown here).

Table 2. Co- treatment of Ascorbic acid, β Carotene, Quercetin, and tocopherol (1 ml/ml) with vinblastin and vincristine (1 ml/ml), resulting in mean erythrocyte fragility (MEF).

SNo	Co-treatment condition	Mean erythrocyte fragility (MEF)		
		MEF25	MEF50	MEF75
1	Control	0.5825±0.002395	0.4425±0.00125	0.2975±0.002394
2	Vinblastin	0.735±0.002041	0.565±0.003536	0.33±0.002394
3	Vinblastin+Ascorbic acid	0.79±0.002041	0.7±0.003536	0.54±0.002394
4	Vinblastin+β Carotene	0.6175±0.01405	0.485±0.024537	0.2425±0.010873
5	Vinblastin+Quercitin	0.685±0.001443	0.565±0.0025	0.44±0.002887
6	Vinblastin + Tocopherol	0.75±0	0.65±0	0.395±0.004787
7	Vincristine	0.6625±0.01375	0.5475±0.015326	0.4425±0.020954
8	Vincristine+ Ascorbic acid	0.63±0	0.56±0	0.5±0
9	Vincristine+β Carotene	0.73±0.003536	0.4375±0.00375	0.3075±0.002393568
10	Vincristine+ Quercitin	0.645±0.001443	0.56±0	0.48±0.002041241
11	Vincristine+ Tocopherol	0.78±0.002041	0.7125±0.004732	0.5725±0.012479149

Cancer is one of the leading causes of death worldwide, more than 6 million new cancer cases are reported every year all over the world. Cancer incidence and mortality are rapidly growing worldwide In 2018, 1,735,350 new cancer cases and 609,640 cancer deaths are projected to occur in the United States (Bray *et al*., 2018). In Kingdom of Saudi Arabia cancer causes death of many people in Saudi Arabia every year Between January 01 and December 31 2014, the total number of newly diagnosed cancer cases reported in Saudi Arabia was 15,807(Saudi Cancer Registry 2014). Overall cancer was more among women than men; it affected 7,462 (472%) males and 8,345 (528%) females. Total of 12,007 cases were reported among Saudi nationals, 3,640 among non-Saudi, and 160 of unknown nationality (Saudi Cancer Registry, 2018). Chemotherapy is the major solution for the treatment of cancer. There are many WHO approved anticancer used to treat different kind of cancer disease or cancer chemotherapy is the very common cause of anemia, which has an adverse impact on quality of life (QOL) of the patient. Drug-induced haemolysis is regarded as an acquired form of this red cell pathology and results as a consequence of clinical treatment. The causes of drug-induced haemolysis have been categorized as either immune or non-immune in origin (Salama, 2009). General assumption in non-immune related haemolysis is that the drug of concern causes lysis by damaging membrane integrity; which may involve direct effects on specific ion transport pathways by particular drugs, drug-

induced oxidative damage of the cell membrane, and/or unpredicted toxic effects of the drug on cell volume control processes, leading to cell swelling (Petz and Garratty, 1975) (Lubran, 1989).

Drug-induced hematological disorders are very common side-effect of many drugs especially anticancer drugs. The most common of them is anaemia. The hemolytic anaemia can be because of many reasons like oxidative stress which causes the membrane damage of the blood cells, megaloblastic change/ dyshaemopoiesis, sideroblastosis or immune related damage of blood cells (Barr*et al.*, 1982). Anticancer drugs have been suggested to cause an oxidative stress to human antioxidative systems and can be the reason for the acute haemolytic anaemia in patients undergoing chemotherapy.

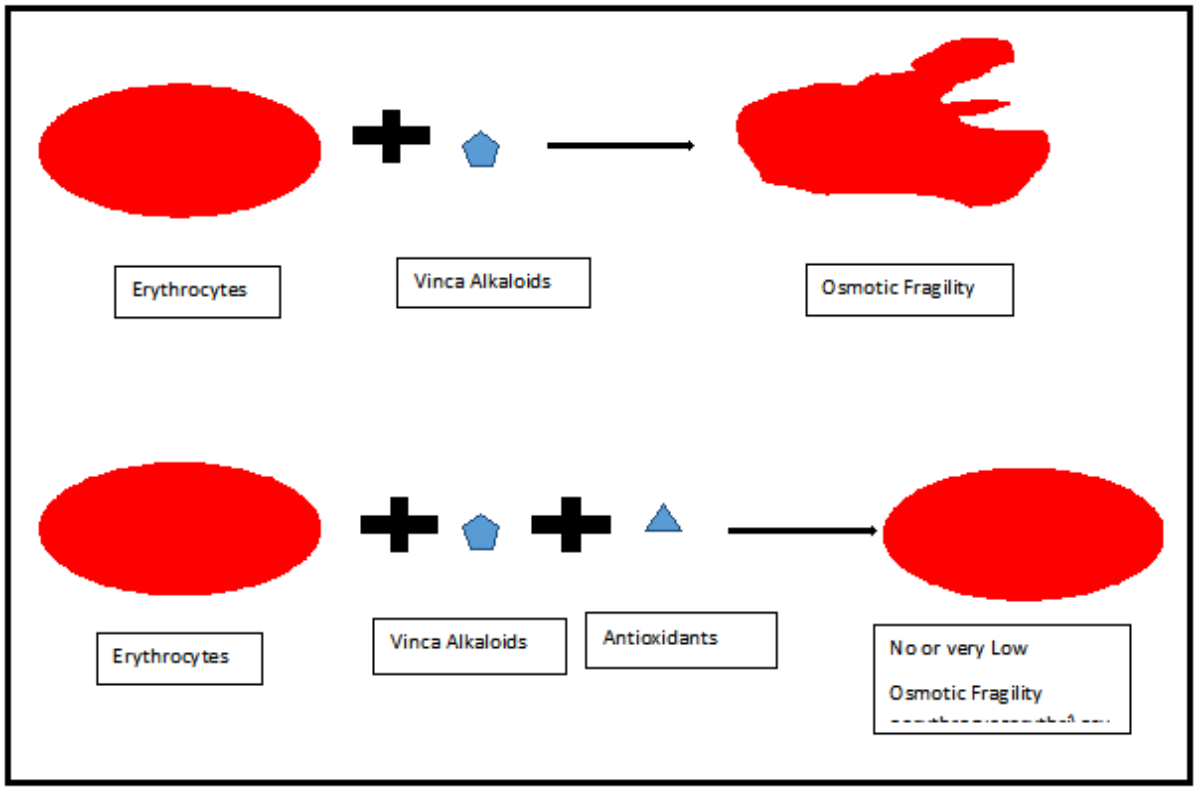

Fig. 1.

The acute haemolytic anaemia causes the damage of erythrocytes because of that the hemoglobin of the patients drops sharply below 10 g/L, leading to blood transfusion in the cancer patients (Quirt *etal.*, 2001).

In several reports, it is shown that many chemotherapeutic agents induce haemolysis in human as well as in primates especially the alkaloids (Blohmer *etal.*, 2005). Vinblastin and vincristine are the example of alkaloid chemotherapeutic drugs.

The antioxidants play key role to protect the cells from the oxidative damage. β-carotene is a class of biological antioxidants and shows radical-trapping antioxidant behaviour.

The data obtained from the present studies indicated that β-carotene reduces the oxidative damage of erythrocytes caused by vinblastin but doesn't play any protective role in vincristine treated erythrocytes in Pre and Co-treatment conditions Other antioxidant had very low or no effect on the vinca alkaloids treated erythrocytes.

Conclusion

The results of the present study significantly indicating that β-carotene reduced the harmful effect

of vinblastin on human red blood cells. Although this is a preliminary study and should be validated by other studies as well but results seem to be promising and can be play crucial role to reduce the chemotherapy related side-effects.

Declaration of conflicting interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

Anitha Sri S. 2016. Pharmacological activity of Vinca alkaloids. Journal of pharmacognosy and phytochemistry **4,** 27-34.

Barr RD, Davidson AR, Jung LK, Pai KM. 1981. Erythrocytotoxicity Induced by Cancer Chemotherapeutic Agents: In Vitro Studies of Osmotic Fragility and Methaemoglobin Generation. Scandinavian journal of haematology **25(4),** 363-368.

Birben E, Sahiner UM, Sackesen C. 2012. Oxidative stress and antioxidant defense. World Allergy Organ Journal **5(1),** 1. http://dx.doi.org/10.1097/WOX.0b013e3182439613

Blohmer JU, Dunst J, Harrison L, Johnston P, Khayat D, Ludwig H, O'Brien M, Van Belle S, Vaupel P. 2005. Cancer-related anemia: biological findings, clinical implications and impact on quality of life. Oncology **68(1),** 12-21. http://dx.doi.org/10.1159/000083129

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre, LA, Jemal A. 2018. Global cancer statistics 2018. GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA: a cancer journal for clinicians **68(6),** 394-424. http://dx.doi.org/10.3322/caac.21492

Budman DR. 1997. Vinorelbine (Navelbiner): A Third-Generation Vinca Alkaloid Cancer investigation **15(5),** 475-490. http://dx.doi.org/10.3109/07357909709047587

De Conti RC, Muggia F, Cummings FJ. 1975. Clinical and pharmacological studies with D-tetrandrine In: Proceedings of the American Association for Cancer Research and ASCO, San Diego, California **16**, 96.

Doucet Jay Zu-hua Gao, Leslie Mac Laren A, Vivian McAlister C. 2004. Modification of xenoantigens on porcine erythrocytes for xenotransfusion. Surgery **135(2).** http://dx.doi.org/10.1016/j.surg.2003.08.013

Erhola M, Toyokuni S, Okada K. 1997. Biomarker evidence of DNA oxidation in lung cancer patients: association of urinary 8-hydroxy-20-deoxyguanosine excretion with radiotherapy, chemotherapy, and response to treatment. FEBS Letter **409(2),** 287–291.

Herman EH, Chadwick DP, Mhatre RM. 1974. Comparison of the acute hemolytic and cardiovascular actions of Ellipticine and some Ellipticineanalogs. Cancer Chemotherapy Reports. **58,** 637–643.

Himes RH, Kersey RN, Heller-Bettinger I, Samson FE. 1976. Action of the vinca alkaloids vincristine, vinblastine, and desacetyl vinblastine amide on microtubules.In-vitro Cancer Research **36(10),** 3798-3802.

Hristozov D, Gadjeva V, Vlaykova T. 2001. Evaluation of oxidative stress in patients with cancer. Archives of Physiology *and* Biochemistry **109(4),** 331–336. http://dx.doi.org/10.1076/apab.109.4.331.4248

Jacobs MH. 1932. Osmotic properties of the erythrocyte III The applicability of osmotic laws to the rate of hemolysis in hypotonic solutions of non-electrolytes. Biological Bulletin **62(2),** 178–194.

Jiahui Liu, Zhichong Wang. 2015. Increased Oxidative Stress as a Selective Anticancer Therapy. Oxidative Medicine and Cellular Longevity. http://dx.doi.org/10.1155/2015/294303

Jordan MA, Thrower D, Wilson L. 1991. Mechanism of inhibition of cell proliferation by Vinca alkaloids. Cancer research **51(8),** 2212-2222. http://dx.doi.org/10.1016/j.bmcl.2018.06.044

Kwame Asare. 2008. Anemia of Critical Illness. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy **28(10),** 1267–1282. http://dx.doi.org/10.1592/phco.28.10.1267

Kufe DW, Pollock RE, Weichselbaum RR, Bast RC, Gansler TS, Holland JF. 2003.6th ed. Hamilton (ON): BC Decker Incorporation;. Holland-Freide cancer medicine.

Lee IP, Dixon RL. 1972. A possible mechanism for Ellipticine induced hemolysis of human red blood cells. Federation proceedings **31,** 554.

Li, YQ, Hu R, Zhong LH. 2016. Synergistic effect of trehalose and saccharosepretreatment on maintenance of lyophilized human red blood cell quality. Tropical Journal of Pharmaceutical Research. **15(3),** 527–533.

Lubran MM. 1982. Hematologic side effects of drugs. The Annals of Clinical & Laboratory Science. **19(2),** 114–121.

Miki M, Tamai H, Mino M. 1987. Free-radical chain oxidation of rat red blood cells by molecular oxygen its inhibition by -tocopherol. Archivesof Biochemistry and Biophysics **258(2),** 373–380. http://dx.doi.org/10.1016/0003-9861(87)90358-4

Moudi M, Go R, Yien CYS, Nazre M. 2013. Vinca alkaloids. International journal of preventive medicine **4(11),** 1231.

Matés JM, Pérez-Góme C, De Castro IN. 1999. Antioxidant enzymes and human diseases. Clinical biochemistry **32(8),** p595-603. http://dx.doi.org/10.1016/s0009-9120(99)00075-2

Niki E, Komuro E, Takahashi M, Urano S, Ito E, Terao K. 1988. Oxidative hemolysis of erythrocytes and its inhibition by free radical scavengers. Journal of Biological Chemistry **263(36),** 19809-19814.

Petz LD, Garratty G. 1975. Drug-induced haemolytic anemia. Clinics in haematology **4(1),** 181-197.

Quirt I, Robeson C, Lau CY. 2001. Canadian Eprex Oncology Study Group Epoetinalfa therapy increases hemoglobin levels and improves quality of life in patients with cancer-related anemia who are not receiving chemotherapy and patients with anemia who are receiving chemotherapy. Journal of Clinical Oncology **19(21),** 4126–4134.

Ruhlmann CH, Herrstedt J. 201. Safety evaluation of aprepitant for the prevention of chemotherapy-induced nausea and vomiting. Expert opinion on drug safety **10(3),** 449-462. http://dx.doi.org/10.1517/14740338.2011.563235

Salama A. 2009. Drug-induced immune hemolytic anemia. Expert opinion on drug safety **8(1),** 73-79. http://dx.doi.org/10.1517/14740330802577351

Vincent PC. 1986. Drug-induced aplastic anaemia and agranulocytosis. Drugs **1,** 52–63, http://dx.doi.org/10.2165/00003495198631010000004