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Research Article

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Evaluation of Anti-Cancer Activity of Brinzolamide with Special Emphasis on Blood Cancer (*Polycethemia Rubra Vera*) *in vitro*

Keval R Rathod[1]* and Tejas H Ganatra[2]

M Pharmacy (Pharmacology), School of Pharmacy, R K University, Kasturbadham, Rajkot, Gujarat, India
Associate Proffesor, Department of Pharmacology, School of Pharmacy, R K University, Kasturbadham, Rajkot, Gujarat, India

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ABSTRACT

Introduction: Polycythemia is an uncommon type of cancer in which bone marrow produces too many red blood cells as well as due to overproduction of white blood cells and platelets. It is caused by neoplastic proliferation and maturation of erythroid, Megakaryocytic and Granulocytic elements to produce Panmyelosis. Looking at the dire to find better & safer alternate, we decided to work on Brinzolamide.
Methodology: *In vitro* cytotoxicity of Brinzolamide was evaluated using Brine shrimp model as well as using healthy human RBC – hemolytic study. In both these models, Methotrexate was taken as standard because of structural & mechanism similarity with brinzolamide.
Result: Brinzolamide significantly reduced number of nauplii count i.e. increased % mortality as well as shown remarkable hemolytic activity in *in vitro* cytotoxicity studies.
Conclusion: From the study conducted, we can conclude that Brinzolamide can serve as one of the alternate for blood cancer treatment specifically in *Polycythemia rubra vera*.

Keywords: Brinzolamide; Brine-shrimp model; Cytotoxicity; Hemolysis

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INTRODUCTION

Blood cancer effects the production and function of Human blood cells. Most of these cancers start in bone marrow where blood is produced. Stem cells presence in bone marrow mature and develop into three types of blood cells: Red blood cells, White blood cells and Platelets. In most blood cancers, the normal blood cell development process is interrupted by uncontrolled growth of an abnormal type of blood cell. These abnormal blood cells prevent blood from performing many of its functions, like fighting of infections or preventing serious bleeding. There are three main types of blood cancers:
Leukemia, a type of cancer found in blood and bone marrow is caused by the rapid production of abnormal White blood cells and it is a cancer that originates in blood forming tissue [1].
Lymphoma, is a type of blood cancer that affects the lymphatic system, which removes excess fluids from body and produces immune cells.
Myeloma, is a cancer of the plasma cells. Plasma cells are white blood cells are white blood cells that disease and infection, fighting antibodies in human body. Myeloma cells prevents the normal production of antibodies, leaving our body's immune system weekend and susceptible to infection [2]. Polycythemia Vera (PV), essential thrombocythemia (ET) and myeloid metaplasia with myelofibrosis (MMM) are clonal disorders arising from hematopoietic progenitors. An internet based protocol for clinical information and biological specimens suggest that a recurrent somatic missense mutation JAK2V617F in granulocyte DNA. Molecular and cytogenetic analysis demonstrated that this mutation were due to duplication of the mutant allele. Treatment for blood cancer depends on the type of cancer, your age, how fast the cancer is progressing, where the cancer has spread and other factors, some common blood cancer treatment include: Stem cell Transplantation, Chemotherapy, Radiation Therapy [3-13]. Because of the need of this treatment we found some alternative approach to satisfy this need and it is Brinzolamide. Brinzolamide is a sulfa drug and its structure contain sulfonamide moiety. It is an inhibitor of carbonic anhydrase – II which are present in red blood cells. Generally it is used in open angle glaucoma and ocular hypertension.

Rationale

Carbonic Anhydrase II is an important enzyme for the human body playing key role in regulation of apoptosis [14]. Literatures revels that Carbonic Anhydrase Inhibitors is induce the apoptosis of the cell and also reduce the cancer cell proliferation [15]. Apart from this, it is well reported that CA II level is raised in myeloid tissues and CA II inhibitors can play significant role in inhibition of progression of myeloid tissue [16]. Sulfonamide is having anti-cancer potential and can inhibit progression of cancer [17]. As Brinzolamide is CA II inhibitor, belonging to Sulfonamide category [17], based on above mentioned rationale we planned to evaluate anti - cancer potential of the same with different experimental models with special emphasis on Polycythemia rubra vera.

MATERIAL AND METHODS

Procurement of Brinzolamide

We procured a brinzolamide as drug of ophthalmic suspension from the market of Rajkot as the brand name of Brinolar.

Study of Effects of Brinzolamide in Brine Shrimp Model [15]

The Brine shrimp egg containing capsules were procured from Amazon (E-Market). First of all store the brine shrimp capsule in tightly closed container it must be free from moisture and stored under cool temperature below 50°F. This capsule containing the small egg of brine shrimp. It's required "V" or coned shaped bottle, continuous fighting source like tungsten filament bulb, etc. Continuous aeration, sea water (water containing 2/3 tablespoon Nacl and pinch of sodium bicarbonate). First fill the coned shaped bottle with sea water with continuous aeration and lighting and then add one capsule of brine shrimp. Continue this method for 24 hr. After 24 hr. close the lighting and aeration and give time to nauplii for settle down in bottom part of bottle. Than use the egg for practical purpose. With help of pippete collect the 10 or 20 alive nauplii into the petri plate. After that it is divided into 5 groups. In this five group, each and individual group give the different dose as Normal, Standard, Control, and test. Normal group having only water, After that in control group add DMSO, in standard group add Methotrexate upto 1 mg/ml, and in test group add three different concentration containing Brinzolamide as a test drug upto 300 µg/ml, 500 µg/ml and 1 mg/ml. During the process of dosing each and individual plate having continuous aeration and lighting is compulsory. After 30 min. of time interval count the each and individual petri plate's nauplii dead or alive. After the all group having all nauplii are dead than finally prepare the observation table regarding the nauplii dead or alive in particular normal, control, standard, and test group (Figure 1). As per some literature review Methotrexate is a general drug for cancer and give effective result on Polycythaemia Vera [16].

Figure 1: Hatching process of brine shrimp egg

Study of Effects of Brinzolamide in Effect on Healthy Human RBC [17]

The hemolytic assay was performed according to Daniel B. Alancer et al. Human blood sample were brought from the Laboratory. Human blood cells prepared by washing them six times with 50 Mm TrisHCL, pH7.4, containing Nacl 0.15 M(TBS). Following the last wash, red blood cells (RBC) were diluted to 1/10 of their volume with TBS. The assay was performed by mixing 0.3 mL of the RBC solution with 1 mg/ml of brinzolamide. According to our chemical requirement we used 1.2 mL of distilled water was set as a negative control 1.2mL of DMSO as a positive control. Methotrexate 1mg/ml we used as a standard drug. The mixture were vortexed, left for 2 hr. at room temperature, and then centrifuged at 5,000 RPM for 10min at 0°C. At last serum was separated then collect this serum and take an absorbance in UV-Visible spectrophotometer. We obtaining the %Absorbance as per following equation:

$$\text{Hemolytic activity} = \frac{Abs_{sample} - Abs_{negative\ control}}{Abs_{positive\ control} - Abs_{negative\ control}} \times 100\%$$

As per some literature review Methotrexate is a general drug for cancer and give effective result on Polycythaemia Vera.

Statistical Analysis

Statistical analysis of results was done by ANOVA test followed by turkey's test for determination of variance. Data were considered significantly different from each other if $p \leq 0.05$ and if $p \leq 0.001$ then the difference between data were consider highly significant.

RESULTS

Brine Shrimp Model:

Brine shrimp model was performed as per mentioned protocol in Material and Methodology and shown following result (Figure 2):

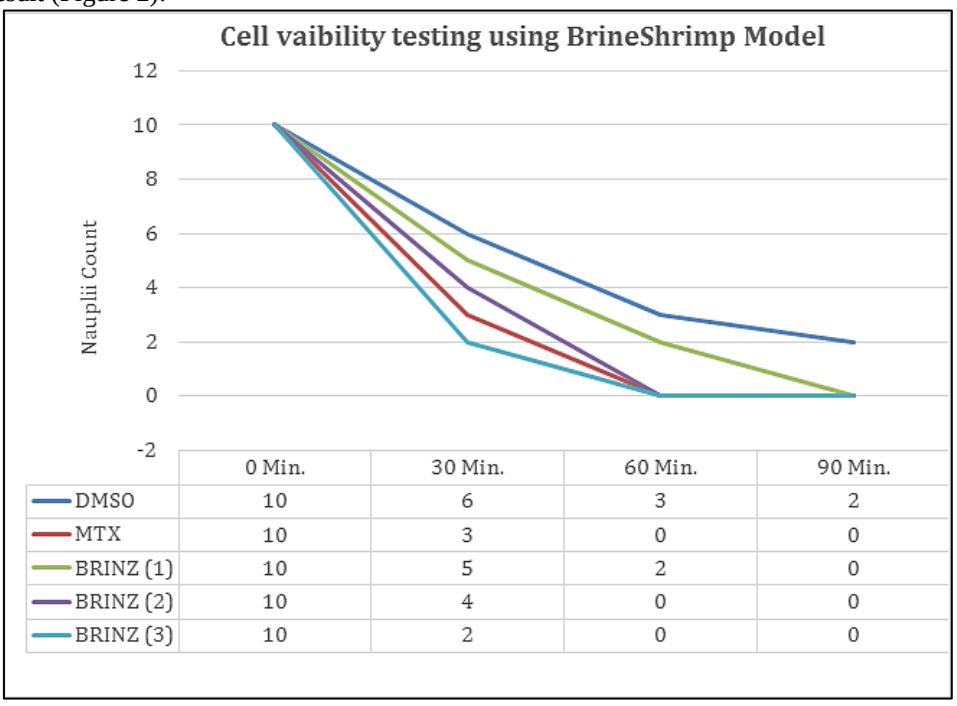

Figure 2: Cytotoxicity model using brine shrimp model

In this model, we used the Methotrexate as a Standard drug and Brinzolamide three different dose as a Test 1, Test 2 and Test 3 drug and DMSO as a normal control. Also the Test 1, Test 2 and Test 3 was according 300µg/ml, 500 µg/ml and 1 mg/ml. The chart above shown that there was significant reduction/increase in nauplii count in standard (3) and Test 1 (5), Test 2 (4), Test 3 (2) at 30 minute compared to that of normal control. Another significant reduction/increase in nauplii count in standard (0) and Test 1 (2), Test 2 (0), Test 3 (0) at 60 minute compared to that of normal control. Another significant reduction/increase in nauplii count in standard (0) and Test 1 (0), Test 2 (0), Test 3 (0) at 90 minute compared to that of normal control.

Effect on Healthy Human RBC:

Hemolytic activity was performed as per mentioned protocol in chapter no.4 and shown following result (Table 1):

Table 1: Absorbance of test sample

Group	Absorbance	%Hemolysis
Negative control (DW)	0.420	---
Positive control (DMSO)	0.522	00%
Standard	0.536	12.66 ± 0.33 %
Test	0.532	10.16 ± 0.477 %

Here, Negative control was Distilled water, Positive control was DMSO, Standard was Methotrexate 1 mg/ml and Test was Brinzolamide 1 mg/ml.

Here, we clearly shown that the %Hemolysis of Standard (12.66 ± 0.33%) and Test (10.16 ± 0.477%) was significantly increase as compared to that of the Positive Control (00%).

DISCUSSION

Blood cancer affect the production and function of Human blood cells. Most of these cancer start in bone marrow where blood is produced. Stem cells presence in bone marrow mature and develop into three types of blood cells: Red blood cells, White blood cells and Platelets. In most blood cancers, the normal blood cell development process is interrupted by uncontrolled growth of an abnormal type of blood cell. These abnormal blood cells, prevent blood from performing many of its functions, like fighting of infections or preventing serious bleeding. Polycythemia Vera (PV), essential thrombocythemia (ET) and myeloid metaplasia with myelofibrosis (MMM) are clonal disorders arising from hematopoietic progenitors. It's also says that it is an abnormal cell growth of Red Blood Cells. An internet based protocol for clinical information and biological specimens suggest that a recurrent somatic missense mutation JAK2V617F in granulocyte DNA. Molecular and cytogenetic analysis demonstrated that this mutation were due to duplication of the mutant allele. Polycythemia Vera is caused by acquired somatic mutation in hematopoietic progenitors, the genetic basis for these disease has not been known, this mutations that result in constitutive activation of a protein tyrosine kinase. These mutant kinases have proven to be good candidates for molecularly targeted therapy [4].

Phlebotomy is one form of treatment. In this removal of blood from the body induce iron deficiency, thereby decreasing the hemoglobin and hematocrit level and reducing the risk of blood clots. This process is typically performed to bring their hematocrit down below 45 for men or 42 for women [11]. Low dose of aspirin (75-81mg daily). It reduce the risk of various thrombotic pain. Chemotherapy with cytoprotective agent (Hydroxyurea) also performed. Injection of radioactive isotopes (Phosphorus -32) is given to suppress the bone marrow. Other therapies include interferon injection and anagrelide may be prescribed. Bone marrow transplantation are rarely undertaken. Erlotinib may be an additional treatment option for this condition.[12] Selective JAK – II inhibitors are being investigated in vitro and in clinical trial [13]. Other treatments include Chemotherapy, Stem cell transplantation and Radiation Therapy. Considering the loopholes associated with current therapy we decided to find one better, safer and cheaper, if possible, alternate of currently existing therapy of cancer and for that we focused on evaluation of anti-cancer potential of Brinzolamide with special emphasize on *polycythemia rubra vera*.

As a part of study, we evaluated cytotoxic potential of brinzolamide using Brine shrimp model. In that model, it is believed that nauplii is rapidly dividing structure which is identical to that of cancerous cell. That's why if a drug can inhibit growth or decrease the count of nauplii than it may be considered as its cytotoxic potential. In this model, standard and test shown significant reduction in nauplii count in respective timeline compared to that of normal control. The difference in nauplii count in test & standard was found statistically significant compared to that of normal control. This finding revealed that Brinzolamide possesses strong cytotoxic potential which can be used to prevent progress of cancer. In Hemolytic study, performed on human blood, was to confirm cytotoxicity study of brinzolamide and study clearly revealed that standard drug – methotrexate and test drug – brinzolamide both shown potent cytotoxic potential and it was clearly suggestible from data i.e. standard and test group shown significantly higher hemolysis compared to that of positive and negative control group.

CONCLUSION

The study carried out clearly revealed that brinzolamide has strong anti-cancer activity. This finding was supported by

In Brine Shrimp model, brinzolamide clearly shown cytotoxic potential;

In hemolytic study, brinzolamide also supported above finding;

From the above findings we can conclude that Brinzolamide has strong anti-cancer potential specifically in *Polycythemia rubra vera* and can subjected for further study to know molecular mechanism of action of brinzolamide as an anti-cancer agent.

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