Protective Effect of Apple Cider Vinegar in Hydroxyurea Treated Mice

R.S. Ayoub T.A. Al-Sandok S.W. Yousif
Department of Dental Basic Science
College of Dentistry - Mosul University

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الخلاصة

يعد انخفاض فعالية نخاع العظم من الامور الهامة في تحديد الجرعة السمية لعقار الهايدروكسي يوريا ، وقد افترض لخل التفاح الطبيعي تاثير كيمياوي حافظ وتاثير منشط نقوي لنخاع العظم . وقد لوحظ ان اعطاء الهايدوركسي يوريا في هذه الدراسة بجرعة 80/ملغم/كغم/يوم عن طريق الفم ادى الى انخفاض معنوي في عدد خلايا الدم الحمر وانخفاض في عدد خلايا الدم البيض والصفيحات الدموية ، وان اعطاء 20مل/كغم /يوم من خل التفاح الطبيعي لمدة (7) ايام بعد العلاج بالهايدروكسي يوريا لم يؤد الى تحسن في عدد خلايا الدم الحمر والبيض والصفيحات الدموية ، وعند اعطاء خل التفاح الطبيعي مع الهايدروكسي يوريا حدثت زيادة معنوية في عدد خلايا الدم الحمر والبيض بينما لم يرجع عدد الصفيحات الدموية الى الحد الطبيعي وان للهايدروكسي يوريا تاثير على مستوى البروتينات في الفئران اذ ادى الى انخفاض معنوي في مستوى البروتين الكلي وانخفاض في مستوى الالبومين والكلوبيولين . وان اعطاء خل التفاح الطبيعي لمدة سبعة ايام بعد الهايدروكسي يوريا لم يؤثر معنويا على مستوى الكلوبيولين والالبومين بينما ازداد البروتين الكلي لكن بشكل غير معنوي . وان اعطاء خل التفاح الطبيعي في الوقت نفسه مع الهايدروكسي يوريا ادى الى وبادة معنوية في مستوى البروتين الكلي والكلوبيولين بينما لم يغير من مستوى الالبومين . وتشير هذه النتائج الى ان لخل التفاح الطبيعي تاثير حافظ لنخاع العظم من التاثير المخفض للادوية المضادة للسرطان .

ABSTRACT

Bone marrow depression is the most serious dose limiting toxicity of hydroxy urea . Natural apple cider vinegar in supposed to have both chemo-protective and myelo -stimulatory effects .

In this study , hydroxy urea at a dose of 80mg/kg/day orally produced significant depression of red blood cell, adecrease in white

blood cells and platelets in mice . 20mg/kg/day of apple cider vinegar seven days after hydroxy urea was administered produced no improvement in red blood cells and white blood cells and platelets counts.

The administration of apple cider vinegar with hydroxy urea produced significant ($P<0.05$) increase in red blood cells and white blood cells while platelets not recovered to normal .

The effect of hydroxy urea in mice on protein was observed as depressent effect significantly ($P<0.05$) on total protein adecrease in albumin and globulin .

The administration of apple cider vinegar seven days after hydroxy urea produced no significant effect on globulin and albumin , yet the total protein increased but not significantly .

The administration of apple cider vinegar at the same time with hydroxy urea significantly increase total protein and globulin , while no change on albumin level .

The results in this study indicate that apple cider vinegar has a protective effect on bone marrow from depressive effect of cyto toxic drug hydroxy urea .

INTRODUCTION

The mechanism of action of hydroxy urea (Hu), an analogue of urea, involve the inhibition of deoxyribonucleic acid (DNA) synthesis . It exerts its lethal effect on cells in S phase by inhibiting the enzyme ribonucleotide reductase , resulting in the depletion of deoxynucleoside triphosphate pools. The major uses are in melanoma , chronic myelogenous leukemia and primary squamous cell cancer of head and neck . The adverse effect of Hu is mainly bone marrow depression (1) . Patients with cancer are usually treated with cytotoxic drugs , which produce variable degrees of myelo suppression (Leuco penia , thrombo cytopenia and anemia) (2) . Natural apple cider vinegar (NACV)contains high concentration of acetic acid and pectin and low concentration of propionic , Lactic and malic acids . In addition , it contains vitamins C, A, B_1, B_2 & B_6 , B_{12} and minerals (Sodium, Potassium, Calcium, Magnesium, Phosphorus, Chlorine , Aluminum, Silicon and Cupper) .
NACV has hypolipidemic and antioxidative effects (3) . Abnormal production of free radical lead to damage of some macromolecules including protein, lipid and nucleic acid (4) , and this is believed to be involved in the etiology of many disease (5,6) cellular defense system against excessive free radicals formation can be accomplished by enzymatic or non-enzymatic mechanism including vit C and E , glutathione (7,8) and NACV (3).

The aim of this study was to expose mice to Hu to induce bone marrow suppression and to evaluate red blood cells (RBCs) , white blood cells (WBCs)& platelets count , total protein, albumin & globulin levels ; and to estimate the stimulatory effect of NACV of these factors .

MATERIALS AND METHODS

Male Swiss mice weighting 25-30gm were housed under controlled conditions of light (14 hrs light and 10 hrs dark)at 25 ± 2 ^{0}C. Commercial pelleted food and water were given and libitum .

EXPERIMENTAL DESIGN

Animals were randomly divided into (4) groups (6 mice each) .

Group I: control receiving no medication 0.2 ml of distilled water was administrated to mice by oral intubation .

Group II: 80 mg/kg of Hu was administrated to mice by oral intubation for 7 days.

Groups III: 20mg/kg NACV (Seafood, New York, 11783)was administrated by oral intubation for 7 days to mice pretreated for 7 day with hydroxy urea.

Groups IV: 80mg/kg Hu and 20mg/kg of NACV were administrated to mice at the same time by oral intubation for 7 days.

At the end of the experimental period blood sample were connected from each mouse in clean centrifuge tube , by puncture of the retro-orbital sinus for determination of total evythrocytic count , total leucocytic count, platelets count using hemocytometer (9) serum was separated for determination of total protein using total protein kit (Randox Co, UK) (Biuret method). Albumin level was measured by using albumin kit (syr Bio- France) .(Bromocresol Green Method). Globulin estimated by subtraction Albumin from total protein (9).

Data were analyzed statistically using one way analysis of variance (ANOVA)and groups differences were determined using Duncan multiple rang test . The results were expressed as mean ± S.E... $P<0.05$ was considered as statistically significant (10) .

RESULTS

Table (1) represents RBC_s , WBC_s and platelets count in different animal groups .

Hydroxy urea produced a significant ($P<0.05$)decrease in RBC_s count as compared to control , while WBC_s and platelets count did not show significant differences as compared to control group, yet they tend to be declined .

Apple cider vinegar administered for (7) days to mice pretreated for (7) days with hydroxy urea . Caused significant ($P<0.05$) decrease in RBC_s count compared to control as well as platelets count with hydroxy urea administered group. While there is no effect on WBC_s count , hydroxy urea and NACV administered to mice at the same time for (7) days produced significant ($P<0.05$) increase in RBC_s and WBC_s count compared with hydroxy urea administered group while a significant ($P<0.05$) decrease in platelets count was observed compared to hydroxy urea and control groups. Table (2) shows total protein , albumin and globulin levels in experimental groups exposed to different medications . Total protein decrease significantly ($P<0.05$) in hydroxy urea treated group compared to control Albumin and globulin decreased compared to control , yet it was not significant .

Apple cider vinegar administrated for (7) days to mice pretreated for (7) days with hydroxy urea caused decrease in total protein , globulin and albumin but not to a significant degree . Hydroxy urea and NACV administrated to mice at the same time for (7) days resulted in a significant ($P<0.05$) increase in total protein compared to hydroxy urea treated group and reached higher level than control group , but not to a significant degree. A significant ($P<0.05$) increase in globulin level was noticed as compared to hydroxy urea treated and control groups . Albumin level not altered significantly with that of control .

Table (1): Effect of apple cider vinegar on RBC_s, WBC_s and platelets counts in hydroxy urea treated mice.

Experimental group	$RBC_s\ 10^6/\mu l$	WBC_s $10^3/\mu l$	Platelets $10^3/\mu l$
Control	9.25 ± 1.05	4.68 ± 0.54	74.60 ± 11.92
Hydroxy urea (80mg/kg) for (7) days	4.21 ± 1.27*	3.02 ± 0.54	65.80 ± 14.36
Hydroxy urea (80mg/kg) for (7) days followed by apple cider vinegar (20mg/kg) for (7) days	2.80 ± 0.50*	3.35 ± 0.17	27.75 ± 5.10*+
apple cider vinegar 20gm/kg + hydroxy urea (80mg/kg)for (7) days	8.79 ± 1.25+	7.08 ± 0.63*+	43.00 ± 2.04*+

Values are mean ± S.E

* Significantly different from control ($P<0.05$).

+ Significantly different from group II ($P<0.05$).

RBC_s: Red blood cells ; WBC_s: White blood cells

S.E: Standard error .

Table (2): Effect of apple cider vinegar on total protein, albumin and globulin levels in hydroxy urea treated mice.

Experimental group	Total protein gm/dl	Albumin gm/dl	Globulin gm/dl
Control	8.57 ± 1.48	4.52 ± 0.80	5.05 ± 1.89
Hydroxy urea (80mg/kg) for (7) days	5.55 ± 1.48*	2.50 ± 0.55	3.05 ± 1.87
Hydroxy urea (80mg/kg) for (7) days followed by apple cider vinegar (20mg/kg) for (7) days	6.35 ± 0.26	2.95 ± 0.62	3.40 ± 0.52
apple cider vinegar 20mg/kg + hydroxy urea (80mg/kg)for (7) days	11.50 ± 1.10+	2.83 ± 0.24	8.66 ± 1.61*+

* Significantly different from control ($P<0.05$).

+ Significantly different from group II ($P<0.05$).

SE: standard error .

DISCUSSION

The results of the present study demonstrated that hydroxy urea produced a marked bone suppression as manifested by a significant decrease in RBC_s , WBC_s ans platelets counts . These results were in agreement with that reported by Herzig etal (2) and WHO (10)and in clinically manifested in human by the occurrence of anemia, decrease in defense mechanism and immunity with an associated increase in bleeding time . The destructive effect of hydroxy urea on bone marrow may be attributed to it's inhibitory effect on DNA synthesis in bone marrow cells (1). Administration of NACV to hydroxy urea treated mice after establishment of the above mentioned changes produced no improvement in bone marrow depression .

Indicating that NACV can not provide protection to the damaging effect of hydroxyurea on progenitor cells in bone marrow .

The combined administration of NACV with hydroxyurea improved significantly RBC_s & WBC_s counts which reflect an improvement in the state of anemia and defense mechanism .

Platelets count remained unchanged and this could explained by the fact that platelets half is about (7-10) days and in this condition there was no chance for the new platelets to develop during the cause of experiment 7 days (12) . Hydroxy urea induced significant decrease in total protein and depress the value of albumin and globulin. These results may be attributed to the destructive effect of hydroxyurea on liver and lymphoid tissues . Herzig et al (2) . Suggested that cytotoxic drugs lead to hepatic dysfunction , Calabresi and Chabner (13)also stated that cyto toxic drug inhibits liver DNA synthesis and repair . Administration of NACV for 7 days after hydroxyurea produced no improvement . These results may indicate that hydroxyurea damaged lymphoid tissues and liver and NACV can not protect and stimulate DNA repair in these tissues. The combined administration of hydroxyurea with NACV significantly restored the total protein to normal globulin level increased significantly which reflect improvement in immunity (3) .

It is concluded that administration of NACV with neoplastic agent such as hydroxy urea improved the depressed bone marrow. Function by cytotoxic agent . It also appeared that repairing effect of NACV is more clear than it's preventing effects and protects liver and lymphoid cellular DNA from damage by chemical substances . It could also be considered as an immuno stimulant agent for both cellular and humoral immunity.

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