DOI: 10.29409/ijcmg.v4i1.53

Iraqi Journal of Cancer and Medical Genetics

The Role of High Dose of Pomegranate on P53 Expression in Healthy and Dacarbazin's Treated Experimental Male Rats

Firas K. Bakir*, Mustafa S. Al-attar*, Goran Q. Othman*

University of Salahaddin-Erbil, College of Science, Biology Dept.

Abstract :

The possible action of high dose of pomegranate (*Punica granatum* L.) powder extract was evaluated in normal healthy and dacarbazine-induced toxicity in albino rats. Twenty-eight male albino rats were used in this study. The animals were divided into three different groups in addition to control group. The first group treated with intravenous injection of (1mg/kg b.w.) dacarbazine. he rats of the second group supplemented with pomegranate juice with concentration (1000mg/l) in drinking water, while the third group was the combination of the two treatments. Samples of blood were collected after a weeks of treating, and the serum was obtained in the laboratory for the evaluation of P53 protein within the serum. The results showed a significant reduction of P53 level in dacarbazine treated rats comparing with control. Whereas, pomegranate juice treated rats had significant increasing of P53 level as compared to control. The level of the combination treatment also was significantly more than control and dacarbazine treatment, while it was significantly lesser than pomegranate juice treated rats. These finding suggests that dacarbazine cytotoxicity is not P53 dependent and high dose of pomegranate has role in P53 protein expression induction.

Key words: *Dacarbazine , Pomegranate , P53.*

Introduction:

Dacarbazine (DTIC) is a DNA-methylating agent with a broad spectrum of antitumor activity in mouse tumor models. Clinical activity of this agent in human malignancies is restricted to melanoma, Hodgkin's disease, and sarcoma. Dacarbazine is used in a curative regimen with adriamycin, bleomycin, and vincristine (ABVD) for Hodgkin's disease and it is the most active single agent used to treat advanced melanoma and soft tissue sarcoma.

It is a purine analogue which continues to be one of the most active drugs in the treatment of malignant melanoma (1).

In vivo, DTIC is metabolically activated by microsomal N-demethylation to 5-(3-methyl-l-triazeno) imidazole-4-carboxamide. 5-(3-methyl-1-triazeno) imidazole-4-carboxamide methylates DNA, producing O6-meG and N7-meG adducts as well as probably other DNA adducts (2, 3).

Dacarbazine causes cell death, which is related to apoptosis (4,5). However, the processes leading to apoptosis appear to be different, reflecting the fact that the DNA lesions produced by this drugs cause different effects on the cellular DNA synthesis. O6-Methylguanine, produced by dacarbazine, would allow progression of the DNA replication forks and, after one round of DNA replication, yield an O6-methylguanine–thymine mispair.

It has been shown that such a mispair, synthesized *in vitro*, can be recognized by the mismatch recognition protein to activate ATR kinase (6). O6-meG is a directly miscoding DNA lesion which, during cell replication, gives rise to G:A mutations, in addition to its mutagenic and carcinogenic potential (7).

Pomegranate (*Punica granatum*, Punicaceae), is an edible fruit cultivated in Mediterranean countries, such as Iraq and the United States. Edible parts of pomegranate fruit (~80% of total fruit weight) comprise 80% juice and 20%

Corresponding Address:
Firas K. Bakir
University of Salahaddin-Erbil / College of Science / Biology Dept.

Email: ***shnafk@yahoo.com***

seed. Pomegranate is a rich source of crude fibers, pectin, sugars and several tannins, its juice and seed oil contain certain species of flavonoids and anthocyanins which provide the fruit potent antioxidant activity (8).

Pomegranate ellagitannins, a group of bioactive constituents of pomegranate juice (PJ) derived from the *Punica granatum* fruit, have received increasing attention for their potential as nontoxic chemopreventive dietary agents.

Recently, the researchers showed that consumption of PJ produced from pressed whole pomegranate fruit prolonged the doubling time of the serum prostate-specific antigen (PSA) tumor marker in patients who had experienced a PSA recurrence after prostatectomy (9).

Interestingly, pomegranate extract (PE) has been shown to inhibit NF-κB in normal human cells, including chondrocytes, epidermal keratinocytes, and vascular endothelial cells (10, 11).

The main aim of this research is to investigate the cytotoxic influences of Dacarbazine in normal cells via determining P53 concentration to know whether it's cytotoxic and apoptotic actions are P53 dependent or not, and also to study the effects of Pomegranate on Dacarbazin's toxic actions in male albino rats besides its own action alone on P53 level in normal experimental animals .

Materials and Methods:

Animals: The experimental animals, Wistar rats, were procured from the animal house of the biology Department, University of Salahaddin.

The animals were maintained under standard conditions of temperature (20 ± 5°C), with a regular 12-h light/12-h dark cycle.

They were allowed free access to standard laboratory food and water ad libitum (13).

Experimental design: Twenty eight adult Sprague Dawley male rats (average body weight range, 250.0±25.0 grams) were randomly assigned to four groups:

Group 1: Control :- The rats of this group received standard rat chow and tap water ad libitum (n=7).

Group 2: Animals were injected with single dose of Dacarbazine intra- veinous (1m/Kg) of body weight (n=7) daily for one weeks. The drug was purchased from Hospira UK Ltd. Mayne Pharma Plc ,Queensway Royal Leamington, Spa Warwickshire CV31 3RW. UK.

Group 3: Animals treated as in group 2 plus pomegranate supplementation (1000mg/L in drinking water) (n=7).

Group 4: Animals supplemented with pomegranate supplementation (1000mg/L in drinking water) (n=7).Pomegranate 100% pure 500 mg powder capsules were purchased from Club natural dietary supplements, USA. The experiment evaluated for one week.

Collection of blood samples: At the end of each experiment, the rats were anesthetized with ketamine hydrochloride(50 mg/kg). Blood samples were taken by cardiac puncture and transferred into chilled tubes with or without ethylene diamine tetraacetic acid (EDTA-K3) (4.5 mM) as anticoagulant (13). Blood sample were centrifuged at 4C° for 15 minutes, the separated plasma and serum samples were stored at (– 80C°) (Sony Ultra low,Japan).

P53 Assay: The animals serum were prepared for the quantification of p53 using pan ELISA. The kit was Purchased from (Roche Diagnostics GmbH) Roche Applied Science Nonnenwald 2 82372 Penzberg Germany.

The assay is based on a quantitative "sandwich ELISA" principle. The biotin-labeled capture antibody is pre-bound to the streptavidin-coated microtiter plate. During one single incubation step the p53-containing sample (specimen or standard) reacts with capture antibody and peroxidase-labeled detection antibody to form a stable immunocomplex. Subsequent to the washing step, the peroxidase bound in the complex is developed by tetramethylbenzidine (TMB) as a substrate.

The photometrically determined color is proportional to the concentration of p53. using Micro-plate reader stat fax 303+ , japan.

Results:

Table (1) shows significant differences at level of significant (P<0.05) and (P<0.01) among the treatments. The highest mean level of P53 was observed in rats treated with pomegranate alone in drinking water with value (140±5.36), while the lowest level of P53 protein was found in dacarbazine treated rats with value (56.3± 3.37).

The current result also shows that serum P53 level reached up to (108±5.36) in combination of dacarbazine and pomegranate treatment, and this P53 level was even significantly (P<0.05) higher than the level of it in control rats (75±4.2).

Comparing with the control P53 value (75±4.2), the results reported that the level of P53 was reduced significantly at level of significant (P<0.05) only in dacarbazine treated rats up to (56.3± 3.37) .

Whereas, the level of P53 in rats treated with pomegranate alone in drinking water was significantly more than control group at both levels of significants (P<0.05) and (P<0.01) as its illustrated in table (1).

Discussion:

The reduction of P53 protein in Dacarbazine's treated rats was very interesting result, because this anticancer drug is considered as cytotoxic agent against normal cells (14) and cancerous cells in addition to it's apoptosis induction (15) .

It is a methylating drug used for chemotherapy in subjects with malignant melanoma, soft-tissue sarcoma, osteogenic sarcoma, neuroblastomas and Hodgkin's disease

(16). According to our results, the mechanism of action of this drug is P53 independent because it doesn't lead to increasing the level of this protein, because as it has been well-known that the level of P53 expression is related to the cell cycle regulation and proliferation of the cells, and it has special mechanism for suppression of tumorogenesis and carcinogenesis process through induction of apoptosis and DNA fragmentation (13).

Our result was consistent with (17) who concluded that P53 and P21/waf 1-protein were not induced by dacarbazine treatment in human lynphobalstoid cells.The proposed mechanism of cytotoxicity and apoptosis induction is related to the conversion of this drug to its active form after administration which subsequently methylate the DNA bases.

The primary metabolic pathway of dacarbazine involves a-hydroxylation at one of its N-methyl residues and conversion to 5-(3-hydroxymethyl-3-methyl)imidazole-4-carboxamide (18). This metabolite is chemically unstable and will lose formaldehyde to generate the corresponding monomethyltriazene, which can release the methyldiazonium ion. Consistent with this proposed pathway, the administration of dacarbazine to animals results in the formation of O6-methylguanine in the DNA of various tissues and in the proportions that would be expected if a methyldiazonium ion was acting as the ultimate methylating agent (19,20).

Dacarbazine causes cell death, which is related to apoptosis (4). However, the processes leading to apoptosis appear to be different from P53 induction, as it affects DNA synthesis through production of DNA lesions. O6-Methylguanine, produced by dacarbazine, would allow progression of the DNA replication forks and, after one round of DNA replication, creating an O6-methylguanine–thymine mispair, which subsequently leads to the death of the cell (5).

The results also revealed that administration Pomegranate extract powder leads to high significant increasing in the level of P53. This result was also unexpected, and according to our review, we didn't find any investigation about the effect of Pomegranate extract on P53 expression in normal cells. The majority of researches reported the efficacy of it for treating and preventing most of the diseases, like its anticancer (21) and antioxidant actions (22) in addition to its induction of fertility (23).

However, the high dose Pomegranate extract has been proposed to be safe and produce only some side effects including nausea and allergic reaction (24), but at molecular level, the genotoxicity of Pomegranate extract was reported by (25) who suggests that administration of Pomegranate extract induced apoptotic DNA fragmentation. This finding support our results as it indicates the P53 induction by apoptosis.

Also (26) studied the action of different concentrations of Pomegranate juice on Vero and Hep-2 cell lines, and they concluded that Pomegranate juice was not cytotoxic only in high doses against the cells.

The toxicity of high dose of Pomegranate extract may be due to the polyphenols which are found within the extract.

Table (1): The mean and standard errors for the serum P53 levels in dacarbazine (i.v.) injection treatment alone , Pomegranate treated rats in drinking water alone and the combination of pomegranate and dacarbazine treatment versus control group of the experiment.

Treatments	control	Dacarbazine (i.v.)	pomegranate (drinking water)	Dacarbazine + pomegranate
Mean	75	56.3	140	108.9
S.E.	4.2	3.37	10.83	5.36
LSD(0.05)	18.33			
LSD(0.01)	24.13			

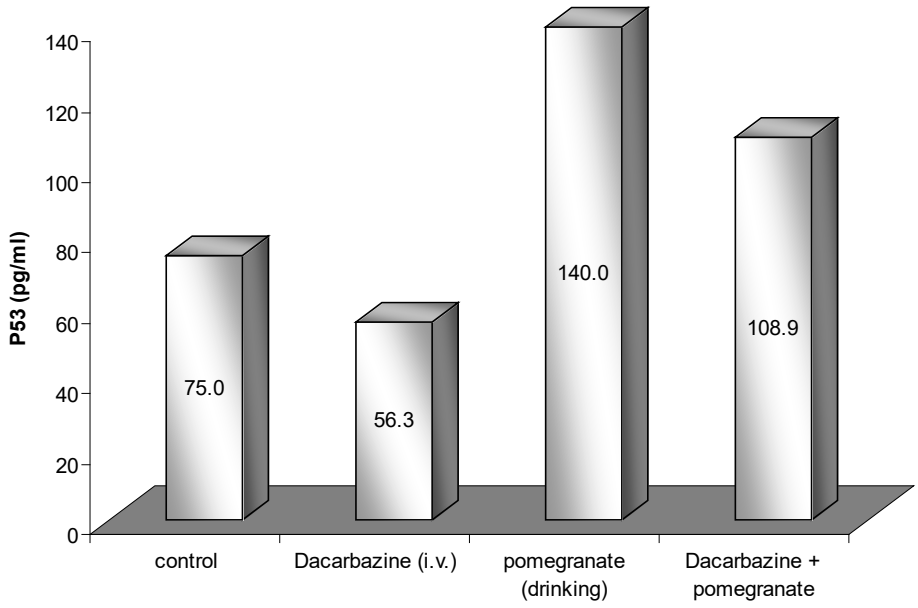

Figure 1: Comparison the levels of serum P53 (pg/ml) in in dacarbazine (i.v.) injection treatment alone , Pomegranate treated rats in drinking water alone and the combination of pomegranate and dacarbazine treatment versus control group of the experiment.

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دور الجرع العالية من قشور الرمان علي تعبير الجين P53 في الجرذان المعاملة وغير المعاملة بمادة Dacarbazin

فراس خالد قاسم*، مصطفى صابر العطار*، طوران قادر عثمان*

قسم علوم الحياة / كلية العلوم / جامعة صلاح الدين - اربيل

الخُلاصة:

أختبرت الفعالية المحتملة للجرعات العالية لمسحوق الرمان (***Punica granatum***) . في الجرذان البيضاء الصحّية وفي الجرذان البيضاء المعاملة بعقار الـ (Dacarbazine) المحفز للسمية فيها. تم استخدام ثمانية وعشرون ذكراً من الجرذان البيض في هذا الدراسة. قُسّمت الحيوانات إلى ثلاث مجموعات مختلفة بالأضافة إلى مجموعة السيطرة (control) .

تم اعطاء عقار ال dacarbazine (1 ملغ / كيلوغرام من وزن الجسم) للمجموعة الأولى بالحقن الوريدي. جرذان المجموعة الثانية زودت بعصير الرمان بالتركيز (1000ملغ / لتر) في الماء الصالح للشرب، بينما المجموعة الثالثة كَانتْ مجموعةَ التداخل التي عوملت بالمادتين معاً في نفس الوقت وبنفس التراكيز.

جُمِعتْ عينات الدمِّ بعد أسبوع واحد مِنْ المُعَالَجَة، وتم تقدير بروتين P53 ضمن المصل. أظهرت النَتائج أنخفاضاً معنوياً لمستوى P53 في الجرذان المعاملة بمادة ال dacarbazine مُقَارَنَة بجرذان السيطرة. بينما الجرذان المعاملة بعصيرَ الرمان كَانَت لديها زيَادَة معنويةَ في مستوى بروتين الP53 بالمقارنة مع جرذان السيطرة. في حين كانت هناك زيَادَة معنوية في مستوى p53 لمجموعةَ التداخل وبشكل ملحوظ أكثر منْه في مجموعتي السيطرة والمعالجة بعقار ال dacarbazine، بينما كَانَت النتائج أقلَ معنويةً وبكثيرً في مجموعة الجرذان التي عوملت بعصير الرمان فقط. يلاحظ من هذه النتائج بأنّ السمية الخلوية لعقار ال dacarbazine غير مقرونة الاعتماد على P53 والجرعات العالية مِنْ الرمان لَها دورُ في حثّ وتعبير بروتين P53 .