The Chemotherapy Drugs Correlation With Red Blood Cells Antioxidants And Plasma Uric Acid

ASIM A.ALSALEHI MUFEED J.EWADH KHOWLA A. SHEMRAN
MOHAMMED R.JODI* ABDUL SAMIE H.ALTAEE

BIOCHEMISTRY DEPARTMENT,COLLEGE OF MEDICINE,BABYLON UNIVERSITY

*ASSIT. PROF. IN HILLA TEACHING HOSPITAL,HILLA

Abstract:

The effect of specified drugs on The red blood cells glutathione level have been studied and the result revealed that mitomycine-c has a large effect at the concentration of o.6 mg /ml which indicate the distraction of cell memdrane and there is no transport system for such drugs .This novel study revealed that we have give an attention to the dose given to the patients.

الخلاصة :

في هذا البحث تم دراسة تأثير بعض المركبات الكيمياوية والتي تستخدم كعلاج لمرض السرطان بكافة أنواعه على مستويات الكلوتاثايون في كريات الدم الحمراء . حيث تبين بان عقار الميتومايسين له تأثير كبير على هذه المستويات بتركيز (0.6mg/ml) مما ادى الى تحطيم جدران الخلايا الحمراء وانخفاض مستوى الكلوتاثايون مما يدل على وجود جهاز انتقال تغير في جدران هذه الخلايا لهذا المركب ويجب تأخذ بنضر الاعتبار الجرع المعطاة إلى المرضى تفاديا" لحدوث مثل هذه الأعراض الجانبية .

INTRODUCTION:

Many chemicals including a wide array of drugs are capable of causing cellular damage depending on the dose of the chemical and the status of certain cellular protection systems. The degree of injury and whether the injury is reversible or irreversible is important in determining the conditions for safe use of drugs and exposure to exogenous chemical in general.

Some of these drugs used to treat the cancer patients which require careful assessment of liver function prior to treatment to determine which drugs may not be appropriate (1) .Also careful should take for the blood cells as well in such treatment especially using the anti tumor antibiotic such as doxorubicine,mitomycin,and mitoxantrone which acts through DNA interaction, alteration of membrane function and free radical formation (2).It is extensively metabolized inside cells and cells antioxidant capacity, including that provided by glutathione production may protect against free radical injury (3).Glutathione (GSH) a ubiquitous cellular antioxidant plays a central role in defense against a verity of disease and both exogenous and endogenous insults (4) .Its functions include the detoxification of xenobiotics,carcinogenes ,free radicals and peroxides and maintenance of protein structure, function and turnover (4,5) .low concentration of GSH have been implicated in numerous pathological conditions including diabetes (6) ,alcoholic liver disease (7) ,Aids (8,9), cataracts (10),xenobiotic-induced oxidative stress and toxicity (11).

Given these essential roles of GSH in cellular protection and homestasis,GSH availability may be a key factor in the maintenance of health and GSH concentration may serve as a useful indicator of disease risk in humans (12).
This study try to evaluate the effects of two compounds used as chemotherapy in cancer patients on the plasma glutathione and uric acid levels which consider as an antioxidant in its normal value.

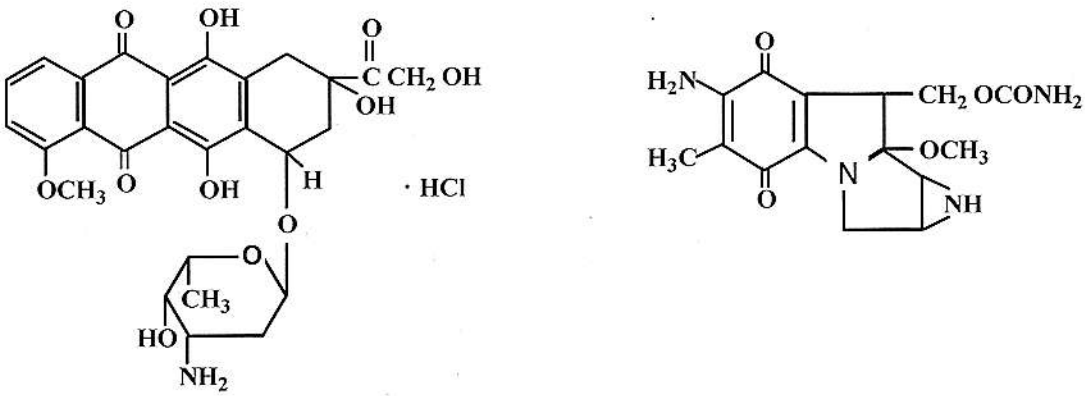

Fig (1): The chemotherapy drugs used in the present study (a) Doxorubicin (b) Mitomycine C

MATERIAL AND METHODS:

All chemicals used are highly pure analytical grade imported from sigma company (USA)

BLOOD COLLECTION:

5 mls of venous blood was collected from the auteaubital vein divided into two parts ,one in a plan tube for serum uric acid estimation and the other part into sterilized tubes containing heparin or EDTA as an anticoagulant for the red blood cells glutathione estimation.

DRUGS PREPATION

For mitomycin –C drug , different concentration were prepared in normal saline which include 0.2 ,0.4 ,and 0.6 mg / ml of blood .Same concentration were consider for the doxorubicin drugs and both drugs influence on the glutathione and uric acid were done by keeping blood samples at 37 C in rotating state in order to allow the drugs in continuous adhesion with red blood sample ,while the control sample contain a normal saline instead of drugs.

RED BLOOD CELLS GLUTATHIONE AND SERUM URIC ACID ESTIMATIONS.

The method used in this study to evaluate the RBCs glutathione levels and the standerzation curve was described by Beutler ,E (13) while the estimation of uric acid was carried out by using the kit supplied by Randox Labortories LTD.

QUALITY CONTROL:

To maintain assay precision and accuracy over long periods, we performed several quality control measures that external calibrators were included on every assay, and the range of concentration in the calibration curve encompassed the range of expected sample GSH values. All samples were run in triplicate and all results expressed as (mean + SD).

RESULTS AND DISCUSSION:

The results shows that the doxorubicin have an invasive effects at the concentration of 0.6 mg / ml on the red blood cells glutathione levels ,while the other concentrations of such drugs shows no significance effects .(Table 1, and Fig. 2).This results indicate the inability of such drug in first two concentration (i.e. 0.2 and 0.4 mg/ml) to penetrate the red blood cells membrane and then could no transport system available for such compound since the transport systems need the interaction between membrane with other group to facilitate the endocytosis of such compound. The action of third concentration show a dramatically decrease of GSH which is could be due to the destruction of red blood cell membrane and the direct reaction of GSH with such drugs to form a glutathione conjucation,however this results agree with results showed by ewadh et.al.,(14)which indicate the effects of other antibiotic on the cell membrane.

The effects of mitomycine-c on the glutathione levels shows no significance effects even at high concentration used in this study which indicate the inability of drugs neither to penetrate nor to distract the cell membrane (Table 2, and Fig. 3) .This results make the mitomycine more safe antibiotic than the others since the levels of GSH stay on its normal value and might show no effects on other blood parameters and so less side effects on the cancer patients than the other drugs.

To prove that the antibiotic doxorubicin has an effects ,the levels of plasma uric acid has been measured that because uric acid consider as an antioxidant in its normal value and the results shows that the level of uric acid not change which reflect the ability of uric acid to prevent even in a small degree the status of antioxidants ,this is supported by the results gained by Marshall (15) which indicate that the level of melatonin which is it the last product of adrenaline oxidation can increase in a response to protect the system from the action of oxidative stress .However the effects of oxidative stress could prevent by the addition of 0.1 m M of cysteine to the reaction mixture in the concentration of 0.6 mg/ml of drugs and the results shows that the addition of the such amino acid reduced the effects byss about 35% of total effects (Fig: 4) ,which agree with the results obtained by the (16 , 17). On this basis we recommend that oncologist should take an attention to the drugs type and the dose used in addition to the type of drugs administration to the patients.

REFERENCES:

1-Isak K G,Zimmeraman H j, Gastroentersl clin north Ann.1995,24,759
2-Farrell.Gc.In drug-induced liver disease.Churchill livingstone .1994
3-Meredith M ,J ,Reed DJ.Biochemparmacol.1983,32,1383.
4-Meister A,Anderson M ,E.Glutathione .Ann Rev. Biochem 1993; 52;711
5-Vina j.In glutathione metabolism,Boca,Roton,Fl:CRC press. 1990,378
6-Murahani K,Kondo T,Ohtsuka Y,Shimada M,Kawakami .Y. Metabolism,1989,38,753.
7-Macdonald CM,Dow J,Moore MR, Biochem pharmacol ,1977;26; 1529.
8-staal Fd;Elasw, Roederer M,Anderson MT,Uerzenberg LA.Lancet.1992;339;909.
9-Kaleb 'C T,Kinter A,Poli G,Anderson M E, Meister.A, fauci A S, Proc Natl Acadsci USA ,1991;88; 986.
10- Harding jj. Journal of Biochemistry ;1970;117;957.
11-Disimplicio P,Dolara P,Lodovici M,Journal Appl.Toxicol.1984;4;227.
12-Lang CA,Naryshkins,schneider DL,Mills BJ,Lindeman RD .Journal of Lab. Clin Med. 1992;120;720.
13- Beutler,E. Journal Lab. Clin.Med.1957,49,84.
14- Ewadh.M J ,Alhammed.N J, Ejam.I.K, Journal of babylon university Journal of Badylon Univarsty 1999,4
15-Marshall,J. Pharmacology and Toxicology;1996,78,5,308.
16-Bannai,S. J.Biol.Chem; 1984;259;2435.
17-Denenke,S.M;Steiger,V. AND Fanburg.B.L. Journal of Appl.Physiol.1987,63,5,1966.

Table 1:GSH levels under the influence of doxorubicine

Time (h)	Blood GSH conc. (mg/dl)			
	C	T1	T2	T3
0	33.7	33.7	33.7	33.7
0.75	36.26	30.56	30.8	22.95
1.5	37.07	34.1	30.49	13.91
2.25	34.63	35.04	31.77	10.44
3	34.22	31.78	34.34	5.56
3.75	30.56	34.63	28.33	5.56

C (control)
T1 (0.2 mg doxorubicine/ml blood)
T2 (0.4 mg doxorubicine/ml blood)
T3 (0.6 mg doxorubicine/ml blood)

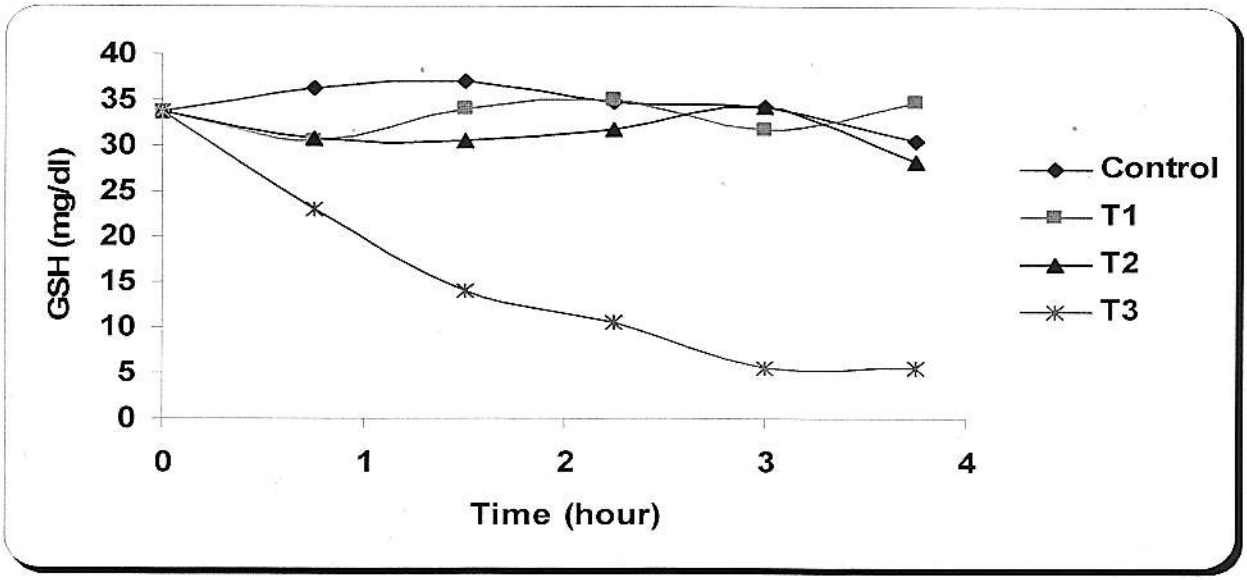

Figure 2:GSH levels under the influence of doxorubicine

Table 2: GSH levels under the influence of mitomycine-c

Time (h)	Blood GSH conc. (mg/dl)		
	T1	T2	T3
0	42.59	42.59	42.59
0.75	42.37	45.78	37.56
1.5	38.29	41.77	40.44
2.25	39.93	46.67	53.44
3	41.97	42.23	41.45
3.75	41.56	46.67	50.54

T1 (0.2 mg mitomycine-c/ml blood)
T2 (0.4 mg mitomycine-c /ml blood)
T3 (0.6 mg mitomycine-c /ml blood)

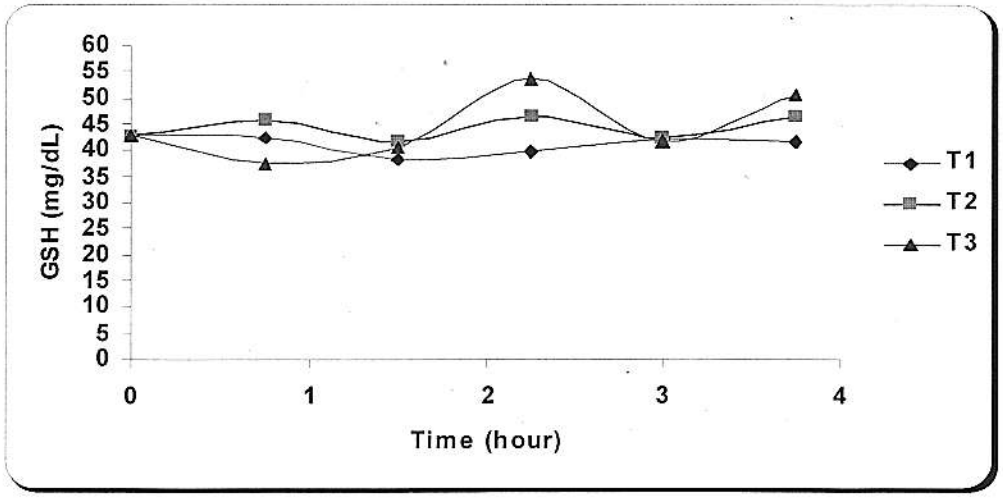

Figure 3: GSH levels under the influence of mitomycine-c

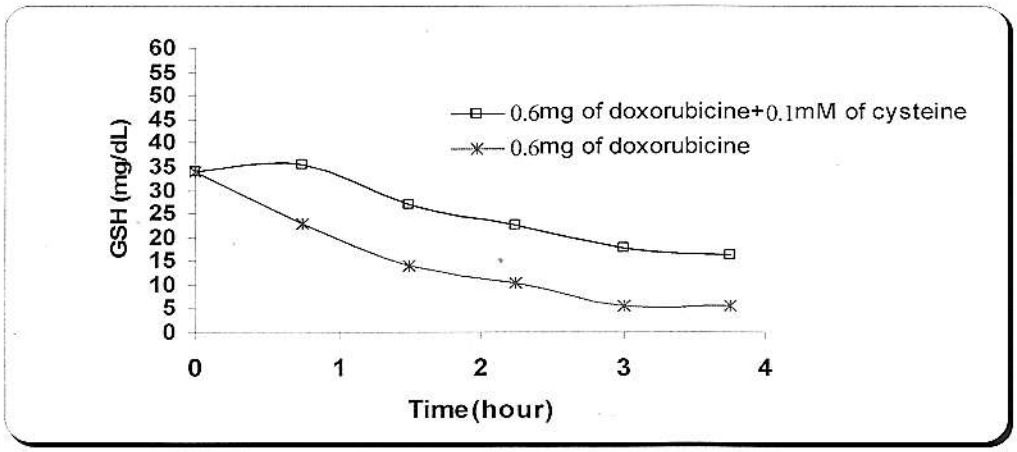

Figure 4:GSH levels under the influence of doxorubicine in the presence of 0.1mM of cysteine