The Internet Journal of Aesthetic and Antiaging Medicine
Volume 1 Number 1

Can a combination of glutathione and granulocyte –colony stimulating factor increase chemoprotection?

S Gad El-Hak, I El-Shawaf, E Ali, M Salama

Citation

S Gad El-Hak, I El-Shawaf, E Ali, M Salama. *Can a combination of glutathione and granulocyte –colony stimulating factor increase chemoprotection?*. The Internet Journal of Aesthetic and Antiaging Medicine. 2007 Volume 1 Number 1.

Abstract

5-FU is a chemotherapeutic agent with myelosuppressive effects, glutathione has been proved to have aprotective action against this myelosuppression. Also granulocyte colony stimulating factor is well known to be a protective agent aginst chemotherapeutics induced bone marrow depression. Whether glutathione (GSH) and granulocyte colony stimulating factor (G-CSF) combination offered more protection on myelosuppression induced by 5-fluorouracil (5-FU) or not was questioned in female mice. The animals were divided into seven groups (10 mice each) . Group 1 received no treatment , group 2 received GSH, group 3 received G-CSF, Group 4 received a single dose of 5-FU, Group 5 received GSH+5-FU, Group 6 received G-CSF + 5-FU and group 7 received daily doses of both GSH and G-CSF + 5-FU. Animals were sacrificed on day 9 i.e. 1 day after 5-FU administration and were evaluated using complete blood count. Surprisingly combination of GSH and G-CSF nullified the protective effects of each other.

INTRODUCTION

5-fluorouracil (5-FU), is an antimetabolite, anticancer agent which is used as an essential part for the treatment of wide range of solid tumors. It has antitumor activity against epithelial malignancies arising in the gastrointestinal tract , breast as well as the head and neck(Malet-Martino and Martino, 2002).As all the anticancer agents, 5-FU leads to several toxicities. Myelotoxicity is the major toxic effect in patients receiving bolus doses (Takimoto and Page, 2004).

The haemopoietic growth factor (granulocyte-colony stimulating factor G-CSF) was proved to shorten the duration and decrease the severity of chemotherapy induced neutropenia (Liang, 2003).

Lamson and Brignall, (2000)demonstrated the cytoprotective effect of glutathione (GSH) against chemotherapeutic agents. Kojima et al.(2003)suggested that glutathione could prevent the 5-FU-induced haemopoietic toxicities and accelerate recovery from such toxicities.

The aim of the present study was to evaluate the effect of combining both GSH and G-CSF on their chemoprotective functions.

MATERIAL AND METHODS

DRUGS

5-fluorouracil (5-FU)5ml ampoules each containing 250mg 5-FU (Biosyn Arzneimittel GmbH Fellbach, Germany).5-FUwere diluted with distilled water to give a final concentration of 10 mg/ml. It was given in a dose 160 mg/kg by intraperitoneal route (Friberg et al.,2000).

L-Glutathione reduced (GSH)powder, 5gm/bottle (SIGMA-ALDRICH,Inc) was obtained from Egyptian International Center Importer Cairo, Egypt. It was stored in the refrigerator.GSHwas dissolved in distilled water to give a final concentration of 80 mg/ml. It was given in a dose 800 mg/kg by intraperitoneal route (Kojima et al., 2003).

Filgrastim Neupogen (Granulocyte- Colony Stimulating Factor “G-CSF”)0.5 ml prefilled syringe of 300µg/ml concentration (F.Hoffmann La Roche Ltd, Basel, Switzerland). It was stored in the refrigerator.G-CSFwas diluted with distilled water to give a final concentration of 10 µg/ml. It was given in a dose 250 µg/kg by subcutaneous route (Lord et al., 2001).

ANIMALS

The study was carried out on 70 adult female mice with their weight ranged 27-32 gm obtained from the Animal House of Mansoura Faculty of Pharmacy. Mice were chosen all

females to alleviate the gender effect on the results (Doeing et al ., 2003). The mice were housed in metallic cages , fed a standard diet and allowed unlimited access to food and water under standard laboratory conditions. After a week of acclimatization to the housing conditions the mice were divided into seven groups housed in separate cages.

Group 1 of animals (10 mice) received no treatment and served as control group , group 2 (10 mice) received GSH (800 mg/kg) by intraperitoneal route in daily doses for the first 7, and group 3 (10 mice) received G-CSF (250 µg/kg) by subcutaneous route in daily doses for the first 7 days. Group 4 (10 mice) received a single dose of 5-FU (160 mg/kg) by intraperitoneal route in the 8th day , Group 5 (10 mice) received GSH in daily doses (800 mg/kg by intraperitoneal route) for the first 7 days followed by a single dose of 5-FU (160 mg/kg by intraperitoneal route) in the 8th day , Group 6 (10 mice) received G-CSF in daily doses (250 µg/kg by subcutaneous route) for the first 7 days followed by a single dose of 5-FU (160 mg/kg by intraperitoneal route) in the 8th day and group 7 of animals (10 mice) received daily doses of both GSH (800 mg/kg by intraperitoneal route) and G-CSF (250 µg/kg by subcutaneous route) for the first 7 days followed by a single dose of 5-FU (160 mg/kg by intraperitoneal route) in the 8th day. Animals were sacrificed on day 9 i.e. 1 day after 5-FU administration.

SAMPLES COLLECTION

Blood samples (2ml, each) were collected in EDTA tubes from the mice cut throats after sacrification. The following haematological values were determined ; haemoglobin (Hb) g/dl , mean corpuscular haemoglobin concentration (MCHC) g/dl , mean corpuscular volume (MCV) Fl (femoliter) , mean corpuscular haemoglobin (MCH) Pcg (pictogram) measured according to the method ofRiedinger and Rodak (1998), haematocrite value (Hct) % measured by microhaematocrit method (Dill and Costill,1974) as well as total (TLC) and differential (DLC) leucocyte count measured according to the method ofEngland and Bain (1976) .

STATISTICAL ANALYSIS

Statistical analysis of data was done by using Excel program (Office 2000) and SPSS (Statistical Package of Social Science) , Windows 98, computer compatible IBM.

The first part of data analysis was descriptive. The mean ± S.D. were used for description of frequency.

The second part of the analysis was analytic: For quantitative data. Student's t test was used for comparison between two groups.Anova t testwas used for comparing more than two groups followed byPost-Hoc testfor testing the significant different one. P value was considered significant if < 0.05.

RESULTS

Haematological parameters and bone marrow cytology: Table (1)

As regards TLC and bone marrow cytology it was found that groups 3 (received G-CSF), and 6 (received G-CSF before 5-FU and killed 1 day later) have higher (i.e. better) values than the control, while other groups have lower values (i.e. worse). TLC was higher also in group 5 (received GSH before 5-FU and killed 1 day later) than the control. The peripheral leukopenia and medullary myelosuppression in this study are detected in mice received 5-FU either alone or with pretreatment of combined GSH and G-CSF. (Table 2)

When comparing TLC and bone marrow cytology, groups 5 (GSH +5-FU) and 6 (G-CSF + 5-FU) were significantly higher than group 4 (5-FU only). Also both groups were significantly higher than group 7 (GSH+G-CSF+ 5-FU).

Group 7 showed no significant difference with group 4 regarding TLC and bone marrow cytology, but showed significantly lower counts than groups 5 and 6 (Table 3).

Figure 1

Table 1: Haematological parameters and bone marrow cytology in all studied groups.

Parameter / Groups	B.M.cytology (/mm³)	Hb (g/dl)	HCT (%)	MCHC (%)	MCV (fl)	MCH (pcg)	TLC (/mm³)
1 Control	19785 ± 130.959	12.133 ± 0.966	37.609 ± 2.994	32.727 ± 0.699	49.872 ± 0.644	32.513 ± 0.981	3810 ± 685.484
2 GSH	18525 ± 208.982	11.117 ± 1.361	34.457 ± 4.221	32.504 ± 0.307	50.251 ± 0.952	31.708 ± 1.121	3800 ± 1293.573
3 G-CSF	2345 ± 554.000	12.181 ± 1.29	38.198 ± 4.079	32.241 ± 0.091	50.661 ± 2.618	31.529 ± 0.545	6950 ± 2867.538
4	1217 ± 288.253	10.328 ± 2.159	31.175 ± 6.011	32.927 ± 2.302	49.971 ± 6.564	30.36 ± 1.685	1980 ± 616.080
5	1925 ± 302.994	11.881 ± 1.226	35.482 ± 3.171	33.467 ± 3.446	47.0940 ± 4.657	29.03 ± 2.568	4170 ± 1310.682
6	2172 ± 233.942	11.871 ± 2.157	36.881 ± 6.069	31.735 ± 2.811	46.512 ± 5.484	29.232 ± 2.365	4670 ± 1095.495
7	1273 ± 191.546	9.8360 ± 2.135	39.802 ± 5.919	32.861 ± 1.752	48.142 ± 7.401	30.225 ± 2.351	1975 ± 300. 924

DLC (%)				
Neutrophils	Lymphocytes	Eosinophils	Monocytes	Basophils
60.8 ± 8.456	34.3 ± 9.684	1.6 ± 1.505	3.5 ± 2.223	0 ±0
53.3 ± 8.731	42.5 ± 8.784	1.1 ± 0.737	2.7 ± 0.948	0.4 ± 0.516
45.6 ± 10.243	50.4 ± 9.754	1.7 ± 1.337	1.4 ± 0.843	0.9 ± 0.567
51 ± 4.320	45.2 ± 5.432	1.1 ± 0.994	1.9 ± 0.875	0.8 ± 0.788
54.1 ± 6.384	41.7 ± 6.815	1.2 ± 1.032	2.7 ± 0.948	0.3 ± 0.674
54.5 ± 6.587	40.4 ± 5.853	2 ± 1.054	2.5 ± 1.080	0.6 ± 0.699
51.7 ± 7.958	44.7 ± 7.846	0.9 ± 0.875	2.4 ± 1.264	0.3 ± 0.483

4 5-FU killed after 1 day, 5 5-FU + GSH killed after 1 day, 6 5-FU + G-CSF killed after 1 day, 7 5-FU + GSH + G-CSF killed after 1 day.

Figure 2

Table 2: Comparison of haematological parameters and bone marrow cytology in all studied groups versus control group.

Parameter / Groups	B.M.cytology (/mm³)	Hb (g/dl)	HCT (%)	MCHC (%)	MCV (fl)	MCH (pcg)	TLC (/mm³)
Gp2 vs control	↓ 0.703	↓ 0.170	↓ 0.172	↓ 0.509	↑ 0.866	↓ 0.141	↓ 1.000
Gp3 vs control	↑ 0.067	↑ 0.996	↑ 0.936	↓ 0.395	↑ 0.541	↓ 0.059	↑ 0.002**
Gp 4 vs control	↓ 0.0001**	↓ 0.336	↓ 0.135	↑ 1.000	↑ 1.000	↓ 0.267	↓ 0.0001**
Gp 5 vs control	↓ 1.000	↓ 1.000	↓ 0.990	↑ 0.998	↓ 0.952	↓ 0.435	↑ 0.987
Gp 6 vs control	↑ 0.723	↓ 0.092	↓ 1.000	↓ 0.983	↓ 0.870	↓ 0.110	↑ 0.346
Gp 7 vs control	↓ 0.0001**	↓ 0.092	↑ 1.000	↑ 1.000	↓ 0.998	↓ 0.198	↓ 0.0001**

DLC (%)			
Neutrophils	Lymphocytes	Eosinophils	Monocytes
↓ 0.18	↑ 0.145	↓ 0.643	↓ 0.458
↓ 0.003**	↑ 0.002**	↑ 0.982	↓ 0.010**
↓ 0.076	↑ 0.039*	↓ 0.977	↓ 0.175
↓ 0.489	↑ 0.001**	↓ 0.977	↓ 0.913
↓ 0.572	↑ 0.646	↑ 0.995	↓ 0.755
↓ 0.126	↑ 0.059	↓ 0.875	↓ 0.650

* significant if P <0.05. ↑ = higher
** highly significant if P <0.01 ↓ = lower

Figure 3

Table 3: Comparison of haematological parameters and bone marrow cytology in mice killed 1 day after receiving: 5-FU (gp 4), 5-FU preceeded by GSH pretreatment (gp 5), 5-FU preceeded by G-CSF pretreatment (GP 6) and 5-FU preceeded by combined GSH and G-CSF pretreatment (gp 7).

	Gp 5 vs gp 4	Gp 6 vs gp 4	Gp 6 vs gp 5	Gp 7 vs gp 4	Gp 7 vs gp 5	Gp 7 vs gp 6
TLC (/mm³)	↑ 0.0001**	↑ 0.0001**	↑ 0.911	↓ 1.000	↓ 0.0001**	↓ 0.0001**
Neutrophils %	↑ 0.987	↑ 0.973	↑ 1.000	↑ 1.000	↓ 0.998	↓ 0.993
Lymphocytes%	↓ 0.977	↓ 0.868	↓ 0.868	↓ 1.000	↑ 0.991	↑ 0.925
Eosinophils %	↑ 1.000	↓ 0.608	↓ 0.338	↓ 1.000	↓ 0.999	↓ 0.338
Monocytes %	↑ 0.913	↑ 0.983	↓ 1.000	↑ 0.995	↓ 1.000	↓ 1.000
Basophils %	↓ 0.662	↓ 0.998	↑ 0.972	↓ 0.662	↓ 1.000	↓ 0.972
Hb (g/dl)	↑ 0.540	↑ 0.549	↓ 1.000	↓ 0.999	↓ 0.189	↓ 0.194
HCT (%)	↑ 0.628	↑ 0.257	↑ 0.999	↑ 1.000	↑ 0.263	↑ 0.070
MCHC (%)	↑ 1.000	↓ 0.948	↓ 0.698	↓ 1.000	↓ 0.999	↑ 0.963
MCV(fl)	↓ 0.942	↓ 0.851	↓ 1.000	↓ 0.997	↑ 1.000	↑ 0.999
MCH (pcg)	↓ 0.837	↓ 0.928	↑ 1.000	↓ 1.000	↑ 0.903	↑ 0.965
B.M cytology (/mm³)	↑ 0.0001**	↑ 0.0001**	↑ 0.411	↑ 1.000	↓ 0.0001**	↓ 0.0001**

* significant if P <0.05. ↑ = higher
** highly significant if P <0.01 ↓ = lower

DISCUSSION

5-fluorouracil has been used for the treatment of cancer for

more than 40 years and is the most commonly prescribed chemotherapeutic agent (Dobritzsch et al.,2001). Chemotherapy-induced neutropenia, the primary dose-limiting toxicity of most cancer chemotherapy agents, is associated with numerous negative consequences, including life-threatening infections (Dale, 2003).

Glutathione is a naturally occurring endogenous antioxidant that is cytoprotective. Thus, treatment with glutathione or its precursors may protect normal cells during cancer chemotherapy(Doolittle et al.,2002)and that is what happened in our study.

Priming has been defined as the administration of a colony stimulating factor prior to chemotherapy. There are several theoretical reasons why this schedule of administration may prove to be advantageous (Lowenberg et al., 2003).Again this was proven in this work.

However, the combined pretreatment with GSH and G-CSF reveal no protection at all denoted by presence of leucopenia in tested group.

These results could be explained by the work ofSattler et al. (1999). They found that haematopoietic growth factors signals through the formation of reactive oxygen species (ROS) and that antioxidants e.g. N-acetyl cysteine (a glutathione precursor) reduced growth and viability of cells.

AlsoGate et al. (2004) have shown that G-CSF was more effective at stimulating proliferation of haematopoietic cells in glutathione transferase (GST) deficient mice than in wild type. This is explained by the fact that GST interacts with and suppresses c-Jun NH2-terminal kinase [an enzyme which is responsible for increased myeloproliferation].

Despite the previous works it may be the first time to test these results in vivo instead of cellular level.

Cascinu et al. (1997), however contradict these findings and this could be explained by the fact that there was a separation between G-CSF and GSH as regards days of administration which could prevent the antagonism between them.

It can be concluded from this work that the chemoprotective effect of GSH and G-CSF is diminished if they are combined during treatment. So, some times one plus one does not equal two but instead a big zero.

References

r-0. Cascinu, S.; Labianca, R.; Alessandroni, P.; Marcellini, M.; Silva, R.R.; Pancera, G.; Testa, E.; Martignoni, G.; Barni, S.; Frontini, L.; Zaniboni, A.; Luporini, G.; Cellerino, R. and Catalano, G. (1997): “Intensive weekly chemotherapy for advanced gastric cancer using 5-fluorouracil, cisplatin, epidoxorubicin, 6S-leucovorin, glutathione and filgrastim: a report from the Italian Group for the Study of Digestive Tract Cancer (GISCAD).” J.C.O., 15: 3313-3319.
r-1. Dale, D.C. (2003):“Optimizing the management of chemotherapy induced neutropenia.” Clinical Advances in Hematology & Oncology, 1: 679-684.
r-2. Dill, D.B. and Costill, D.L. (1974):“Calculation of percentage changes in volumes of blood, plasma and red cells in dehydration.” J. Appl. Physiol., 37: 247-248.
r-3. Dobritzsch, D.; Schneider, G.; Schnackerz, K.D. and Lindqvist, Y. (2001): “Crystalstructure of dihydropyrimidine dehydrogenase, a major determinant of the pharmacokinetics of the anti-cancer drug 5-fluorouracil.” The EMBO Journal, 20: 650-660.
r-4. Doeing, D.C.; Borowicz, J.L. and Crockett, E.T. (2003):“Gender dimporphism in differential peripheral blood leukocyte counts in mice using cardiac, tail, foot and saphenous vein puncture methods.” B.M.C. Clinical Pathology, 3: 3-13.
r-5. Doolittle, N.D.; Abrey, L.E.; Ferrari, N.; Hall, W.A.; Laws, E.R.; McLendon, R.E.; Muldoon, L.L.; Peereboom, D.; Peterson, D.R.; Reynolds, C.P.; Senter, P. and Neuwelt, E.A.(2002):“Targeted Delivery in Primary and Metastatic Brain Tumors.” Clinical Cancer Research, 8: 1702-1709.
r-6. England, J.M. and Bain, B.J. (1976):“Total and differential leukocyte count.” Br. J. Haematol., 33: 1.
r-7. Friberg, L.E.; Freijs, A.; Sandström, M. and Karlsson, M.O. (2000):“Semiphysiological Model for the Time Course of Leukocytes after Varying Schedules of 5-fluorouracil in Rats” J.P.E.T., 295 (2): 734-740.
r-8. Gate, L.; Majumdar, R.S.; Lunk, A. and Tew, K.D. (2004):“Increased myeloproliferation in glutathione s-transferase π-deficient mice is associated with a deregulation of JNK and Janus Kinase/STAT pathways.” J. Biol. Chem., 279: 8606-8616.
r-9. Kojima, S.; Takaba, K.; Kimoto, N.; Takeda, T.; Kakuni, M.; Mizutani, M.; Suzuki, K.; Sato, H. and Hara, T.(2003):“Protective effects of glutathione on (5-FU)-induced myelosuppression in mice.” Archives of Toxicology, 77: 285-290.
r-10. Lamson, D.W. and Brignall, M.S. (2000):“Antioxidants and cancer therapy II: quick reference guide.” Altern. Med. Rev., 5:152-163.
r-11. Liang, D.C. (2003):“The Role of Colony-Stimulating Factors and Granulocyte Transfusion in Treatment Options for Neutropenia in Children with Cancer.” Pediatr. Drugs, 5 (10): 673-684.
r-12. Lord, I.B.; Woolford, L.B. and Molineux, G. (2001):“Kinetics of neutrophil production in normal and neutropenic animals during the response to filgrastim (r-metHu G-CSF) or filgrastim SD/01 (PEG-r-metHu G-CSF).” Clinical Cancer Research, 7(7):2085-2090.
r-13. Lowenberg, B.; van Putten, W.; Theobald, M.; Gmür, J.; Verdonck, L.; Sonneveld, P.; Fey, M.; Schouten, H.; de Greef, G.; Ferrant, A.; Kovacsovics, T.; Gratwohl, A.; Daenen, S.; Huijgens, P. and Boogaerts, M. (2003):“Effect of priming with granulocyte factor on the outcome of chemotherapy for acute myeloid leukemia.” N. Engl. J. Med., 349:743-752.
r-14. Malet-Martino, M. and Martino, R. (2002) :“Clinical Studies of Three Oral prodrugs of 5-Fluorouracil (Capecitabine, UFT, S-1): A Review.” The Oncologist, 7 (4): 288-323.
r-15. Riedinger, T.M. and Rodak, B.F.(1998): Quantitative

laboratory evaluation of erythrocytes. In: Clinical Hematology Principles, Procedures, Correlations. Steine-Martin, E.A.; Lotspeich-Steininger, C.A. and Koepke, J.A. (Eds.), 2nd ed., Lippincott, Philadelphia, New York, Pp.106-124.

r-16. Sattler, M.; Winkler, T.; Verma, S.; Byrne, C.H.; Shrikande, G.; Salgia, R. and Griffin, J.D. (1999):"Hematopoietic growth factors signal through the formation of reactive oxygen species." Blood, 93: 2928-2935.

r-17. Takimoto, C. and Page, R. (2004) rinciples of chemotherapy. In : Cancer Management A Multidisciplinary Approach. Pazdur, R.; Hoskins, W.J.; Coia, L.R. and Wagman, L.D. (Eds.), 8th ed., CMP Healthcare Media. Pp. 21-38.

Author Information

Seham A. Gad El-Hak
Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Mansoura University

Ibrahim El-Shawaf
Department of Pathology, Faculty of Medicine, Mansoura University

Essam M. Ali
Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Mansoura University

Mohamad M. Salama
Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Mansoura University