

EFFECTS OF DIFFERENT DOSES OF VITAMIN C ON STRESS AND LYMPHOCYTES PARAMETERS IN LIBRATORY RATS.

Nowfel H.Jassim,

Department of physiology, College of Veterinary Medicine, University of Basrah,Basrah, Iraq.

(Received 26 january 2009, Accepted 18 february 2009)

Key words:Leuckocytes,Vit.C,Metabolism.

ABSTRACT

The studied of effects of vitamin C administration on the leukocyte counts and testing of exercise of male rats. Rats were divided into 4 groups; the first group contain the control and the other group contain the administration with different doses of ascorbic acid (35mg, 75 mg, and 120 mg/day; respectively. The doses were giving by intra-peritoneal injection. Physiological solution was given to the control group (CON) via the same procedure. Exercise performance was based on swim time to fatigue. Blood samples were taken and evaluated at day 10, 20 and 30 days. The lymphocyte percentage was 55.3 ± 5 % for CON on day 28. For all C groups, the range of the lymphocyte percentage was 54.5 %- 64.1 % ($p < 0.001$). Swim time was 1.6 ± 0.3 min at day 30. This value was increased to 5.1- 8.4 min for the C groups ($p < 0.001$).

INTRODUCTION

Little research was studied the effects for vitamin C on stress and some of blood parameters and stress on animals, In laboratory was only few studies have been done using of Vitamin C as methods in resistance the stress . The Vitamins are organic micro food materials that are needed for optimal cell tissue, organ and body functions. Its have effect on some enzymes activate which have important roles in synthesis and metabolism of protein, carbohydrate and fat metabolisms (1 , 2). An important water-soluble vitamin is vitamin C, which has diverse functions including being an antioxidant, exerting positive effects on lipid and iron metabolism (3), and promoting improved immune function (4).

The aim of this study is to describes the relationship between vitamin C administration and effects on number of lymphocytes and stress which resulted by swim .

MATERIALS AND METHODS

Adult forty rats (223 ± 9 g) were randomly divided into 4 equal groups in the first group was receiving standard rats feed, and isotonic water given as 120 mg/day intraperitoneal injection, second group was injected by 40 mg/day vitamin of C, While the third group 75 mg/day vitamin of C was intraperitoneal injection by 75 mg/day vitamin of C, the four group was intraperitoneal injection by 120 mg/day of vitamin C

The vitamin C source was ampules containing 500mg/5mL vitamin C (5).

Blood samples were taken at days 10, 20 and 30 the blood smears were achieved and stained with giemsa stain. Lymphocyte, monocyte and neutrophil counts were done using standard manual count and formula methods (6).

For exercise performance measurements, a swimming test was applied to the rats at the end of the 4th week. Mice swam alone in pools with 100 cm length and 45 cm diameter dimensions. Swim time was recorded as the time to the first sign of an inability to maintain the head above water.

STATISTICAL ANALYSIS

The different between of groups was determined by CRD test ANOVA and revised L. S. D. test according to - (7).

RESULTS

Table (1) showed that the Lymphocyte percentages of each groups increased significantly, whereas neutrophil percentages decreased compares with the control group.

Swim time (Stress) was significantly increased in all experimental groups, and also increased with increasing dosages of vitamin C (Table 2). The vitamin C adminlymphocyte was showed that ,increase of monocyte and neutrophl percentages of blood obtained at blood smear from experimental groups compare with control at the end of days 10, 20 and 30 Table (1) .also there were recorded differences in lymphocyte, monocyte and neutrophil The results percentages across days for each group.

Table (1) rates (%) of Lymphocytes, Monocytes, and Nutrophile in different groups after injection by Vitamin C

<i>groups</i>	<i>35mg.day</i>	<i>75mg.day</i>	<i>120mg.day</i>	
<i>lymphocyte %</i>				
<i>10</i>	<i>55.6 ± 5</i>	<i>57.1±7</i>	<i>59.8±6</i>	<i>67.1±5</i>
<i>20</i>	<i>48.5±4</i>	<i>56.6±6</i>	<i>51.3±7</i>	<i>64.3±5</i>
<i>30</i>	<i>49.2±6</i>	<i>57.5±5</i>	<i>61.1±4</i>	<i>67.1±4</i>
<i>monocyte %</i>				
<i>10</i>	<i>6.2±3</i>	<i>7.4±3</i>	<i>4.8±3</i>	<i>6.8±3</i>
<i>20</i>	<i>8.4±2</i>	<i>7.4±2</i>	<i>6.1±2</i>	<i>6.4±2</i>
<i>30</i>	<i>8.5±3</i>	<i>6.1±3</i>	<i>5.3±2</i>	<i>6.4±2</i>
<i>neutrophil%</i>				
<i>10</i>	<i>61.2±7</i>	<i>50.1±8</i>	<i>56.5±9</i>	<i>47.1±5</i>
<i>20</i>	<i>60.1±5</i>	<i>57±7</i>	<i>53.6±7</i>	<i>50.5±6</i>
<i>30</i>	<i>60.3±7</i>	<i>57.4±8</i>	<i>54.6±5</i>	<i>47.7±5</i>

Table 2. rate the stress (Swimming time) of the groups on the 30th day after injection by Vitamin C

treatment	<i>35mg.day</i>	<i>75mg.day</i>	<i>120mg.day</i>	<i>Main Effect</i>
<i>Swim time (min)</i>	<i>2.3±0.3</i>	<i>5.6±1.2</i>	<i>6.3±1.7</i>	p<0.001

DISCUSSION

(10). reported that endotoxin shock decreased the chemotaxis of lymphocytes and increased free radical production. According De la Fuente’s study, N-acetylcysteine and vitamin C prevented these changes in lymphocyte functions (11). In this study, it was also reported that lymphocytes were important targets of oxidative stress. (12). reported that immune cells needed proper antioxidant levels in oxidative stress conditions. They also reported lymphocytes stored vitamin C, and that these stores were used by macrophage (4,5).(12) showed that vitamin C prevented the entrance of Ca⁺⁺ formed as a result of oxidative stress, and by this mechanism prevented intracellular damage (13). Higher number of lymphocytes observed in the groups administrated with vitamin C (regarding control group) indicated a relationship between vitamin C and lymphocyte numbers. Also, the identification of the dose-response relation between vitamin C and lymphocytes is noteworthy.

The additional topic of investigation in our study was the effect of vitamin C on exercise. There are several past studies on this topic (14-22). In fact it is known that intake of vitamin C in amounts lower than the necessary detrimentally affects exercise performance (7). It has also been reported that intake vitamin C intake must be increased with increases in physical exercises (19). Several authors reported that there was no affect of excessive vitamin C supplementation on sportsmen or normal people (2, 16, and 18). For example, no significant benefit of vitamin C supplementation was noted in several placebo-controlled studies (17-18). In fact, in the earlier noted study using greyhounds, vitamin C supplementation actually decreased performance (12).

The other result obtained in these studies was the increase in leukocytes (especially lymphocytes) and plasma C vitamin levels after exercises (7, 23). It is thought that this result indicates the release of vitamin C from the organs as a defence because of the stress formed as a result of the exercise.

The different studies explained increasing plasma vitamin C levels as a result of increasing dehydration or the reflection of the vitamin excreted from the lymphocytes (23). Another explanation for increased plasma vitamin C after exercise is a release of vitamin C from the adrenal gland (24). In a study similar to ours, it was also reported rats fed with vitamin C and vitamin E increased growth and exercise performance (25).

The our data show clear benefits of vitamin C administration in rats. In addition, there were no differences in pathological examinations of the tissues obtained from liver, kidney, spleen, stomach, heart, tail, furry skin, brain and leg muscles both control group and experimental groups.

The results of the study must be evaluated by considering that rats are organisms that can synthesize vitamin C in their body in contrast to the human being.

CONCLUSIONS

Higher levels of lymphocyte were observed in the groups administrated with vitamin C compared to control. By increasing the dose of administrated vitamin C, lymphocyte levels and swimming time increased.

تأثير اعطاء جرعة مختلفة من فيتامين C على الاجهاد ومستوى كريات الدم البيضاء في الجرذان المختبرية

نوفل حمادي جاسم

فرع الفلسفة، كلية الطب البيطري، جامعة البصرة، البصرة، العراق

الخلاصة

أستهدفت هذه الدراسة معرفة تأثير إعطاء فيتامين سي على عدد كريات الدم البيضاء وممارسة التمرين على الجرذان المختبرية. استخدمت فيها 40 جرذ بالغ وبوزن 223 غم قسمت إلى أربع مجاميع متساوية المجموعة الأولى اعتبرت كمجموعة سيطرة إما المجاميع الثلاثة الأخرى فتم إعطاءها حامض الاسكوربيك (120 غم، 75 غم، 120 غم) يوميا على التوالي عن طريق الحقن داخل الخلب. أعطي المصل الفسلجي إلى مجموعة السيطرة بنفس الطريقة. إما وقت السباحة كان مستند على التعب في أداء التمرين، ثم أخذت عينات الدم وجربت بعد (10، 20، 30) يوم. نسبة الخلية اللمفية كانت (2 و 50) لمجموعة السيطرة في اليوم 28

مدى نسبة الخلية اللمفية (57% - 67,1%) وقت السباحة كان (0,31,6) دقيقة في اليوم (28) لمجموعة السيطرة، هذه القيمة زادت إلى (4,6 - 8,8) دقيقة. نستنتج من ذلك إن إعطاء فيتامين سي يزيد من مستوى الخلية اللمفية بالدم ويزيد وقت السباحة قبل التعب.

REFERENCES

1. Kayaalp SO. *Rasyonel Tedavi Yönünden Tıbbi Farmakoloji* 2. cilt, Hacettepe – Taş Kitapçılık, Ankara 9. Baskı 2000.
2. Arslan C , Gönül B ,Güreşçilerde C Vitamini Kullanımının Max VO₂ Ve İstirahat Kan Laktik Asit Düzeylerine Etkisi , Doktora Tezi .Ankara.1994.
3. Anbarasu K, Chandran MR. Effect of Ascorbic acid on The Immune Response of The Catfish, *Mystus gulio* (Hamilton), to Different Bacterins of *Aeromonas hydrophilia*, *Fish & Shellfish Immunology*, 2001;11(4):347-351.
4. Victor VM, Guayerbas N, Peurto M, De la Fuente M, *Free Radic Res*. 2001; 35(6):907-916.
5. Victor VM, Guayerbas N, De FM. Changes in the Antioxidant Content of Mononuclear Leukocytes from Mice With Endotoxin-Induced Oxidative Stress. *Mol Cell Biochem*. 2002; 229(1-2):107-111.
1. 6.Yavuzer S. , *Fizyoloji Pratik Klavuzu* , 2. Baskı, Antıp A. Ş. Tıp Kitapları ve Bilimsel Yayınlar Ankara.1999;20.
6. Brauns F., Saris N., How vitamins effects performance., *J.Sport.Med*. 1980; 29(4):400-404.
7. Bender AE, David A, *Vitamins in Medicine* ,William Helleman Medical Books Ltd London 1982; 2:116. *Vitamin C, Leukocytes and Exercise Performance* 105.
8. Peters EM, Goetzsche JM, Grobbelaar B, Noakes TD, Vitamin C supplementation reduces the incidence of post-race symptoms of upper-respiratory- tract infection in ultramarathon runners. *Am.J.Clin.Nutr*.1993; 57: 170-74.
9. Anbarasu K, Chandran MR, Effect of Ascorbic acid on The Immune Response of The Catfish, *Mystus gulio* (Hamilton), to Different Bacterins of *Aeromonas hydrophilia*, *Fish & Shellfish Immunology*. 2001;11(4):347-355.
10. De la Fuente M. Victor VM. Ascorbic acid and N-acetylcysteine improve in vitro the function of lymphocytes from mice with endotoxin-induced oxidative stress., *Free Radic Res*.2001; 35(1): 73-84.
11. Marshall RJ, Scott KC, Hill RC, Lewis DD, Sundstrom D, Jones GL, Harper J. Supplemental vitamin C appears to slow racing greyhounds. *J Nutr*. 2002;132(6 Suppl 2):1616-1621.
12. Öztürk G. Mulholland CW. Hannigan BM. Vitamin C Decreases Intracellular Calcium Level In Human Lymphoid Cells., *J.Physiology and Pharmacology*. 2001; 52(2):285-292.

13. **Driskell VA. Herbert WG.** Pulmonary function and treadmill performance of males receiving ascorbic acid supplements. *Nutr.Rep.İnt.* 1985; 32:443-451.
14. **Keren G. Epstein Y.** The effect of high dosage vitamin C on aerobic and anaerobic capacity.*J.Sport Med.Phy. Fitness.*1980; 20:145-148.
15. **Bailey DA. Carron AV. Teece RG.,** Effect of vitamin C supplementation upon the physiological response to exercise in trained and untrained subjects. *Int.J.Vit.Nutr.Res.*1970; 40:435-441.
16. **Gey GO. Cooper KH. Bottenberg RA.**Effect of ascorbic acid on endurance performance and athletic injury. *J.A.M.A* 1970; 211:105.
17. **Rasch PJ. Arnheim DD. Klafs CE.** Effects of vitamin C supplementation on cross country runners. *Sportärztl.Paris* 1962; 5:10-13.
18. **Guyton AC. Hall JE.** *Textbook of Medical Physiology.* 9. Edition.(Çev: H.Çavuşoğlu), İstanbul 1996; 898.
19. **Samanta SC. Biswas K.** Effect of supplementation of vitamin C on the cardiorespiratory endurance capacity of collage women. *Snipes J.*1985; 8:55-59.
20. **Chatterjee P.Chatterjee S. Das S.Maita SR.**Effects of vitamins B1 and C on the physical fitness of athletes. *Ind.J.Physiol. Pharmacol.* 1977; 21:276.
21. **Howald H. Segesser B. Körner WF.** Ascorbic acid and athletic performance. *Ann NY Acad.Sci.*1975; 258:458-464.
22. **Senger H. Israel S S.** Kozenstrationsveränderungen der Asorbinsäure İm Blut und Harn während und nach körper licher Belastung.*Med. Sports.*1975;15:105-110 .
23. **Gerster H.**The role of vitamin C in athletic performance. *J. Am. Col. Nut.*1989; 8(6): 636-643.
24. **Pablos JL, Pina illar-Patino G, Diaz-Cruz A, Avila-Gonzalez E, Guinzberg R, E.,** Effects of dietary supplementattion with vitamin C or vitamin E on cardiac lipid peroxidation and growth performance in broilers at risk of devoloping ascites syndrome. *Am J Vet Res.* 2002;63(5):673-676.