

EFFECT OF TOCOPHEROL ON PATHOGENESIS OF GASTRIC STRESS
ULCERATION AS A RADICAL SCAVENGER IN RATSGASTRİK STRES ÜLSERİ PATOGENEZİNDE TOKOFEROLÜN ETKİSİ
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ÖZET: Yakın zamanda yapılan çalışmalara göre serbest oksijen radikalleri birçok patolojik süreçte rol oynamaktadırlar. Yazarlar gastrik stres ülserlerinde serbest oksijen radikallerinin patojenik rolünü araştırmış ve tokoferolün (vitamin E) terapötik potansiyelini değerlendirmişlerdir. 40 dişi Wistar Albino rat dört eşit gruba ayrılmıştır (n=10). Grup 1 de hiçbir işlem yapılmamış, diğer gruplarda ise stress oluşturulmuştur. Grup 2 tedavi edilmemiş, Grup 3'e sadece ranitidin verilmiş, Grup 4'e ise ranitidin ve tokoferol (vitamin E) kombinasyonu uygulanmıştır. 24 saat sonra tüm hayvanlar sakrifiye edilmiş, biyokimyasal analizler, patolojik inceleme ve kemilüminesan ölçümler için örnek alınmıştır. Glukoz düzeyleri dışında biyokimyasal analizler açısından 4 grup arasında anlamlı fark bulunamamıştır. Grup 3 ve 4 'de grup 2 'ye göre anlamlı olarak düşük ülser indeksleri ve kemilüminesan değerler bulunmuş ancak aynı değerler açısından Grup 2 ve 3 arasında anlamlı bir fark bulunamamıştır. Bizim çalışmamız serbest oksijen radikallerinin ülserojenik etkilerini göstermiştir ancak tokoferolün gastrik stres ülserinde tedavi edici etkisini göstermek için daha detaylı bilgilere ihtiyaç vardır.

Anahtar kelimeler: Tokoferol, serbest oksijen radikalleri, radikal scavenger

SUMMARY: Recent studies have demonstrated that free oxygen radicals were involved in many pathologic processes and significant improvements were gained by using radical scavengers in numerous human diseases. The authors surveyed the pathogenic role of free oxygen radicals in gastric stress ulceration and evaluated the therapeutic potency of Tocopherol (Vitamin E) as a radical scavenger in the management of this disease. Forty female Wistar albino rats were randomized into four equal groups (n=10). Nothing was done to group 1. The other three groups were stressed. Group 2 was not treated, group 3 was given ranitidine alone and group 4 was administered a combination of ranitidine and tocopherol (vitamin E). Twenty four hours later, all the animals were sacrificed and samples were taken for biochemical analysis, pathologic-anatomic examination and chemiluminescence measurements. No significant differences were found among the four groups in terms of the results of biochemical analysis except the glucose levels. Significantly lower ulcer indexes and chemiluminescence values were found in both Group 3 and 4 in regard to Group 2, but the difference between Group 2 and 3 was not significant in terms of the same values. Our study has demonstrated the ulcerogenic effects of free oxygen radicals but more detailed data are required for evaluation of the ameliorating potency of tocopherol as a radical scavenger in gastric stress ulceration.

Keywords: Tocopherol, Free oxygen radicals, radical scavenger

INTRODUCTION

Free Oxygen Radicals (FORs) have emerged as a major final common pathway of tissue injury in a wide variety and recently, these toxic metabolites have been implicated in the pathogenesis of many human diseases (1,2,3,4,5,6).

These toxic oxygen metabolites are generated normally by aerobic metabolism in cells and this production can significantly increase in certain pathologic conditions. When endogenous antioxidant defense capacities are

exceeded by this oxydant flux, tissue injury occurs. FORs are capable of reversibly or irreversibly damaging compounds of all biochemical classes, including nucleic acids, protein and free aminoacids, lipids and lipoproteins, carbohydrates and connective tissue macromolecules. Most of the organs such as brain, lungs, stomach and bowels are affected by all these reactions and consequential complications generate serious vital problems (5,6,7,8,9,10,11).

Critically ill patients are at an increasing risk for abundant upper gastrointestinal bleedings caused by gastric mucosal erosions. We still do not possess enough knowledge to explain how or why this clinicopathologic process which we called "Gastric Stress Ulceration or Acute Erosive Gastritis" occurs. However, there is a widespread belief that reduced mucosal blood flow of the stomach is not

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capable of eliminating back diffused hydrogen ions effectively and thus, serial pathologic events induced by mural asidosis cause cell injury(12,13,14,15,16,17,18,19).

Itoh et al.(14) have shown the role of FORs and the effects of radical scavengers (RSs) in the gastric stress ulceration (GSU) induced by hemorrhagic shock in rats. Perry et al.(21) have obtained similar results in GSU induced by local gastric ischemia in cats.

Despite the fact that peptic ulcers are treated with an increasing success recently, there is not any satisfactory reduction in the morbidity and mortality of GSU. At the same time, RSs are used as a supplementary therapeutic agent in many human diseases(22,23).

We conducted this experimental study to find out if FORs really played a role in the pathogenesis of GSU and we also wanted to realize if tocopherol could be used as a RSs to support the current therapy of this complication.

MATERIALS AND METHODS

In this experimental study conducted at The Center for Experimental Medical Research Application (DETAM) of Istanbul University, forty female Wistar Albino rats weighing 200-240 gr were used. For hormonal standardization, the rats in the same menstrual period were selected by making vaginal smear examination.

The rats were randomly divided into four equal groups (n=10). Nothing was done in Group 1 (the control group). In the other three groups, a modified stress model that resembles the Brodies' one was performed. Of these, Group 2 (the stress group) wasn't treated, Group 3 (The ranitidine group) and Group 4 (The vitamin group) were given ranitidine immediately and at 6 hours intervals at a daily dose of 5 mg/kg (totally 1mg for each subject) intramuscularly. Group 4 was administered di-alpha tocopherol asetat (Vitamin E) immediately and at 12 hours intervals at a daily dose of 50 IU/kg (totally 10 IU for each animal) intramuscularly in addition to ranitidine.

Stress Model: The animals were fixed to do deliberately prepared (T) shaped woods by taping with medical plasters in a suitable position for easily breathing and allowed neither to move nor to feed. All the animals were sacrificed by making cervical dislocation twentyfour hours later. Blood samples were obtained through the inferior caval vein for measurement of the serum concentrations of glucose, SGOT, SGPT, amylase and alchaly phosphatase. These analysis were done at the Biochemistry Department of Taksim Hospital.

All of the stomach were removed totally and opened by cutting length of their great curvature. After washing in solution of %0.9 NaCl gently, the erosions were evaluated by macroscopic examination and the ulcer indexes were determined according to Dekansky scoring (17,24). Four tiny samples (nearly 50 mg) were taken from different anatomic parts of each gastric specimen for chemiluminescence measurement. These tissue samples were conveyed in an ice box to the Biochemistry Department of the Faculty of Medicine of Marmara University within one hour

for chemiluminescence measurement. The measurements were realized by using a scintillation counter (Tricarb 1500; Packard instruments, inc IL USA) in out of coincidence mode with a single active photomultiplier tube. The tissue samples were incubated with Kreps Hepes buffer maintained at body temperature (37°C) for 30 minutes and then gently transferred to glass scintillation vials. Luminescence was recorded at room temperature after the addition of lucigenin (bis-N-methylacridinium nitrate 0.2 mM) and Luminol (5-amino-2,3-dihydro-1,4-pyrazolinedione, 0.2 mM) and other additions (final volume 3ml). Lucigenin was used to estimate the superoxide radical and luminol was added to estimate the peroxynitrate, hydroxyl and hydrogen peroxide radicals. The counts were obtained at 1-min interval and the results were expressed as cpm/mg wet weight for the maximum points. The remnant gastric tissues were examined microscopically at the Clinic of Pathology of Haseki Hospital.

Data were presented as median and analysed at the Biostatistics Department of Cerrahpaşa Faculty of Medicine of Istanbul University. The significance of differences was estimated by Student's t test and the differences were accepted significant when the probability was less than 0.05 ($p < 0.05$).

RESULTS:

The serum concentrations were found within normal limits in Group 1. In the other groups; SGOT and SGPT concentrations rose, glucose concentrations decreased. However, no significant difference was found among these groups in terms of these concentrations except glucose ($p > 0.05$) (Table 1).

The stomachs were normal in Group 1. The ulcer indexes were significantly lower in both Group 3 and 4 in regard to Group 2 ($p < 0.05$), however, the difference between Group 3 and 4 was not significant ($p > 0.05$) (Table 2) (Figure 1).

Deep erosions including all layers of the mucosa and severe submucosal inflammations were observed by microscopic examination in Group 2. More superficial erosions and milder submucosal inflammations were seen in both Group 3 and 4, however the findings were slighter in Group 4 seeing the Group 3 (Figure 1).

The chemiluminescence values obtained by both luminol and lucigenin were significantly lower in Group 1 compared with the other Groups ($p < 0.05$). We also found significantly lower chemiluminescence values in both Group 3 and 4 by comparison to Group 2 ($p < 0.05$), but, the difference between Group 3 and 4 was not significant ($p > 0.05$) (Table 2) (Figure 2).

DISCUSSION

FORs have been implicated as important pathologic mediators in many clinical disorders. These toxic metabolites (such as; superoxide, hydroxyl, hydrogen peroxide) are exceedingly produced in ischemic conditions and cause cell injury (5,7,8,9,10).

Gastric stress ulcerations is a challenging complication characterized by subsequent massive gastrointestinal hemorrhage and which is a responsible factor for mortality in critic ill patients. Precise understanding of the pathogenesis remains unclear and certain aspects of management remain controversial, however, most authorities would generally agree on the correlation between these ulcerations and ischemic tissue injury caused by FORs (15,18,19). In ischemic conditions, the back diffusion of acid increases secondary to the disruption of mucosal barrier and cell injury occurs (5,11,19,25,26).

It has been shown that experimental stress significantly reduced gastric mucosal circulation in rats (9). There are many other studies that verify the causative effects of FORs and the benefits of RSs in GSU (17,21,22,23,24).

Tocopherol is described as a liposoluble low molecule weight radical scavenger which is protective against FORs mediated cell injury in lipid phase and moreover, its metabolites also have the same scavenging potency (21,27,28,29).

We used tocopherol as a RSs in experimental GSU in rats

The glucose levels were found significantly lower in the three experimental groups (Table 1). It could be accepted due to fasting. The chemiluminescence values concerning the Group 1 were significantly lower compared with the other three groups ($p < 0.05$) (Table 3) (Figure 2). This may be interpreted that there is a significant correlation between FORs and GSU. It has also been observed that the chemiluminescence values were lower in both Group 3 and 4 in regard to Group 2 ($p < 0.05$). There is no significant difference between Group 3 and 4. Although more abortive aspect of GSU was observed by both macroscopic and microscopic examination in Group 4 (Figure 1) (Table 2), this difference could not be evaluated statistically because of not having satisfactory quantitative data. Ultimately, we could not verify any significant advantage of the combined therapy with tocopherol in regard to the treatment with ranitidine alone.

In conclusion, our results suggest that FORs have a meaningful role in the pathogenesis of GSU, but, tracer studies and more quantitative data are required to confirm the ameliorating potency of Tocopherol in GSU as a RSs.

Table-1 The serum concentrations (average values)

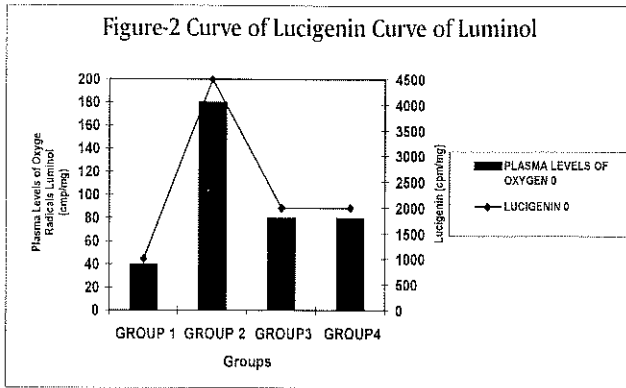
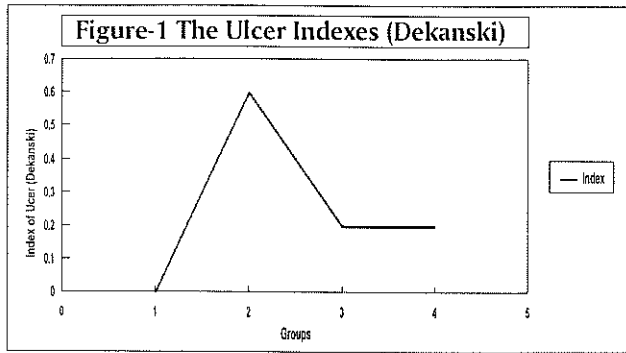
| | Group 1 | Group 2 | Group 3 | Group 4 |
|---------------------------|---------|---------|---------|---------|
| Glucose(%mg) | 109 | 48 | 47 | 35 |
| SGOT(IU/L) | 52 | 205 | 185 | 186 |
| SGPT(IU/L) | 56 | 752 | 731 | 635 |
| Alchaly Phosphatase(IU/L) | 211 | 285 | 290 | 305 |
| Amylase(IU/L) | 2.070 | 3.381 | 3.505 | 2.850 |
| Calcium(mg/dl) | 10,8 | 12,3 | 11,2 | 11,3 |

Table-2 Pathologic-anatomic findings

| | Ulcer indexes (Av.) | Depth of erosion | Submucosal inflammation |
|---------|---------------------|------------------|-------------------------|
| Group 1 | | | |
| Group 2 | 0,62 | Entire mucosa | Evident reaction |
| Group 3 | 0,21 | Half of mucosa | Moderate reaction |
| Group 4 | 0,18 | Third of mucosa | Vague reaction |

Table-3 Average values of chemiluminescence(cpg/mg)

| | Group 1 | Group 2 | Group 3 | Group 4 |
|-----------|---------|----------|----------|---------|
| Luminol | 45,28 | 189,6 | 88,34 | 86,48 |
| Lucigenin | 323,34 | 4.045,42 | 2.281,22 | 2.432,8 |



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