ROLE OF VITAMIN E IN ISCHAEMIC SKIN FLAP SURVIVAL IN RATS

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SUMMARY: The efficacy of free radical scavenger - Vitamin E (VE) in salvage of ischaemic skin flaps was studied in an experimental rat skin flap model. The animals were divided into two groups - a control group (N=15) who did not receive VE and a test group (N=15) who received VE starting one week preoperatively. No significant difference was found in flap necrosis between the control group (38.365% ± 1.398% SEM) and the test group (35.288% ± 0.635% SEM), which received therapeutic dose of VE (100 mg/kg/day. 1 mg = 1.49 I.U.). Further research is needed to establish the role of vitamin E as an agent of ischaemic skin flap salvage.

INTRODUCTION

Cell death following ischaemia is the culmination of a process involving a complex interplay of multiple local and systemic factors. Free radicals have been shown to play major role in ischaemic reperfusion injury. Superoxide radicals (O_2^-) and its derivatives, hydroxyl radicals (OH) and hydrogen peroxide (H_2O_2), have been implicated as essential mediators in many such processes. Presently, there exists a growing body of evidence showing the importance of free radicals in ischaemic skin flap necrosis. It has been shown that ischaemia induced oxygen free radicals are responsible for damage to the flap microvasculature. This has evolved into experimental use of a class of pharmacologic agents called “free radical scavengers” (FRS) to salvage ischaemic flaps. At the cellular and intracellular level, vitamin E (VE) along with glutathione peroxidase (GP) and superoxide dismutase (SOD) are the naturally active FRS protecting the cell from the harmful effects of peroxidant molecules. Many studies have shown the beneficial effects of SOD in ischaemic skin flap salvage. Glutathione and VE (alpha-D-tocopherol) have been demonstrated to be beneficial in a study which used an acute pedicled skin flap model in the rat. Our study was aimed to evaluate the role of VE in salvage of ischaemic flaps in a random flap model in rat and to establish its relationship with serum and liver VE levels in VE treated rats. Distal portion of a random flap represents an incompletely ischaemic state, with tissue damage resulting from lipid hydroperoxides and related free radical formation and is ideally suited to test the efficacy of a free radical scavenger.

MATERIAL AND METHODS

Male Wistar rats weighing between 275-375 gms. were used. The rats (N=30) were divided into 2 groups, Group A was the control group (n=15). Group B (the test group; n=15) consisted of rats started on VE (Evion paediatric drops - Merck) one week prior to surgery and continued until the 7th postoperative day. VE in oil was mixed with standard rat food so as to deliver a dose of 100 mg/kg/day orally. The rats were all individually housed in cages in an air-conditioned room. Each rat had its own source of water and standard rat food.

Surgical Technique

Rats were anaesthetised using sodium pentothal 30 mg/kg intra peritoneally. A caudally based 3 x 10 cm rectangular dorsal skin flap was raised. The raised flap was reaproximated to the skin edges with 4-0 nylon continuous sutures. No electrocautery or haemostatic agent was used.

For all animals the flaps were assessed at the end of the first postoperative week. Flap necrosis is known to stabilize at 1 week postoperatively. Under anaesthesia, the flaps were traced out on acetate paper and the area of necrosis marked. The necrotic flap area (NFA) and the total flap area (TFA) were measured using a digitizer to feed the tracings to a computer employing the ‘AUTOGRAPH’ program. For each rat the result was expressed as percentage necrosis (i.e. NFA/TFA x 100).

To document objectively the difference in the systemic levels of VE between the test and control group, serum levels (mg/100 ml) and wet liver VE concentration (mg/gm wet weight) were measured.
at the time of sacrifice of the rats using ultraviolet spectrophotofluorometry as described by Duggan. It was not feasible to submit all the rats' samples for VE analysis and hence first 5 control rats and first 10 test rats were selected consecutively from their respective groups.

**Statistical Methods**

Cochran's modified t-test was used for testing the significance of the flap necrosis data from the two experimental groups. Students t-test was used for calculating the statistical significance of the difference in serum VE and liver VE levels of the two groups. Correlation coefficient (r) was calculated using standard statistical formulae.

**RESULTS**

In the test group B the range of percentage flap necrosis (%fn) was 29.872% - 38.616% with a mean of 35.288% ± 0.635 (SEM) and SD of 2.3378%. For the control group A the range of %fn was 28.969% - 47.374% with a mean of 38.365% ± 1.398% (SEM) and SD of 5.232%. Analysis of the data revealed a slight difference in the means of two groups (Viz: X1 - X2 = 3.0769) indicating a trend towards lesser flap necrosis in the VE treated rats. However, this difference was not statistically significant (p < 0.05). On the analysis of the data on VE levels in serum and liver of rats in the two groups, we arrived at the following conclusions (Table 1).

**TABLE 1 : ANALYZED DATA ON SERUM AND LIVER VE CONCENTRATION**

<table>
<thead>
<tr>
<th>Statistical Parameters</th>
<th>% Flap Necrosis</th>
<th>Serum Level**</th>
<th>Liver Concentration**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group (N=5)</td>
<td>(mg/100 ml)</td>
<td>(mg/gm wet weight)</td>
</tr>
<tr>
<td></td>
<td>Control*</td>
<td>Test*</td>
<td>Control*</td>
</tr>
<tr>
<td>Mean</td>
<td>36.533</td>
<td>36.050</td>
<td>0.394</td>
</tr>
<tr>
<td>SD</td>
<td>7.182</td>
<td>1.924</td>
<td>0.065</td>
</tr>
<tr>
<td>SEM</td>
<td>3.591</td>
<td>0.641</td>
<td>0.033</td>
</tr>
</tbody>
</table>

* 5 rats from control group and 10 rats from test group were chosen consecutively for VE sampling.

** Mean serum and liver VE concentrations in test rats were significantly greater than control rats with p < 0.01.

* 'r' calculated for serum p < 0.01 level VE %fn and liver concentration VE % for the test group rats.

(i) Mean Serum level (mg/100 ml) in group B rats (0.596 ± 0.045 SEM) was significantly higher (P < 0.01) than group A rats (0.394 ± 0.033 SEM).

(ii) Mean liver VE concentrations (mg/gm wet weight) in group B rats (1.743 ± 0.106 (SEM) was significantly (p < 0.01) greater than mean liver VE concentration in the control rats (0.6691 ± 0.1232 SEM).

(iii) Despite the significant differences in VE levels in both serum and liver in the two groups no significant correlation could be seen between the serum level or liver concentration with regard to flap necrosis in the test group. The correlation coefficient 'r' was found to be -0.455 and -0.322 for serum level versus percentage flap necrosis and liver concentration versus percentage flap necrosis respectively. Both values fall short of the critical value of ± 0.6297. The negative values of r indicate that there is a trend towards decreasing percentage flap necrosis with increasing VE levels and vice-versa though it is not statistically significant.

**DISCUSSION**

Experiments support the contention that depleted levels of high energy phosphate stores and the build up of free radicals, during ischaemia and at the time of revascularisation, are two of the most important changes in ischaemic skin flaps which ultimately lead to flap failure. Oxygen derived free radicals are believed to be the cause of cell death by initiating a chain reaction that leads to damage of cell membranes in tissues subjected to partial ischaemia. This irreversible process occurs primarily by promoting the peroxidation of polyunsaturated fatty acids (PUFA) which are essential constituents of the cell membrane. Previous studies with free radical scavengers like super oxide dismutase, liposomal SOD and N-acetylcysteine have improved survival of island skin flaps in rabbits and rats, even after 10-11 hrs of complete ischaemia.

The superoxide radical (O2-), hydroxyl radical (OH-) and singlet oxygen (O3) act on PUFA of membrane phospholipids to disrupt their chemical structure. The hydroxyl radicals (OH-) are highly reactive and they initiate the peroxidation of polyunsaturated lipid (LH) leading to formation of fatty acyl radical (L+) and peroxyl radical (LOO*). The chain breaking reaction with VE (alpha tocopherol) is as follows:

(i) VE (alpha tocopherol) + LOO* ----> LOOH + alpha T* (VE free radical)pa

(ii) Alpha T* + LOO* ----> LOO* - alpha T.

From the above it is obvious that VE acts as a chain breaking antioxidant by trapping the chain.
propagating free radical (i.e. LOO). VE is effective in vitro, inhibits lipid peroxidation in vivo, and is a major chain breaking antioxidant in blood. In nature VE exists in a bond from with PUFA helping to stabilize the membrane structure. This role of VE in protection of cell membranes against lipid peroxidation has been well documented.

Experimental observations indicate that components within blood contribute to tissue damage in the low flow state that is found in the distal portion of a random skin flap. Red and white cells are potential sources of free radicals. Fibrins, platelet products and vasoactive amines also may be involved. It is known that in animals, supplemented VE affords protection against various drugs, metals and chemicals that can initiate free radical formation. The antioxidant property of VE helps stabilize erythrocyte membranes against oxidative stresses and improves their survival.

It has been shown in a study employing rat abdominal wall pedicled skin flaps based on the inferior epigastric artery, that VE can be of survival benefit for an acutely ischaemic flap. Our study does not confirm this finding.

It differs from the above study in two ways. We used the usually quoted therapeutic dose of alpha-D tocopherol in experimental rats, which is 100 mg/Kg/day (1 mg = 1.49 IU) in comparison to a dose of 1000 IU/Kg/day used in the previous study. We also demonstrated a direct correlation between serum levels and oral administration of the drug. It is possible that VE in therapeutic doses, is unable to salvage random ischaemic skin flaps.

The second crucial difference is in the rat model used by us. It is a random rectangular skin flap and not a pedicled island flap. The reason why this model was chosen has been outlined before.

It has been shown experimentally that xanthine oxidase (XO) may not be a major source of free radical generation during ischaemia - reperfusion injury in rat, pig or human skin. Another study has shown that although reactive oxygen species were involved in both post ischaemic necrosis of island flaps and distal necrosis of random flaps, XO was significant in producing free radicals only in the former group. This would explain why flap survival is not remarkably altered despite the membrane stabilising activity of VE which stunts the release of XO from injured endothelium.

Our results do not show any statistically significant improvement in flap survival in VE treated rats although there is a tendency towards improved flap survival. The possibility of type II error cannot be ruled out. Therefore, it would be prudent to do further studies in rats and other experimental models to clarify VE's role in ischaemia and ischaemia reperfusion injury. Future research should focus on the XO-independent pathways of free radical generation especially in the random skin flap models to further delineate the role of free radical scavengers in skin flap salvage.

REFERENCES


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