



The role of lipid peroxidation in the genesis of vasospasm secondary to subarachnoid hemorrhage.

Caner, H ; Orugkaptan, H ; Bolay, H ; Kiling, K ; Senaati, S ; Benli, K ; Ayhan, A

(Citation)

The Kobe journal of the medical sciences, 37(1):13-20

(Issue Date)

1991

(Resource Type)

departmental bulletin paper

(Version)

Version of Record

(URL)

<https://hdl.handle.net/20.500.14094/E0034080>



THE ROLE OF LIPID PEROXIDATION IN THE GENESIS OF VASOSPASM SECONDARY TO SUBARACHNOID HEMORRHAGE

Hakan CANER*, Hakan ORUÇKAPTAN*, Hayrunnisa BOLAY**, Kamer KILINÇ***
Sassan SENAATI****, Kemal BENLİ*, Ayşe AYHAN*****

Departments of Neurosurgery*, Neurology**, Biochemistry***, Radiology****,
and Pathology*****

Hacettepe University School of Medicine
Ankara - Turkey

INDEXING WORDS

lipid peroxidation; subarachnoid hemorrhage, vasospasm.

SYNOPSIS

The aim of this study is to find out the possible trigger role of lipid peroxidation in vasospasm. Haemoglobin-free washed erythrocyte membranes (erythrocyte ghost) corresponding to 2.5 mg membrane protein was mixed with 0.5 μ mol NADPH 3 μ mol ADP, 4 μ mol ferrous sulphate and injected into the cisterna magna of cats to stimulate lipid peroxidation in vivo (Group 1). The second group was injected 1ml/kg whole blood and the control group 1 ml/kg saline. angiographic studies revealed significant vasospasm in five cats in group 1. Severe vasospasm was seen in six cats in group 2. A significant increase in lipid peroxidation was observed in groups 1 and 2, compared to the control group. These results may suggest that free radical products may play an important role in the complex genesis of vasospasm in subarachnoid haemorrhage.

INTRODUCTION

The mechanism of cerebrovascular spasm secondary to subarachnoid haemorrhage is still a controversial topic in neurosurgery. Asano et al.^{1,10,17,20} suggested that cerebral vasospasm might be caused by free radical reactions and subsequent lipid peroxidation initiated by clot lysis. When bleeding occurs in the subarachnoid space, after haemolysis, oxyhaemoglobin is liberated. It's conversion to methaemoglobin releases superoxide anions and subsequently some free radical chain reactions start^{2,12,22,23}. Free radicals are important deleterious factors of ischaemia, trauma, and inflammation^{2,3,23}. Sasaki et al.¹⁸ demonstrated that intratecal injection of 15-hydroperoxyeicosatetraenoic acid caused prolonged vasospasm in dogs.

Measurement of thiobarbituric acid reactants have been the best index of lipid peroxidation. It was found that these reactants had increased in the cerebrospinal fluid of patients with vasospasm^{1,17,19}.

Received for publication : February 14, 1991

Iron is a stimulator of lipid peroxidation and this stimulation is more effective in the presence of an electron donor, such as dihydronicotinamide adenine dinucleotide phosphate (NADPH 16).

In this study; degradation products of erythrocytes such as NADPH, adenosine diphosphate (ADP), iron (Fe^{++} , ferrous state) and washed erythrocyte membranes were given into the cerebrospinal fluid in order to initiate in vivo lipid peroxidation. Since neither other erythrocyte degradation products nor other blood contents were present, their effects on the aetiology of vasospasm could be eliminated.

MATERIALS AND METHODS

Cats ranging in weight from 3-4 kg were anaesthetised with 35 mg/kg sodium pentobarbital administered intraperitoneally. An 18 gauge catheter was advanced in the right brachial artery and a baseline digital subtraction angiography was performed using 20 ml of radioopaque dye (Omnipaque 350ml/l). After the angiographic procedure, the cisterna magna was punctured by a 16 gauge needle. Erythrocyte ghosts were prepared from fresh arterial blood. Prior to injection, 1 ml. of erythrocyte ghosts; corresponding to 2.5 mg membrane proteins was mixed with 0.5 μmol NADPH, 3 μmol ADP and 4 μmol ferrous sulphate. The mixture was injected over a period of 2 minutes into the cisterna magna of seven cats as the first group. The other seven cats, in group 2, received 1ml/kg autologous blood obtained from the artery. The last seven cats served as a control group that received only 1 ml/kg saline solution to the cisterna magna. The cats were turned to head down position for 20 minutes to maintain the distribution of the blood within the basal cisterns and around the basilar artery.

A second angiography was performed 2 days later to demonstrate the presence and severity of cerebral vasospasm. Thereafter the cats were deeply anaesthetised and transcatheterial infusion was performed to wash out the blood from the cerebral circulation. Craniectomy was performed brain and the basilar artery was dissected out.

The amount of lipid peroxides on each brain homogenate were measured by means of thiobarbituric acid reactive substances using the method described by Uchiyama and Mihara ²⁴).

Light microscope examinations of the basilar arteries were performed to demonstrate the pathophysiological changes of the arterial wall using hematoxylin-eosin and elastin stains.

RESULTS

Second angiographic studies revealed significant vasospasm in five cats and moderate vasospasm in two cats in group 1 (Fig.1). Severe vasospasm was seen in six cats and no vasospasm in 1 in group 2. There was no vasospasm in the control group.

Clinical observation revealed drowsiness, hypoactivity and loss of appetite after 48 hours of injection in the cats with vasospasm (in groups 1 and 2).

The levels of lipid peroxidase in the cortex are as follows Group 1: 560 ± 69 nmol thiobarbituric acid reactive substance (TBAR)/gr wet tissue, Group 2: 470 ± 57 TBAR/gr wet tissue, Control Group: 275 ± 48 TBAR/gr et tissue.

LIPID PEROXIDATION AND VASOSPASM

The levels of lipid peroxidase in the cerebellum are, 365 ± 72 , 360 ± 55 , 340 ± 63 TBAR/gr wet tissue respectively (Fig: 2). A statistically significant increase in the levels of lipid peroxidase was found in the cerebrum of blood or erythrocyte ghosts-injected animals ($p < 0.05$). The level of lipid peroxidation was higher in erythrocyte ghosts-injected animals than blood-injected ones.

Light microscope studies revealed significant narrowing of the diameter of spastic basilar arteries with folding and corrugation of the lamina elastica. There were no significant structural changes in the muscular layer but accumulation of inflammatory cells was observed around the outer adventitia in five cats of group 1 and a few inflammatory cells were seen two others (Fig 3 and 4). But more profound inflammatory cell accumulation was seen in all animals in group 2.

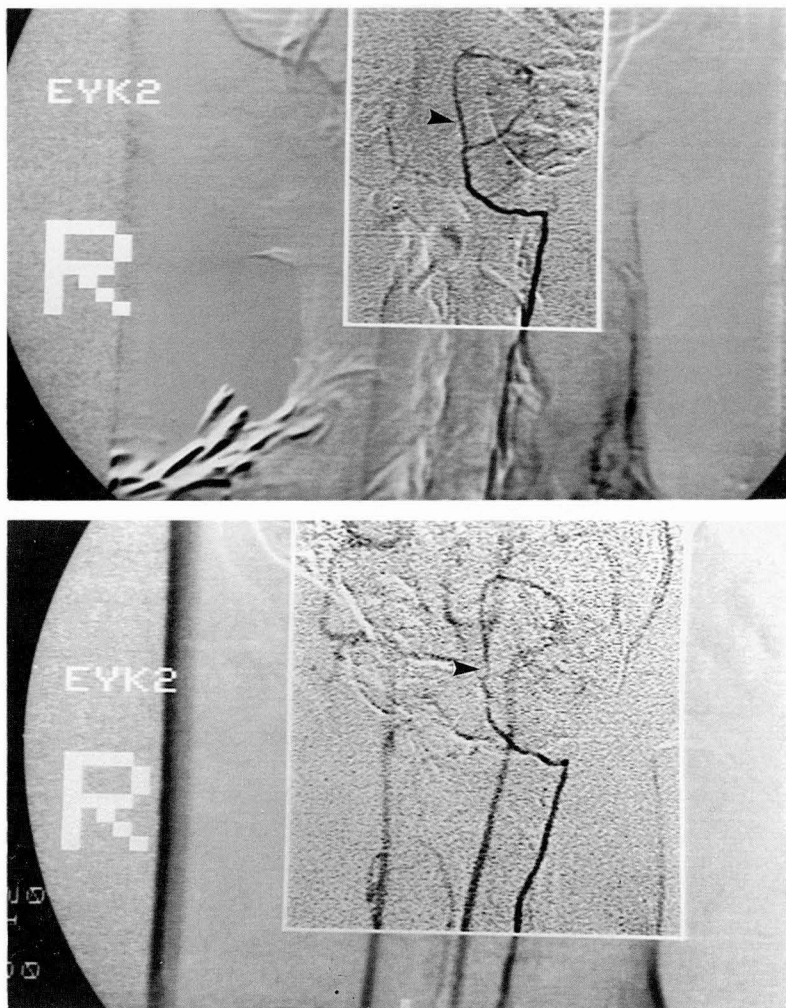


Fig. 1: Pre-injection (a) and post-injection (b) angiography of a cat in group 1. Arrow indicates basilar artery. Spasm of basilar artery is seen after injection of erythrocyte ghosts corresponding to 2.5 mg membrane proteins was mixed with 0.5 μ mol NADPH, 3 μ mol ADP and 4 μ mol ferrous sulphate.

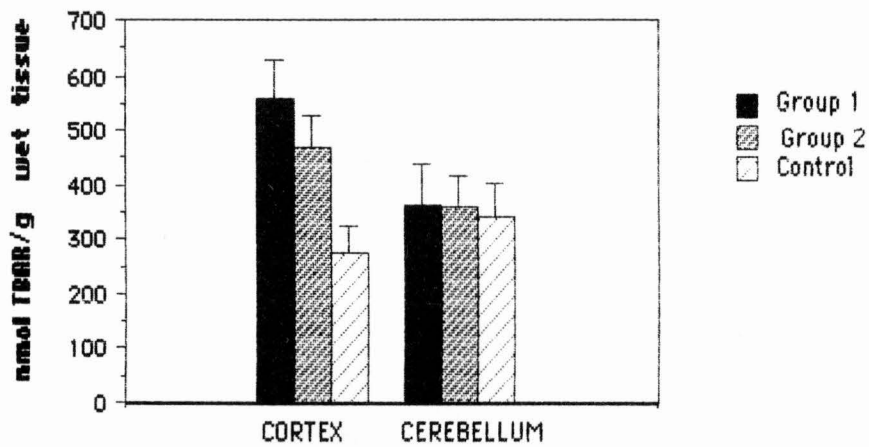


Fig. 2: The level of lipid peroxidation in the cortex and cerebellum in group 1, group 2 and the control group. TBAR: Thiobarbituric acid reactive substance. Each group has 7 cats.

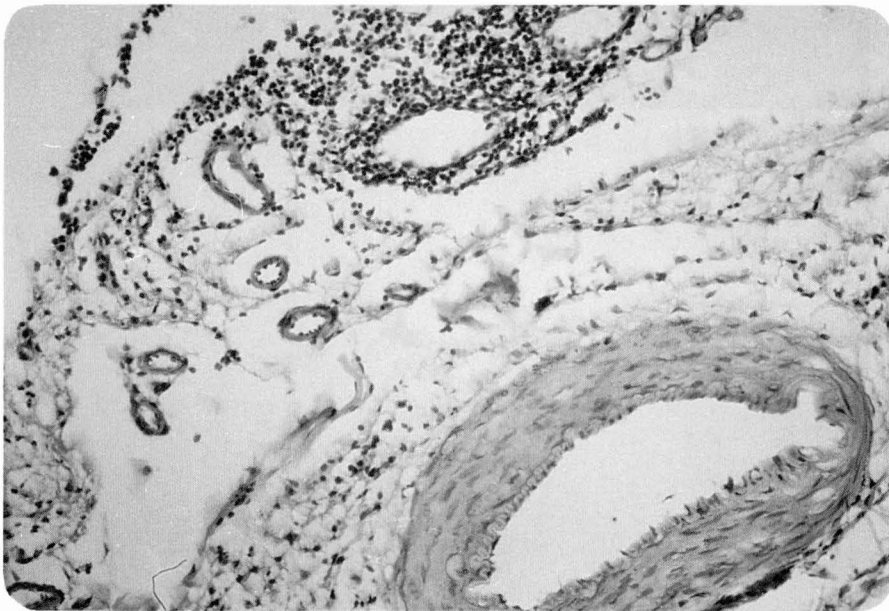


Fig. 3: (a) Light microscope appearance of a basilar artery of a cat in group 1.

LIPID PEROXIDATION AND VASOSPASM

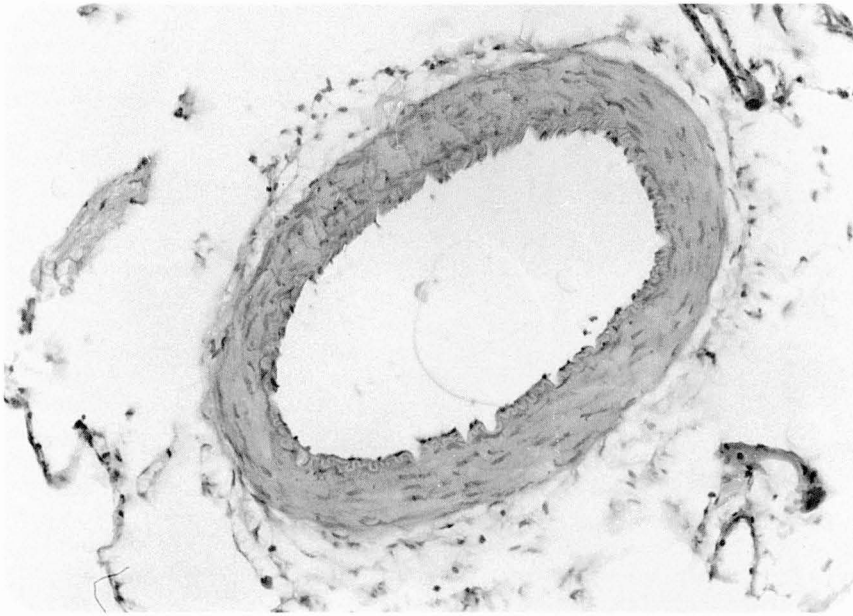


Fig. 3: (b) in the control group. (230X hemotoxylin-eosin staining). Narrowing of the diameter of spastic basilar artery with corrugation of lamina elastica. Accumulation of inflammatory cells was seen in group 1.

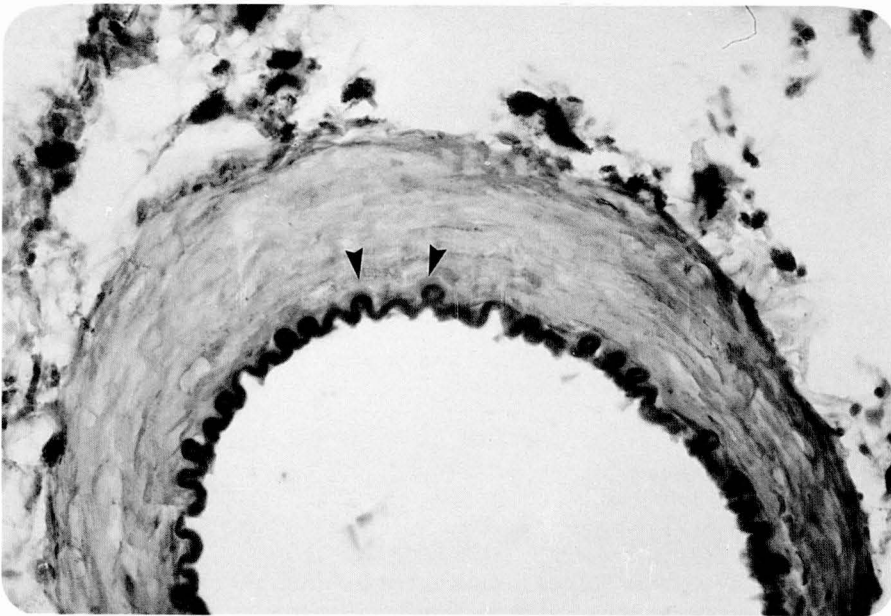


Fig. 4: Higher magnification of a basilar artery of a cat in group 1. Note corrugation of the lamina elastica (Arrows). (460X Elastin staining).

DISCUSSION

Cerebral vasospasm after subarachnoid haemorrhage is still a challenging subject, the pathophysiology of which is still unclear. So far the only agreement among different researchers is that blood is essential within the basal cisterns for the development of vasospasm after subarachnoid haemorrhage 6). But there is no agreement upon the mechanism of vasospasm. Many studies of the pathophysiology of vasospasm have already demonstrated that many different factors may contribute to this pathology 5,7,8,9,11). One of the attractive hypotheses is activation of the lipoxygenase pathway influenced by free radicals. It is suggested that free radical reactions, especially lipid peroxidation, are initiated by clot lysis in the cerebrospinal fluid. Each constituent of free radical reactions (such as lipid hydroperoxidase) has a vasoconstrictile capacity¹⁾. Furthermore they have toxic effects on the endothelium and media of the artery, and increase the permeability of the endothelium which causes penetration of plasma constituents into the arterial wall. As a result, the state of chronic vasospasm with histological changes is observed in this vasospastic artery. Although the effects mentioned above are well known and increased lipid peroxidation has been demonstrated in subarachnoid haemorrhage, it is still speculative whether the increased amount of free radical products and lipid peroxidation are trigger mechanisms to start the complex vasospasm pathophysiology in subarachnoid haemorrhage or results of cellular damage due to ischaemia secondary to vasospasm. In animal experiments injection of blood into the cisterna magna is a standard method for studying subarachnoid haemorrhage and subsequent cerebral vasospasm. In this study, we introduced a new method to study the possible role of lipid peroxidase in the genesis of vasospasm, which is based on the *in vivo* stimulation of injected lipids. Since the injected compounds are released products of degenerated erythrocytes, the chemical environment around the cerebral arteries is similar to that of subarachnoid haemorrhage.

Iron plays an important role in the initiation and propagation of free radical reactions, in which the hydroxyl radical, the most effective agent of lipid peroxidation, is produced. Therefore, most polyunsaturated fatty acids in the cerebrospinal fluid (either from injected ghosts or degenerated erythrocytes) undergo peroxidation. Supporting this idea, we observed a significant increase in the levels of lipid peroxidase of group 1 and 2. The level of lipid peroxidation was higher in erythrocyte ghosts-injected animals than blood-injected ones. This is not surprising, since erythrocyte ghosts were injected together with a radical producing system: ADP, Fe^{++} and NADPH.

The interesting point is that the levels of lipid peroxidase in the cerebellum of all groups were approximately similar. Although this observation needs more investigation for an explanation, one possibility is that the tentorium of cats is bone and this different structure may interfere with the free transportation of cerebrospinal fluid between the cerebellum and the cerebrum.

Histopathological changes observed in cats of group 1 were similar to those of group 2 except for the severity of accumulation of inflammatory cells in the adventitia layer. Although electron microscopy and transmission electron microscopy were not used for further study, the same observation

LIPID PEROXIDATION AND VASOSPASM

on the vasospastic basilar artery of cats after subarachnoid haemorrhage was mentioned with light microscopy by Seifert et al²⁰). Several investigators have hypothesized that chronic cerebral vasospasm may be related to an aseptic inflammatory response, initiated by inflammatory cells^{4,7,13,15}). But in group 1, although inflammatory cells were observed in the adventitia layer, the severity of accumulation was less than in group 2 especially in two cats but no significant difference was seen at angiography. These results suggested that inflammatory cells may not be the origin of vasospasm but may contribute to the histopathological changes.

In conclusion, although subarachnoid haemorrhage is presently considered as multifactorial, production of oxygen free radicals and subsequent lipid peroxidation may play an important role in the genesis of vasospasm in subarachnoid haemorrhage.

REFERENCES

1. Asano, T., Tanishima, T., Sasaki, T., Sano, K., in Wilkings RH ed Cerebral arterial spasm 2nd Workshop. Williams and Wilkings Co. Baltimore 190/201. Possible participation of free radical reactions initiated by clot lysis in the pathogenesis of vasospasm after subarachnoid haemorrhage.
2. Cadet, J., L., J., Neuroscience 40: 13-18 1988 Free radical mechanisms in the central nervous system an overview intern.
3. Cao, We., Carney, J., M., Duchon, a., floyd, R., A., Chevion, M., Neuroshemia and reperfusion injury to brain.
4. Chyatte, D., Rusch, N., Sundt, T., M., Jr., J Neurosurg. 59: 925-932 1983 Prevention of chronic experimental cerebral vasospasm using ibuprofen and high dose methyl prednisolone.
5. Chyatte, D., Sundt, M., T., in Cerebral vasospasm ed Wilkings Ravess press New York 1988, 357/365 Cerebral vasospasm: Evidence supporting an inflammatory etiology.
6. Handa, Y., Weir, B., K., A., Nosko, M., Mosewich, R., Tsuji, T., Grace, M., J., Neurosurg 67: 558-564, 1987. The effect of timing of clot removal on chronic vasospasm in a primate model.
7. Hoshi, T., Shimuzu T., Kito K., et al. Immunological study of late cerebral vasospasm in subarachnoid hemorage. Neural Med Chir (Tokyo) 24:647-654, 1984.
8. Kim P., Yaksh, T., L., Romero, S., D., J., Neurosurg 58: 18-26 1983 Production of uric acid in CSF after experimental subarachnoid hemorrhage is associated with blood components within the arterial wall.
9. Koide, T., Neichi, T., Takato, M., J., Pharmacol Exp Ther 221: 481-488, 1982 Possible mechanism of 15-hydroperoxy, arachidonic acid induced contractions of canine basiler artery in vitro.
10. Kontos, H., A., Wei, E., P., Christman, C., W., Levasseur, J., E., Povlishock, J., T., Elliss, E., F., Physiologist 26: 156-169, 1983. Free oxygen radicals in cerebral vascular response.
11. Lee, T., J., F., McIlhany, M., P., Sarwinski, S., J., Cereb Blood Flow Metab 4: 474-476, 1984, Eritrocyte extracts enhance neurogenic vasoconstriction of dog cerebral arteries in vitro.
12. Nagarajan, S., Theodore, D., R., Abraham, J., Balasubramanian, A., S., Neurochemical research 13: 193-201, 1988 Free fatty acids, lipid peroxidation and lysosomal enzymes in experimental focal cerebral ischemia in primates: loss of lysosomal latency by lipid peroxidation.

13. Nazar, G., B., Kassel, N., F., Povlishock, J., T., Lee, J., Hudson S., in Cerebral vasospasm ed Wilkins RH Raven Press New York 1988. 343/356. Subarachnoid hemorrhage causes adherence of white blood cells to the cerebral arterial luminal surface.
14. Nukui, H., Sasaki, H., Toyota, O., Mitsuka, S., Horikoshi, S., in Cerebral vasospasm ed Wilkins Ravess press New York 1988. 327/334. Changes of blood coagulability and effects of a platelet aggregation inhibitor in experimental subarachnoid hemorrhage in dogs.
15. Parkinson, D., Stephenson, S., Surg Neurol 21: 132-134, 1984. Leukocytes and subarachnoid hemorrhage.
16. Poli, G., Dianzani, M., U., Cheeseman, K., H., Biochem J., 227: 629-638, 1985. Speration and characterization of the aldehydic products of lipid peroxidation stimulated by carbon tetrachloride on ADP-iron in isolated rat hepatocytes and rat liver microsomal suspension.
17. Sano, K., in Cerebral Vasospasm ed. Wilkings JR., Raven Press. New York 1988, 285/295 Cerebral vasospasm as a deficiency syndrome.
18. Sasaki, T., Wakai, S., Asano, T., Watanabe, T., Kirino, T., Sano, K., J., Neurosurg. 54: 357-365 1981. The effect of a lipid hydroperoxide of arachidonic acid on the canine basiler artery.
19. Sasaki, T., asano, T., Sano, K., Neurol Med Chir (Tokyo) 20: 145-153, 1980 Cerebral vasospasm and fre radical reactions.
20. Seifert, V., Stolke, D., Reale, E., Acta Neurochir (Wien) 100: 164-171, 1989. Ultrastructural changes of the basiler artery following experimental subarachnoid haemorrhage a morphological study on the pathogenesis of delayed cerebral vasospasm.
21. Seifert, V., Stolke, D., Kunz, U., Resch, K., Neurosurgery 23: 313-321, 1988. Influence of blood volume on cerebrospinal fluid levels of arachidonic acid metabolities after subarachnoid haemorrhage. Experimental study on the pathogenesis of cerebral vasospasm.
22. Southorn, P., Powis, G., Mayo clin prc 63: 381-388, 1988 Free radicals in medicine. I. Chemical nature and biologic reaction.
23. Southorn, P., Powis, G., Mayo clin. proc 63: 390-408, 1988. Free radicals in medicine. II.involvement in human disease.
24. Uchiyama, M., Mihara, S., Anal Biochem. 86: 271-278 Determination of malonaldehyde precursor in tissues by thiobarbituric acid test.