EFFECTS OF DEFEROXAMINE OF LIPID PEROXIDATION IN FOCAL CEREBRAL ISCHAEMIA AND REPERFUSION

(PART II)

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SUMMARY:

In a reversible middle cerebral artery occlusion model in rats, the possible efficacy of deferoxamine on radical level and infarct size was investigated.

Deferoxamine was injected intravenously immediately after the occlusion at a doses of 100 mg/kg. It significantly lowered lipid peroxidation in the focal ischemic or reperfused rat brain ($p<0.03$), reduced the rate of infarction areas ($p<0.05$) and improved neurological grades ($p<0.05$).

KEY WORDS:

Cerebral ischaemia, deferoxamine, free radicals, iron, lipid peroxidation, rat.

INTRODUCTION:

The acute stage of cerebral ischaemia is of therapeutic interest for the high incidence of infarction in stroke patients and prevention of tissue damage by iatrogenic temporary occlusion of a vessel during neurosurgical intervention.

In recent years, it has been established that free radical induced peroxidation plays a major role in the tissue damage seen in ischaemia, reperfusion and traumatic injury (5, 9, 10, 16, 17, 20, 22, 25). A major source of hydroxyl radical in biological systems is the reactive sequence of hydroxyl radical with hydrogen peroxide in the presence of iron ion; this reaction has been called the iron catalysed Haber-Weiss reaction or the superoxide driven Fenton reaction (13):

$$O_2^- + Fe^{3+} \longrightarrow Fe^{2+} + O_2$$

$$H_2O_2 + Fe^{2+} \longrightarrow OH\cdot + Fe^{3+} + OH^-$$

$$O_2^- + H_2O_2 \longrightarrow OH\cdot + OH^- + O_2$$

In addition to the oxygen free radicals generated by ischemic or traumatic processes, the transfer of electrons between free ions of metals such as copper and iron can also initiate the formation of oxygen radicals (10, 20). Some investigators have shown that addition of exogenous iron is enough to initiate lipid peroxidation (20, 26).

The brain is known to be rich in iron content and a linear relationship had been found between endogenous iron content of brain and the ability to produce lipid peroxides (2,20,26,27).

Deferoxamine (DFO) is an iron chelating agent used in iron overload, especially in Thalassemia. Several experimental studies have shown that it reduced the rate of lipid peroxidation in rat or rabbit heart during ischaemia and reperfusion (22, 25). It also has been experienced in brain oedema in cats, in vasospasm prophylaxis in a rabbit model of subarachnoid haemorrhage, exogenous iron induced lipid peroxidation in rats, recovery of cerebral blood flow and function in cardiac arrest in dogs or in some in vitro studies (6, 8, 12, 20, 23). However, DFO has not previously been investigated in focal cerebral ischaemia.

A reversible middle cerebral artery occlusion (MCAO) model was chosen in order to investigate the possible efficacy of DFO on radical level and infarct size in the treatment of focal cerebral ischaemia and reperfusion injury.

MATERIALS and METHOD:

Animal Groups Studied: Thirty-one male Wistar rats weighing 200-+20 gr were used. They were divided into MCAO+DFO and reperfusion+DFO groups. The MCAO+DFO group consisted of 18 rats: 10 animals for lipid peroxide assays and 8 for quantification of the infarct size and neuropathology. The Reperfusion+DFO group consisted of 13 animals; 5 for biochemical assay and 8 for quantification of the infarct size and neuropathology. Previously reported MCAO and Reperfusion groups served as control groups (21).

Surgical Procedure: The surgical method for MCAO and reperfusion has been reported previously (21). Intravenous injection of DFO (Desferal 500 mg-Ciba-Geigy) (100 mg/kg) was performed immediately after MCAO via the dorsal penis veins.

Neurological examination: Neurological examination was performed at the second and 24th hours prior to sacrificing the rats. A neurological grading system described by Bederson (3) was used.

Tissue preparation and lipid peroxide assay: After one hour of survival, 5 rats from each group and after two hours of survival, 5 other rats from each group, were sacrificed by decapitation. Samples from the ischemic right frontoparietal cortex and the lateral segment of the caudate putamen and its corresponding area at the left hemisphere, were prepared and transported in a cold chain as described previously (21). Lipid peroxidation was measured by a thiobarbuturic acid test (21).

Quantification of Ischemic Brain Damage: Five rats from each group were sacrificed at 24 hours. After decapitation, the brains were immediately and carefully removed from the skulls. The position of the intraluminal thread was confirmed each time. Within 2 minutes of sacrifice a 2mm thick coronal slice was made at 5mm from the frontal pole, stained with TTC and later analyzed for the quantification of damage and histology (21).

Neuropathology: Three rats from each group were sacrificed at the second hour of occlusion; brains were immediately removed and stored in 10 % formalin. With the addition of the TTC immersed slices used in quantification of the infarction area, all specimens were embedded in paraffin wax and sectioned. 5 mmicron thick sections were stained with hematoxylin and eosin and examined by light microscopy.

Statistical Analysis: Data is presented as real or mean [+- standard deviation (SD)]. Mann-Whitney U and Wilcoxon-Signed Ranks tests were used to assess significance and ($p<0.05$) was considered statistically significant.

RESULTS :

All rats exhibited right Horner's syndrome. At two hours, only 2 animals in the reperfusion+DFO group showed mild paresis of the right upper extremity. In the MCAO+DFO group, left hemiparesis with the upper extremity dominant was observed. At 24 hours, reperfused animals recovered completely and the MCAO+DFO group showed improvement. The neurological grades of the DFO administered group were better than those of the control group ($p<0.05$) (Table 1).

The position of the intraluminal thread was confirmed in each case of the MCAO+DFO group, and the tip was found to be at the

anterior cerebral artery in all. No subarachnoid haemorrhage was observed in any case.

TABLE 1

GROUP	NO	2hrs	NEURO. GRADE 24 hrs	ISCHAEMIA PERCENT
MCAO	1	3	1	28
"	2	3	1	29
"	3	3	2	34
"	4	2	1	26
"	5	2	0	24
REPERF.	1	1	0	0
"	2	1	0	0
"	3	1	0	0
"	4	1	0	0
"	5	2	0	0
MCAO+DFO	1	2	0	19
"	2	3	1	20
"	3	2	1	19
"	4	2	0	18
"	5	2	1	21
REPERF+DFO	1	1	0	0
"	2	0	0	0
"	3	0	0	0
"	4	1	0	0
"	5	0	0	0

In the control groups, MDA levels at the ischemic or reperfused areas were significantly higher than the corresponding area in the left hemisphere ($p<0.03$); in the DFO administered groups, there was no significant difference between frontoparietal cortex and lateral caudate putamen of both hemispheres ($p<0.7$) (Table 2).

The mean value of MDA of the control groups at the ischemic or reperfused areas was 11.93 (SD: 2.06) nmol/gr (n=15) and DFO lowered this rate to 8.09 (SD: 1.89) nmol/gr (n=15), which means a 32.2 % reduction ($p<0.03$). (Figure 1).

DFO, while lowering the MDA levels also reduced the ischemic area ratio ($p<0.05$) (Figure 2 and 3 A-B) and improved neurological grades (Table 1).

TABLE 2

GROUP	NO	TIME (hr)	NEURO. GRADE 2hrs	MDA RIGHT	LEVEL LEFT
MCAO	1	1		15.17	12.66
"	2	1		12.90	5.88
"	3	1		12.13	10.82
"	4	1		14.32	12.05
"	5	1		15.64	8.06
"	1	2	3	10.68	3.58
"	2	2	3	11.66	8.37
"	3	2	3	13.33	8.00
"	4	2	2	10.09	8.67
"	5	2	2	12.58	8.47
REPERF.	1	2	1	8.28	7.51
"	2	2	1	9.53	8.62
"	3	2	1	9.84	5.02
"	4	2	1	10.48	8.53
"	5	2	2	12.35	6.91
MCAO+DFO	1	1		10.35	12.19
"	2	1		9.01	14.55
"	3	1		11.00	7.33
"	4	1		9.54	9.56
"	5	1		8.61	9.01
"	1	2	1	4.99	8.95
"	2	2	2	6.84	7.28
"	3	2	2	8.82	6.38
"	4	2	2	5.33	4.41
"	5	2	2	6.24	6.00
REPERF+DFO	1	2	0	7.19	10.90
"	2	2	1	9.96	15.33
"	3	2	0	7.47	10.09
"	4	2	0	5.86	9.12
"	5	2	1	10.05	16.55

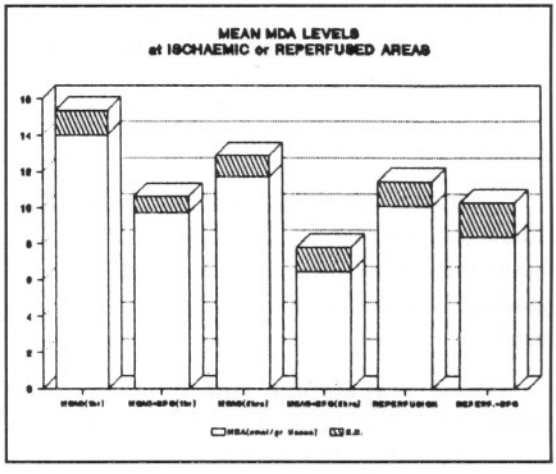


Fig. 1 :

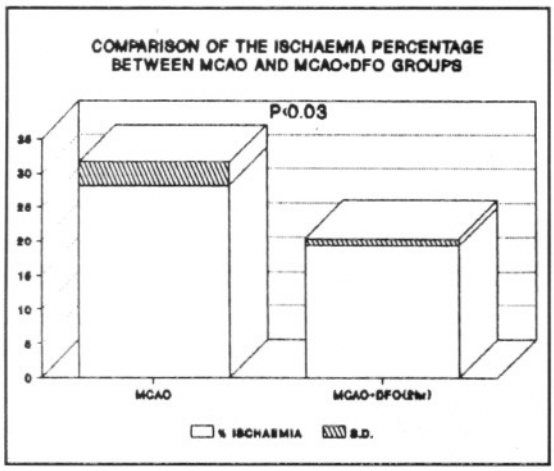
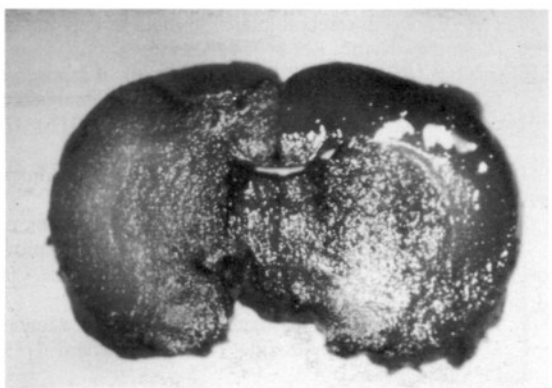


Fig. 2 :



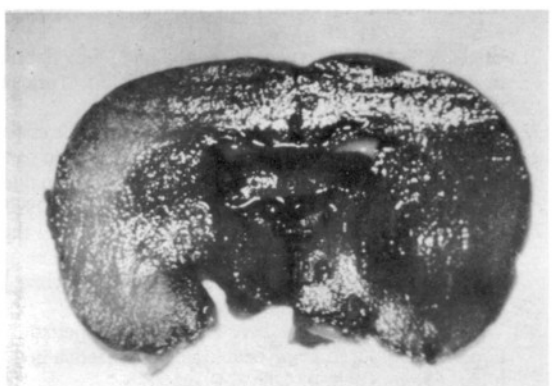


Fig. 3 : Photographs of TTC immersed coronary slices at 5 mm to the fontal pole in control (A) and treated (B) rats following MCAO. Unstained areas reveals infarcted areas.

On histological examination of the reperfusion+DFO group at the 24.th hour, as compared to the control group, resolution of the oedema was seen. In the MCAO+DFO group, both at the second and 24.th hour, the ischemic findings were similar to the histology of the control groups (21).

DISCUSSION:

Our aim was to investigate the efficacy of DFO on lipid peroxidation and infarct size in focal cerebral ischaemia and reperfusion injury. To achieve this goal, we used a rat model of intraluminal MCAO without craniectomy in order to minimize the artifacts complicating physiological and biochemical data analysis.

The auto-oxidation of unsaturated fatty acids has been suggested as one of the primary factors in the acute stage pathology of the ischemic or hypoxic brain (14). In our previously reported study, we also found a significant positive correlation between lipid peroxide level and infarction rate in focal cerebral ischaemia (21).

Ischemic infarction causes decompartmentalization of iron compounds present in the brain tissue followed by the release of free metal, which, undergoing univalent redox reaction, is supposed to initiate and propagate lipid peroxidation into the lipid rich neural parenchyma by stimulation of oxidative free radical formation (4, 8).

During reoxygenation, a burst of superoxide radical production occurs as a by product of the oxygenation of hypoxanthine and arachidonate metabolism via cyclooxygenase and lypooxygenase (6, 22, 24, 25, 28). Superoxide radicals promote the release of iron from ferritin, probably causing the increase in brain concentrations of free iron that occur during reperfusion after cardiac arrest. Free iron catalyses the conversion of superoxide radicals into highly reactive hydroxyl radicals; free iron is also necessary for the initiation and propagation of membrane lipid peroxidation, which does not occur if iron is absent or chelated (6).

Oxygen free radical processes have an intracellular location and treating agents must cross cell membranes (7, 12). DFO is an extensively used drug in the treatment of iron load. It has a high affinity for iron, especially in the ferric form (22). The molecular weight is low and it is known to cross the cell membranes and blood-brain barrier (11, 15, 23, 25).

Iron chelated by DFO is chemically inert and cannot participate in the Fenton or Haber-Weiss reactions (22). Other chelators such as EDTA or ADP have much lower affinity constants for iron and do not prevent the iron from catalyzing the Haber-Weiss or Fenton reactions. They may even augment the reactions because of the transient formation of ferric ions in the transition between bound and low bound states (22). DFO does not allow the availability of ferric ions because of its very high affinity in this state (1). DFO can also act as a direct scavenger of superoxide anions, which are then not available for conversion to hydrogen peroxide, the substrate in the Fenton reaction (18, 19).

The present study showed that DFO significantly lowered lipid peroxidation in focal ischemic or reperfused rat brain, reduced the rate of infarction areas and improved neurological grades. However, more studies concerning parametric time course, dose response and toxicity analysis are needed.

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REFERENCES :

1. Ambrosio G, Zweier JL, Jacobus WE, et al. Improvement of postischemic myocardial function and metabolism by administration of deferoxamine at the time of reflow: the role of iron in the pathogenesis of reperfusion injury. Circulation 76: 906-915, 1987
2. Aruoma OI, Halliwell B: The iron binding and hydroxyl radical scavenging of anti-inflamatory drugs. Xenobiotica 18: 459-470, 1988
3. Bederson JB, Pitts LH, Tsuji M, et al: Rat middle cerebral artery occlusion: Evaluation of the model and developement of a neurological examination. Stroke 17: 472-476, 1986
4. Biemond P, Swaak AJG, Beindorf CM, et al: Superoxide dependent and independent mechanisms of iron mobilization from ferritin by xhantine oxidase. Biochem J 239: 169-173, 1986
5. Braughler JM, Hall ED: Central nervous system trauma and stroke: I: Biochemical considerations for oxygen radical formation and lipid peroxidation. Free Radic Biol Med 6: 289-301, 1989
6. Cerchiari EL, Hoel TM, Safar P, et al: Protective effects of combined superoxide dismutase and deferoxamine on recovery of cerebral blood flow and function after cardiac arrest in dogs. Stroke 18: 869-878, 1987
7. Chan PH, Chen SF, Yu ACH: Induction of intracellular superoxide radical formation by arachidonic acid and by polyunsaturated fatty acids in primary astrocytic cultures. J Neurochem 50: 1185-1193,1988
8. Ciuffi M, Gentilini G, Franchi-Michelli S, et al: Lipid peroxidation induced "in vivo" by iron carbohydrate complex in the rat brain cortex. Neurchem Res 16:43-49, 1991
9. Hall ED, Braughler JM: Central nervous system trauma and stroke: II: Physiological and pharmacological evidence for involvement of oxygen radicals and lipid peroxidation. Free Radic Biol Med 6: 303-313, 1989
10. Halliwell B, Gutteridge JMC: Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts. Arch Biochem Biophys 246: 501-514, 1986
11. Ikeda Y, Ikeda K, Long DM: Protective effect of iron chelator deferoxamine on cold induced brain edema. J Neurosurg 71: 233-238, 1988
12. Ikeda Y, Ikeda K, Long DM: Comparative study of different iron chelating agents in cold induced brain edema. Neurosurgery 24: 820- 824, 1989
13. Ikeda Y, Long DM: The molecular basis of brain injury and brain edema: The role of oxygen free radicals. Neurosurgery 27: 1-11, 1990
14. Imaizumi S, Kayama T, Suzuki J: Chemiluminescence in hypoxic brain: The first report-Corrolation between energy metabolism and free radical reaction. Stroke 15: 1061-1065, 1984
15. Imaizumi S, Tominaga T, Uenohara H Yoshimota T, Suzuki J, Fujita Y: Initiation and propagation of lipid peroxidation in cerebral infarction models: Experimental studies. Neurol Res 8: 214-220,1986
16. Imaizumi S, Woolworth V, Fishman RA, et al: Liposome entrapped superoxide dismutase reduces cerebral infarction in cerebral ischaemia in rats. Stroke 21: 1312-1317, 1990
17. Kontos HA: Oxygen radicals in cerebral vascular injury. Circ Res 57: 508-516, 1985
18. Menasch P, Grousset C, Gandual J, et al: Prevention of hydroxyl radical formation: A critical concept for improving cardioplegia. Protective effects of desferoxamine. Circulation 76 (Supp): 180- 185, 1988
19. Sinaceur J, Ribiere C, Nordmann J, et al: Desferrioxamine. A scavenger of superoxide radicals? Biochem J 33: 1693-1694, 1984
20. Subbarao KV, Richardson JS: Iron dependent peroxidation of rat brain: A regional study. Journal of Neuroscience Research 26:224- 232, 1990
21. Ünal ÖF, Döşoğlu M, Diler AS, et al: Lipid peroxidation in focalcerebral ischaemia and reperfusion. Turkish Nurosurgery (In Press)
22. Van Jaarsveld H, Potgieter GM, Kuyl JM, et al: The effect of desferal on rat heart mitochondrial function, iron content, and xantine dehydrogenase/oxidase conversion during ischaemia- reperfusion. Clin Biochem 23: 509-513, 1990
23. Vollmer DG, Hongo K, Ogawa H, et al. A study of the effectiveness of the iron chelating agent deferoxamine as vasospasm prophylaxis in a rabbit model of subarachnoid haemorrhage. Neurosurgery 28: 27-32, 1991
24. Watson BD, Busto R, Goldberg WJ, et al: Lipid peroxidation in vivo induced by reversible global ischaemia in rat brain. J Neurochem 42: 286-274, 1984
25. Williams RE, Zweier JL, Flaharty JT: Treatment with desferoxamine during ischaemia improves functional and metabolic recovery and reduces reperfusion-induced oxygen radical generation in rabbit hearts. Circulation 83: 1006-1014, 1991
26. Willmore LJ, Rubin JJ: Effects of antiperoxidants on FeCl2-induced lipid peroxidation and focal edema in rat brain. Experimental Neurology 83: 62-70, 1984
27. Willmore JL, Triggs WJ, Gray JD: The role of iron induced hippocampal peroxidation in acute epileptogenesis. Brain Research 382: 422-426, 1986
28. Zaleska MM, Floyd RA: Regional lipid peroxidation in rat brain in vitro: Possible role of endegenous iron. Neurochem Res 10: 397-410, 1985