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OXYGEN FREE RADICAL GENERATION AND LIPID PEROXIDE LEVELS IN ACUTE BRAIN INJURY

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Objective: Determine the role of oxygen free radical (OFR) generation and lipid peroxidation in acute brain injury patients.

Setting: Postgraduate Institute of Medical Education and Research, Chandigarh, India.

Subjects: Seventy five consecutive severe closed head injury patients, presenting within 4 hours of trauma and not requiring surgery.

Design: Blood samples obtained at admission, after 48 hours and 4-5 days were analyzed for OFR activity in neutrophils by chemoluminescence (CL) and lipid peroxide malonaldehyde (MDA) levels.

Results: The CL values showed a peak value within 4 hours, remained elevated after 48 hours and returned to normal 4-5 days after trauma. MDA levels showed maximum elevation within 4 hours, which had reduced at 48 hours and returned to normal after 4-5 days of trauma. CL levels showed a significant correlation with MDA levels at all times.

Conclusion: Marked elevation of CL response, indicative of OFR generation continued up to 48 hours of trauma. MDA levels, indicative of cellular damage, were also elevated up to 48 hours after trauma. The correlation between CL response and MDA levels indicate their synergistic role in OFR mediated cell membrane damage following head injury.

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Oxygen free radicals (OFR) are organic compounds that possess an unpaired (or free) electron[1]. When one, two or

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three electrons react with oxygen, the result is the formation of superoxide radical (O_2.-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH.) respectively. Normally the cells are well protected against these highly reactive and damaging intermediaries by a number of antioxidants, which include superoxide dismutase (SOD) and glutathione peroxidase. OFR are capable of reversibly or irreversibly damaging compounds of all biochemical classes, including nucleic acids, proteins, lipids, carbohydrates and connective tissue macromolecules[2]. Exposure of the cell membrane and vascular endothelium to OFR stimulates lipid peroxidation which proceeds through a free radical mediated chain reaction.

OFR mediated mechanisms have increasingly been suggested to play an important role in the primary and secondary process of acute brain injury[3,4]. Recent experimental studies have demonstrated that OFR may be important mediators of pathological processes as the molecular basis of neural injury[5-8].

The present study was undertaken to determine the role of OFR generation and lipid peroxidation in patients with acute brain injury.

METHODS

Seventy five consecutively admitted patients with severe closed head injury (GCS < 8), in the age group of 25-45 years, presenting within four hours of trauma and not requiring surgery, were studied. Patients with shock, alcohol intoxication, overt sepsis, associated significant musculoskeletal injury and those receiving corticosteroids were excluded.

Blood samples were obtained at the time of admission, after 48 hours and 4-5 days for measuring OFR activity in neutrophils by chemoluminescence (CL) and serum lipid peroxides, with the end product as malonaldehyde (MDA), by the method of Yagi et a[19,10]. CL was measured in all the patients while MDA levels could be done in 35 patients only. Ten normal controls were also studied.

RESULTS

The generation of OFRs in neutrophils measured by CL, using latex as the triggering agent, showed a peak response within 4 hours of sustaining trauma with a mean value of 98.94 (range 34-196) x 103 cpm million-1 cells. After 48 hours, the CL response decreased to a mean value of 57.84 (range 12-93) x 103 cpm million-1 cells. The CL response was suppressed, nearer control levels, after 4-5 days with a mean value of 15.36 (range 4-28) x 103 cpm million-1 cells (Table 1).

Table 1. Peak CL response in Neutrophils in response to triggering with latex in severe head injury (GCS < 8)

Time after injury	Head injury (mean SE)* (Range) n=75		Controls (mean SE)* (Range) n=10		'p'
< 4 hours	98.94 (34 - 196)	0.001 >	0.59 9.9 (8.7 - 10.5)		11.4
48 hours	57.84 (12 - 93)	0.001 >	0.68 9.2 (8.2 - 10.3)		3.06
4 - 5 days	15.36 (4 - 28)	0.01 >	0.68 9.2 (8.3 - 9.78)		0.707

* cpm x 10^3 million^{-1} cells

Serum lipid peroxide (MDA) levels were estimated as indicative of cellular damage in the brain. The MDA levels were highest within 4 hours of sustaining trauma with a mean value of 7.52 (range 5-13) nmoles ml^{-1}. These levels decreased to a mean value of 4.82 (range 3-7) nmoles ml^{-1} after 48 hours and returned to normal after 4-5 days with a mean value of 2.49 (range 1.1-3.8) nmoles ml^{-1} (Table 2).

Table 2. Serum lipid peroxide (MDA) levels in severe head injury (GCS < 8)

Time after injury	Head injury (mean ± SE)* (Range)	Controls (mean ± SE)* (Range)	'p'
	n=35	n=10	
< 4 hours	7.52 ± 0.15 (5 - 13)	3.25 ± 0.32 (2.9 - 3.5)	0.001 >
48 hours	4.82 ± 0.56 (3 - 7)	3.12 ± 0.19 (2.23 - 3.5)	0.01 >
4 - 5 days	2.49 ± 0.56 (1.1 - 3.8)	3.14 ± 0.13 (2.65 - 3.44)	0.01 >

* nmoles ml^{-1}

There was a significant correlation of CL response with MDA levels in these patients within 4 hours ($r = 0.4$; $p < 0.01$), at 48 hours ($r = 0.75$; $p < 0.001$) and at 4-5 days ($r = 0.9$; $p < 0.001$) following trauma.

DISCUSSION

Concepts of the process of posttraumatic brain injury are constantly changing with increasing experimental evidence implicating OFR mediated lipid peroxidation and destruction of cell membranes. The central nervous system (CNS) is especially prone to OFR induced tissue damage as (a) the membrane lipids are especially enriched in cholesterol and polyunsaturated fatty acids, (b) the neurones contain large numbers of lysosomes, (c) the brain is poor in catalase activity and has only moderate amounts of SOD and glutathione peroxidase, (d) the brain is rich in iron - the most likely initiator of OFR in brain injury, and (e) the presence of high concentration of ascorbic acid in both gray and white matter of the CNS, which itself generates large quantities of OFR in the presence of copper and iron[6].

OFR generation continues for at least one hour, and possibly longer, after brain injury and induces sustained arteriolar dilatation, reduced vasoconstrictor responses to arterial hypotension, reduced vessel wall oxygen consumption, focal lesions in endothelium and smooth muscles of blood vessels, and a decrease of electrical resistance of pial venules resulting in increased endothelial cell and ionic permeability causing vasogenic brain oedema. These abnormalities can be inhibited by pretreatment with indomethacin or SOD[7,11,12].

Muizelaar presented evidence that ischaemia-reperfusion injury probably played an important role in the pathogenesis of head injury in human being and that it was active through OFR mediated mechanisms[13].

The precise site of OFR generation in CNS is unknown. The capillaries, brain parenchyma, meninges and formed elements of blood are all potential sources. Kontos and Wei showed that OFR were produced not only in walls of central blood vessel but probably also by leukocytes and macrophages that accumulate in the brain, starting 3-4 hours and reaching a peak at 24 hours, after experimental brain injury[14]. Neutrophils are a potential source of OFR possessing reduced NADPH-oxidase on their surface which is responsible for the production of superoxide (O_2) radical[15,16]. Evidence of recruitment of neutrophils into regions

of traumatic brain injury resulting in endothelial damage by OFR generation has been provided by several workers[17-20]. Zhuang et al demonstrated significantly greater accumulation of neutrophils in both injured and uninjured hemispheres in a porcine model of focal cryogenic brain injury and haemorrhagic shock[21]. They hypothesized an association between neutrophil accumulation and secondary brain injury, possibly through lipid peroxidation. In the present study, CL response of peripheral blood neutrophils from patients with acute brain injury was at peak level within the first 4 hours following trauma. Since the CL response varies directly with the amount of OFR generation, these activated neutrophils produce increased quantities of OFRs when appropriately challenged.

Exposure of the cell membrane to excessive amounts of OFRs stimulates the process of lipid peroxidation which proceeds through a free radical mediated chain reaction to produce a variety of products, including malonaldehyde (MDA)[22,23]. The lipid peroxides formed in brain tissue may easily enter the blood stream following post injury disruption of the blood brain barrier. Measurements of MDA in serum could, therefore, be of clinical and prognostic significance. Lipid peroxides (MDA) in the present study showed the highest levels within 4 hours of trauma, gradually reducing 48 hours later and returning to normal by 4-5 days.

There was a significant correlation between CL responses with MDA levels at all time intervals in the head injury patients studied, indicating their synergistic role in membrane damage.

Functional recovery from neural injury could be facilitated by a therapy that interrupts the molecular processes involved in OFR mediated secondary destruction of the neurones. Numerous pharmacological agents which have shown significant antioxidant efficacy in experimental and clinical studies include 21-aminosteroids, SMA-SOD, SOD, PEGSOD, methyl prednisolone. Vitamin E, selenium and deferoxamine[5,13,17,24-29]. The blunting of neutrophil's response by either 21-aminosteroids or 2-methyl aminochromans may decrease OFR levels and thus decrease secondary brain injury.

CONCLUSION

Further studies on OFR generation and lipid peroxidation in the clinical setting and therapeutic intervention with OFR scavengers and antioxidants could help prevent neuronal damage and improve the outcome of brain injured patients.

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