

Ozone therapy in Ethidium Bromide induced demyelination in rats possible protective effect

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introduction

- Multiple sclerosis is a complex disease with several pathophysiological processes: inflammation, demyelination, oxidative stress, axonal damage, and repair mechanisms that participate in this disorder. Since (ROS) play a pivotal role in the initial phase as well as the chronic stage of MS, antioxidant therapy might be an attractive approach to limit disease progression.

- Notably there are number of disadvantages to the use of exogenous antioxidant for MS treatment ,as most antioxidant compounds do not efficiently cross the BBB and have a narrow therapeutic window
- Steroids are postulated to positively impact MS at the molecular level by many mechanism including reduction of adhesion molecules ,pro-inflammatory cytokines and circulating T-lymphocytes .

- Ozone is a paradoxical inducer of antioxidant enzymes ,an immunomodulator ,metabolic enhancer ,an inducer of endothelial nitric oxid synthetase ,and possibly an activator of stem cells with subsequent neovascularization and tissue reconstruction .
- It is considered to be of great value in management of MS

Aim of the work

- The study aimed to investigate the possible protective effect of ozone therapy in Ethidium Bromide EB induced demyelination in rats either alone or in combination with corticosteroids in order to decrease the dose of steroid therapy

Material and Methods

- Rats were anesthetized with pentobarbital
- Pole is done in the right bregma using orthodontic motor
- Hamilton syringe was used to inject EB solution 3mic of .1% in cisterna point the dura matter left open and the skin is sutured

Study design

- On the seventh day after EB injection rats are divided into 7 groups .
- Group(1)receive normal saline as control group
- Group (2) injected intracranial with saline
- Group (3) received vehicle oxygen
- Group (4) EB treated rats injected with EB 3mic .1% intracranially

- Group (5) EB treated rats received ozone (0.5mic /kg/day) rectally for 5 days in the first week and (1mic/kg/day) for 5 days in the second week .
- Group (6) EB-treated rat received 30mg/kg solu-medrol intraperitoneal .
- Group (7) EB treated rats received half the dose of solu-medrol concomitant with ozone 0.5mic/kg /day . for 5 days in the first week and 1mic/kg/day for 5 days in the second week

Methods

Grid walk test to assess motor behavior

By allowing rats to navigate across 1 m-long runway with irregularly assigned gaps

Oxidative stress

It is estimated by measuring thiobarbituric reactive substances (TBARS) in brain samples and paraoxonase (PON1).also glutathione is evaluated and expressed as mic/g weight brain tissue .

Brain cytokine levels

Brain TNF alpha and interferon were determined by enzyme linked immune-sorbent assay

Results

Grid walk test to assess the motor behavior

- EB- treated rats showed significant increase in the number of footfalls in the grid walk test compared to sham operated rats
- Comparison between groups showed significant improvement with administration of ozone
- Best amelioration was achieved by combining half dose of steroids with ozone in EB treated rats

Oxidative stress markers

Brain paroxonase PON1 activity were found to be significantly lowered in EB treated rats in comparison to sham –treated rats (placebo) saline treated.

Ozone treatment revealed an enhancement in PON1 activity and Glutathione

Brain cytokine level

Experimental demyelination showed a significant elevation in brain TNF alpha ,IL-B1 and interferon in comparison to other groups sham saline treated group .

Discussion

- Multiple sclerosis is characterized by demyelination of axons which lead to a deficiency or complete loss in transmission on nerve impulses
- The present study aimed to investigate the effect of ozone and or corticosteroid therapy on demyeliation pathology following local injection of EB in rats

- Our result revealed that glutathione and paroxone enzyme activity were depressed whereas TNf-alpha and Interferon-gamma were elevated in EB –treated rats as compared to sham –operated ones ,as the proinflammatory mediators are increased with the insult .
- these results are in agreement with those obtained in patients with MS where oxidative stress parameters in CSF were increased while antioxidant defenses were found to be decreased

- Glucocorticoids are potent anti-inflammatory drugs and are commonly used in autoimmune diseases .they may have promoted spontaneous CNS remyelination by their anti-inflammatory effects due to inhibition of t-lymphocyte activation or by interfering with locally secreted cytokines.
- our results revealed that solu-medrol enhanced rat ability to perform the grid walk test which is reflected in decrease number of footfalls.

- Ozone has been considered as pro-drug which at certain nontoxic dose can induce a rearrangement of the biochemical pathway with the activation of a second messenger in a cascade with multisystem action.

- Our results revealed that combination of ozone and half dose of solu-medrol succeeded in nearly attaining the ameliorating effect of steroids alone.
- This coincides with other data that evidenced the combined intradiscal and preganglionic injection of ozone alone or with steroids has a cumulative effect that enhance the overall outcome of treatment of lumbar disc herniation

In other studies

They demonstrated that ozone exposed rats had increased levels of plasma corticosteroid which could suggest another mode of action as a stress factor in animals

conclusion

- In conclusion ,ozone therapy approach could be considered as a positive complement to the actual pharmacological therapies addressed to neurodegenerative disorder such as MS ,promoting the maintenance of an adequate cellular redox balance together with considerable immune-modulatory effect .

Thank you