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A combination of radiotherapy, nitric oxide and a hyperoxygenation sensitizing protocol for brain malignant tumor treatment

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Summary Brain malignant tumor such as glioblastoma is a challenging medical and surgical problem. In spite of surgery, radiotherapy and chemotherapy, the prognosis is still very poor. The limitations of currently available treatment modalities to cure or significantly prolong and improve the quality of life should stimulate rigorous research and studies to combat brain malignant tumors. While precision radiotherapy to reduce tumor size and ameliorate symptoms is still the standard of care, tumor sensitivity to radiation is compromised by low oxygen tensions and a necrotic tumor center. We propose to take advantage of the fact that elevated oxygen increases sensitivity of tumor cells to radiation. A specific application of hyperbaric oxygen (HBO_2), using nitric oxide (NO) donors and inducers (such as L-arginine, dinitrite or tocopheryl succinate) and ascorbic acid to dilate blood vessels, should permit oxygen tensions in the range of 1000 mmHg to diffuse into the cells and thus increase sensitivity to radiation. This should permit doses that are low enough to cause the death of tumors cells yet minimize injury to brain tissue near the tumor and induced neurological sequelae.

Background

Brain tumors are either primary or secondary. Whole brain radiation therapy, surgery, radiosurgery, and chemotherapy are used for treatment of brain metastasis [1]. Brain tumors respond differently to treatment with surgery plus radiotherapy or radiotherapy alone, combined with or without chemotherapy [2]. Treatment of malignant brain tumors with conventional approaches is largely unsuccessful because curative doses generally cannot be delivered without excessive toxicity to normal brain [3].

Glioblastoma multiform is an incurable disease that can only be managed in a palliative way. The glioblastoma multiform accounts for approximately half of all newly diagnosed primary brain tumors with an incidence of 2—3 cases per 100,000 people each year [4]. Glioblastoma multiforme is the most frequent primary brain tumor, accounting for approximately 12—15% of all intracranial neoplasms and 50—60% of all astrocytic tumors. In

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most European and North American countries, incidence is approximately 2—3 new cases per 100,000 people per year. Glioblastoma multiforme refers to a malignant neoplasm with abundant glial pleomorphism, numerous mitotic figures and giant cells, vascular hyperplasia, and focal areas of necrosis. Occurring most commonly in the fifth through seventh decades. It grows as an irregular mass in the white matter and infiltrates the surrounding parenchyma by coursing along white matter tracts, frequently involving the corpus callosum and crossing the midline to produce the characteristic ''butterfly'' appearance. Of the estimated 17,000 primary brain tumors diagnosed in the United States each year, approximately 60% are gliomas. Glioblastoma multiforme is by far the most common and most malignant of the glial tumors. The prognosis is very poor. Mean survival length after diagnosis is 8—10 months with less than 10% survival after two years. Only about one out of every four patients with this type of tumor survives two years.

Radiation therapy in addition to surgery has been shown to prolong survival in patients with glioblastoma multiforme compared to surgery alone. Any period of response is short-lived because the tumor typically recurs within 1 year, resulting in further clinical deterioration and the appearance of an expansible region of contrast enhancement. The optimal chemotherapeutic regimen for glioblastoma is not defined at present. However, several studies have suggested that more than 25% of patients obtain a significant survival benefit from adjuvant chemotherapy. Because these tumors cannot be cured with surgery, the surgical goals are to establish a pathological diagnosis, relieve mass effect, and, if possible, achieve a gross total resection to facilitate adjuvant therapy. No specific medications exist to treat glioblastomas.

Hypoxia and radiotherapy

There is strong evidence that regions of hypoxia characterize solid tumors and that oxygen levels within the tumor microenvironment play an important role in determining the tumor grade and response to radiotherapy [5]. Hypoxia in tumors is associated with malignant progression, metastatic spread, and increased resistance to radiotherapy and chemotherapy [6,7].

Hypoxia is a major cause of failure of radiotherapy. The mechanisms of tumor hypoxia are still poorly understood and effective ways for its correction or targeting are not obtained. Although anemia has long been focused on as an important parameter related to tumor hypoxia, differences in vascular density may also affect the intratumoral access of hemoglobin. The vascular density was significantly higher in the tumor periphery as compared to inner areas [8].

Various treatment strategies aimed at increasing tumor oxygenation in head and neck cancer patients (including HBO_2 and hypoxic cell radiosensitizers) have been studied. The results showed that hypoxia adversely affects the radiosensitivity of cells. In vitro studies with conventional photon radiation therapy under normoxic conditions have shown an effectiveness of 2.5—3.0 times greater than that achieved under anoxic conditions [9].

Oxygen and vasoconstriction

High arterial blood oxygen causes vasoconstriction in healthy human [10—12]. Although hyperoxic vasoconstriction was first reported at least 90 years ago, the mechanisms for this phenomenon in healthy humans is poorly understood. Several animal models of hyperoxic vasoconstriction suggest that oxygen tension may influence one or more of the endothelium-derived factors that contribute to the maintenance of vascular tone, such as NO, endothelin, and vasoactive prostaglandins [13,14]. Endothelial cells release O_2^-, which is converted to H_2O_2 or reacts with NO to generate the strong oxidant, peroxynitrite. By reacting rapidly with NO, extracellular O_2^- should decrease biologically available NO, which diffuses from endothelium, erythrocytes, and vascular nerves to smooth muscle.

Hyperbaric oxygen and the brain

HBO_2 has been proposed to reduce tumor hypoxia by increasing the amount of dissolved oxygen in the plasma. Radiotherapy after HBO_2 can be used to enhance the efficacy of clinical treatments. However, it is well known that HBO_2 induces vasoconstriction in systemic organs including the brain [15,16]. Furthermore, HBO_2 produces a variety of biochemical changes in the brain, such as inactivation of intracellular enzymes, formation of lipid hydroperoxides, depletion of GABA, and a decrease in mitochondrial respiratory rate [17,18]. The mechanism of oxygen-induced cerebral vasoconstriction has been sought for more than a century. Extracellular oxygen production reduces the

influence of NO on guanylate cyclase in smooth muscle [19].

It was hypothesized that the cerebral blood flow is reduced at HBO_2 due to inactivation of NO by superoxide anions [20]. Oxygen at a pressure of 4 ATA induces cerebral vasoconstriction in intact animals and decreases blood flow by 11—18% during 60-min exposure to HBO_2 [20]. HBO_2 exposure elevates brain PO_2 and production of reactive oxygen species such as O_2. These hyperoxic events can decrease the half-life of NO, modify its diffusion, and interrupt basal relaxation of cerebral vessels, which may lead to vasoconstriction. It has been found in vitro that superoxide-generating agents rapidly neutralize endothelium-derived relaxing factor, whereas superoxide dismutase increases the half-life of NO and prevents this inactivation.

On the other hand, studies have shown that elevated partial pressure of oxygen increases the steady-state concentration of NO by stimulating neuronal NO synthase activity [21]. Authors observe an increase in arginine levels and a decrease in arginase activity under HBO_2 conditions, implicating an increase in NO. It has been shown that as HBO_2 exposure continues there is a subsequent large increase in cerebral blood flow that was attenuated by NO inhibitor.

Proposed strategy for brain tumor treatment

HBO_2 increases the amount of dissolved oxygen in the plasma and may reduce tumor hypoxia. HBO_2 is used to overcome hypoxia of brain tumor; this will reduce its compromising effects on radiotherapy or chemotherapy. The main problem is cerebral vasoconstriction that followed HBO_2, which would reduce blood flow and availability of oxygen. However, the HBO_2-induced vasoconstriction could be ameliorated with use of NO donors (such as L-arginine) and ascorbic acid. This would increase NO bioavailability in brain tumor, induce vasodilatation, increase neovascularization and blood supply and enhance radiotherapy and chemotherapy (Fig. 1). NO dilates cerebral blood vessels; it should, under HBO_2 conditions, increase blood flow to the brain area and thus increasing oxygen delivery. Further, NO may help to hamper tumor growth because it can induce cytotoxicity and apoptosis of tumor cell and increase immunity against tumor. Low tissue NO increases radioresistance while NO donors increase tumor radiation sensitivity. L-Arginine causes cerebral vasodilatation, elevates GABA and reduces seizures. Ascorbic acid is antioxidant that prevents hyperoxic vasoconstriction. Therefore the addition of these modalities to radiotherapy may improve treatment outcome.

Rationales for the hypothesis and supporting documents

Hyperbaric medicine

HBO_2 means breathing of pure (100%) oxygen under increased atmospheric pressure. HBO_2 induces high oxygen partial pressure in all tissues, causes activation of fibroblasts and macrophages, stimulates angioneogenesis and has a bacteriostatic and bacteriocidic effect. Under 100% oxygen at 3 ATA, the arterial oxygen tension would be over 2000 mmHg, or an increase of about 6 vol% of arterial oxygen, which should provide enough oxygen to tissues, even in the total absence of hemoglobin [22]. HBO_2 exposure transiently suppresses stimulus-induced pro-inflammatory cytokine production. HBO_2 decreases COX2 RNA, and prostaglandins and may increase NO [23]. HBO_2 can help preserve ischemic tissues and promotes healing in challenging wounds. Adjunctive HBO_2 treatment is a new approach to the management of radionecrosis [24]. It has been used widely for treatment of CO poisoning, bends, wound infections, arterial gas embolism and various diseases accompanied by impaired oxygen delivery. The most common side effect is reversible myopia due to oxygen toxicity on the lens of the eye, but others include headaches, seizures, reversible barotrauma and pulmonary symptoms.

New applications of HBO_2 included migraine headaches, chronic fatigue syndrome, fibromyalgia, post injury treatment and rehabilitation, peripheral vascular disease, peripheral neuropathy, post-stroke therapy, multiple sclerosis, myocardial infarction, facial palsy and cerebral palsy [24].

Hyperbaric oxygen and tumor hypoxia

It has been found that HBO_2 and hyperbaric carbogen improved tumor oxygenation [25]. HBO_2 has been shown to improve the radiation response of many solid tumors in rodents and in patients. Treatment with a perfluorochemical emulsion, Fluosol-DA, plus HBO_2 (3 ATA) significantly increases the radiation response of the malignant cells in these solid tumors. In addition, the combination of Fluosol-DA and HBO_2 decreases the proportion of severely hypoxic cells in the tumor to less than 1.5% of the original value [26]. HBO_2 gives a 6.6% improvement in local control and hypoxic cell sensitizers [27].

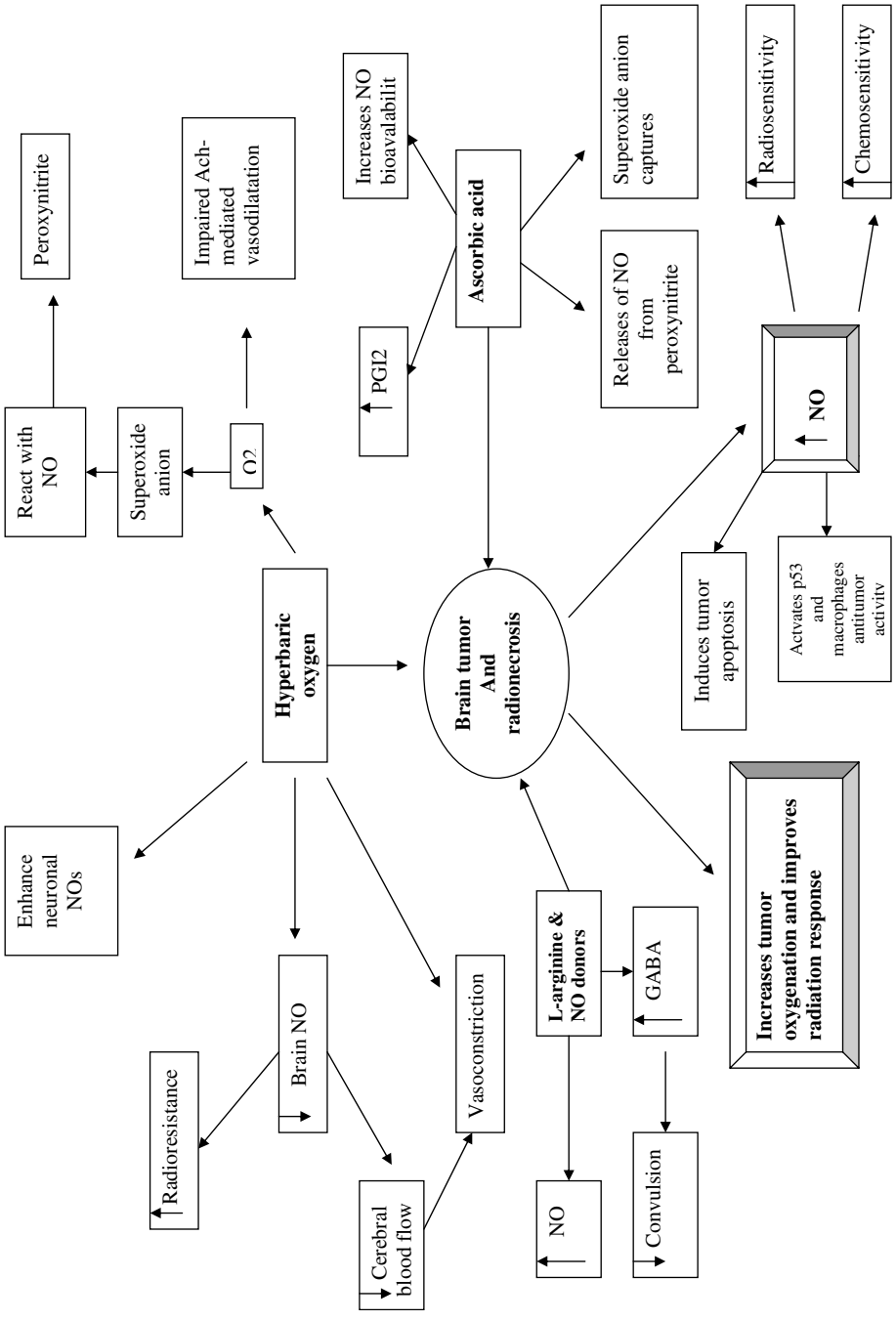

Figure 1 Hypothesis; combination of HBO_2, ascorbic acid and NO donor in the brain malignant tumor treatment.

In brain tumors, it was postulated that the resistance of gliomas to treatment with radiation and antineoplastic drugs might be attributed in part to the effects of severe hypoxia presented in these tumors [28]. Radiotherapy after HBO_2 combined with interferon-β and nimustine hydrochloride was applied for supratentorial malignant gliomas; the results suggested that this therapy could be applied to patients with poor prognostic factors [29].

Hyperbaric oxygen and radiotherapy of tumors

The original rationale for the use of HBO_2 in radiotherapy was developed in the UK and based on the work of Gray et al. and work Dr Churchill-Davidosn [30,31]. Other works formed the basis for the original ongoing work [32—38].

Radiation complications are infrequent, but can be either life threatening or significantly diminish the quality of life. A systematic review of the 74 publications reporting the results of HBO_2 therapy in the treatment and/or prophylaxis of delayed radiation injury showed that all but seven of these publications report a positive result when HBO_2 is delivered as treatment for or prevention of delayed radiation injury [39].

HBO_2 was used in 27 patients with radiation-induced wounds, 9 of them underwent bony reconstruction of the mandible [40]. Results showed that HBO_2 is a very helpful tool in the management of problem-wound-healing, assisting the classical surgical principles. The effect of radiotherapy after HBO_2 in experimental tumors using a tumor growth delay assay was studied [41]. A significant growth delay in the treated animals was obtained within 30 min after HBO_2, and the tumor growth delay time was prolonged 1.61 times as that in radiotherapy alone. It was concluded that radiotherapy after HBO_2 is effective for tumors with hypoxia. The time lapse from decompression to irradiation is an important factor in improving radiosensitivity.

Treatment with oxygen plus oxygen, carbogen (95% O_2/5% CO_2), or HBO_2 increases the effects of radiation on the tumors [40]. HBO_2 significantly improves both survival and local tumor control after radiotherapy for both head and neck tumors and for advanced carcinoma of the cervix. In carcinoma of the bronchus there is some improvement in survival [42—44]. The approach to radiosensitization has been evaluated in the treatment of 61 patients with bladder carcinoma using radical radiotherapy [45]. Patients receiving carbogen showed better response to radiotherapy. It was reported that radiation under HBO_2 increases tumor response [46]. Four-day-old artificial pulmonary micrometastases of murine fibrosarcomas shows increased sensitivity to ionizing radiation by a factor of 1.13 when animals were exposed to oxygen breathing before and during irradiation [47].

Hyperbaric oxygen and brain radiotherapy

Brain radionecrosis is the most serious complication associated with radiation therapy and it can result in significant morbidity and mortality (Fig. 2). The incidence of brain radionecrosis has been reported to be as high as 40—50% in patients exposed to certain types of radiation therapy [48] Three principal mechanisms have been suggested for the development of brain radionecrosis: inflammatory response initiated in brain, direct damage to endothelial cells, and demyelination of oligodendrocytes.

Treatment includes high doses of steroids to reduce edema or nonsteroidal anti-inflammatory drugs to decrease vascular permeability, and anticoagulation therapy [49—51]. Surgery may be needed when medical treatment failed. HBO_2 therapy has been studied and is an accepted standard of care for adjunctive treatment of radiation injury to bone and soft tissue [52—57]. In 1993, Fontanesi et al. reported a positive outcome for a patient with brain radiation necrosis after treatment for a glioblastoma multiforme tumor using HBO_2 [58]. Doctors and researchers at the Brain Radionecrosis Center have used HBO_2 therapy for brain radionecrosis through the development of an accelerated air-break treatment protocol. The results show that the treatments are safe and effective with a high success rate [59]. Radionecrosis develops because of impaired revascularization. Radiation is delivered at greater dosages and, thus, creates greater cellular damage at the center of the radiation port than at the periphery. HBO_2 helps to trigger the biochemical response required for angiogenesis [60,61]. HBO_2 induces angiogenesis, promotes healing and reverses injury in irradiated soft tissue and bone [62—73].

Nitric oxide and nitric oxide donors

Nitric oxide (NO) is a small gaseous molecule generated in a wide variety of cells as a product of the conversion of L-arginine into L-citrulline by the enzyme NO synthase (NOs). NO plays important roles in diverse physiological processes, such as neurotransmission, vasodilatation, and inhibition of platelet aggregation [74,75]. NO has been proposed as a possible active agent for enhancing wound healing. In addition, NO increases cytosolic concentration of free calcium ion and it affects

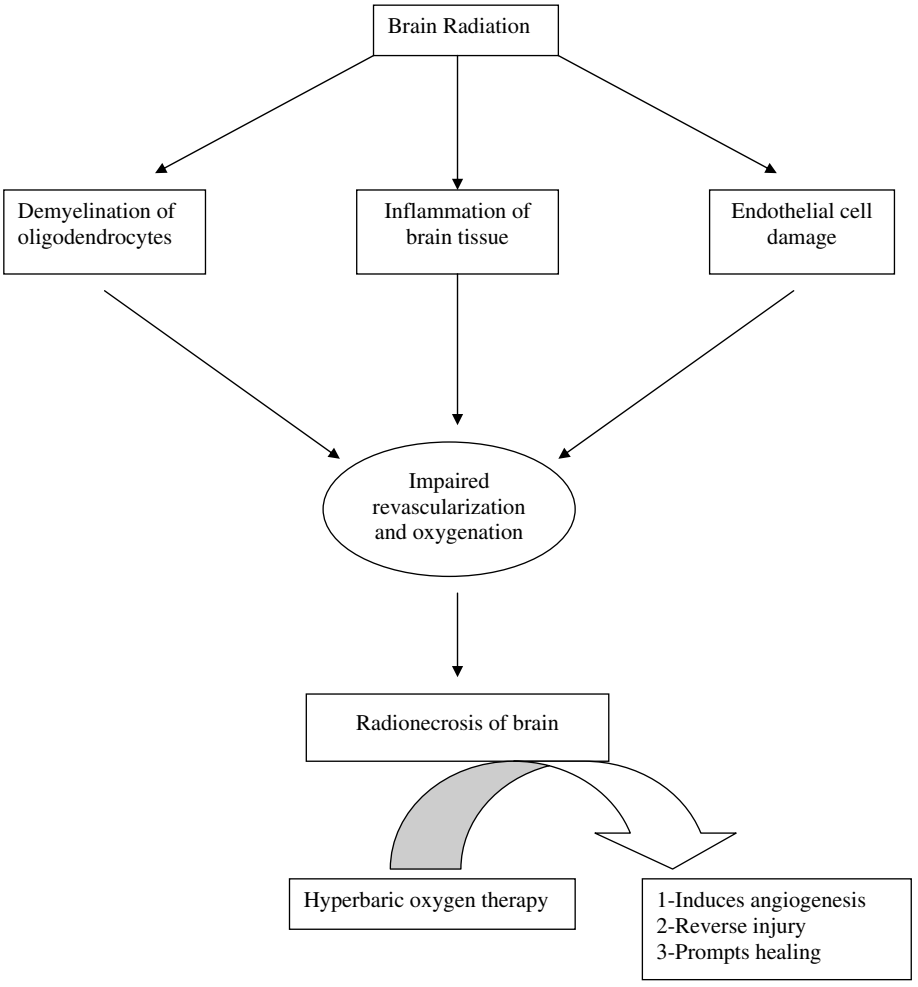

Figure 2 Effects of radiation on brain tissue and role of hyperbaric oxygen therapy.

functions of various enzymes [76,77]. It plays a major role in immunity, inflammation and tumor pathophysiology.

Nitric oxide and tumor

Molecular oxygen is required for the cellular production of NO by the enzyme NOs, and NO may block components of the adaptive response to hypoxia. It was hypothesized that hypoxia increases drug resistance in tumor cells by inhibiting endogenous NO production. NO mediates chemosensitivity in tumor cells. The lack of production of endogenous NO, resulting from a reduction in the oxygen supply to the cell or from pharmacologic inhibition of NOs, can lead to increased resistance to chemotherapy. Very low doses of an NO mimetic can produce a statistically significant reduction in this resistance phenotype and, therefore, may have a potential therapeutic role. Direct stimulation of NO synthesis in tumor cells through the L-arginine/iNOs pathway represents a novel approach to increase radiosensitization. It was found that NO synthesis represents a significant macrophage antitumor mechanism [78]. Tumor growth can be promoted by continuous low NO concentration, while cytotoxicity and apoptosis to tumor cells can be induced by high NO concentration. NO is a potent activator of the p53 tumor suppressor protein. NO enhances markedly the ability of low-dose ionizing radiation to elicit apoptotic killing of neuroblastoma cells expressing cytoplasmic wild-type p53.

Nitric oxide and radiation

Gamma-irradiation of mongrel mice at a sublethal dose (700 Roentgen) enhanced the formation of NO in the liver, intestine, lung, kidney, brain, spleen or heart of the animals. Nitro-L-arginine, NO synhtase inhibitor, increased radiation resistance 3- to 5-fold, consistent with the induction of tumor hypoxia [79]. Tumor energy metabolism may be altered through drug-induced modification of NO availability, and that these changes are sufficient to modify tumor sensitivity to X-rays. It

was found that irradiation dose-dependently induced the activation of the proangiogenic NO pathway in endothelial cells through increases in endothelial NOs abundance and phosphorylation. Inhibition of NO production suppresses these provascular effects of irradiation highlights new potentials for the coordinated use of antiangiogenic strategies and radiotherapy in clinical practice [80]. Administration of the NO donor, isosorbide dinitrate, improved the tumor pO_2 concomitant with an increase in tumor blood flow. It was also demonstrated an increase in tumor radiation sensitivity after isosorbide dinitrate administration, which was similar to the effect of carbogen breathing in the same tumor model [81].

Nitric oxide and the brain

NO acts as a powerful dilator of cerebral blood vessels, and inhibition of NOs prevents vasodilator responses to CO_2. Inhibition of NOs also decreases cerebral blood flow under basal conditions. Cerebral NO is synthesized in vascular nerves and neuronal and endothelial cells and diffuses rapidly in all directions. Extracellular NO concentrations have been proposed to be stable, in the nanomolar range, at steady-state conditions. Because NO is a relatively long-lived molecule, it can diffuse away from its site of synthesis and reach critical targets in the brain at distances up to 200 μm [82].

NO half-life depends on its rate of autooxidation, its reaction with oxygen, scavenging by metal-containing compounds, e.g., hemoglobin and other hemoproteins, and its binding to cellular thiols [83]. NO that escapes degradation in the brain can activate guanylate cyclase, which catalyzes the formation of cGMP and triggers a number of cellular responses, including smooth muscle relaxation and cerebral vasodilatation [19]. NO participates in the coupling of function activity to blood flow and support the hypothesis that NO is one of the mediators responsible for functional hyperemia in the central nervous system.

L-Arginine

L-Arginine is a amino acid that is involved in many important physiological processes such as protein, NO, agmatine, putrescine, urea, L-ornithine or creatine synthesis and is essential for posttranslational arginylation of protein. It is becoming apparent that the synthesis of NO from L-arginine not only explains endothelium-dependent vascular relaxation, but also is a widespread mechanism for the regulation of cell function and communication.

Topical application of $10(-6) - 10(-4)$ mol/L L-arginine-induced dose-dependent brain arteriolar vasodilatation. Exogenously administered L-arginine may decrease the oxidative stress in the liver and brain [84]. L-Arginine, which increases cerebral blood perfusion and improves vasomotions of microvessels by enhancing NO levels and decreasing endothelin-1 levels in blood, exerts a protective effect on secondary cerebral ischemic injury following experimental subacute hemorrhage [85].

It was found that L-arginine significantly prolonges the latent period to onset of seizures [86]. A convulsion induced by picrotoxin is inhibited by pretreatment with L-arginine [87]. L-Arginine and sodium nitroprusside increase NO, which might be an endogenous anticonvulsant agent [88,89].

Another study demonstrates that NO synthesized from systemically administered L-arginine inhibits convulsions by increasing the concentration of GABA in the brain [90]. High NO concentrations in the brain following L-arginine administration may increase the permeability of blood—brain barrier to peripheral GABA. Administration of L-arginine (2000 mg/kg b. wt.) increases GABA concentration in the brain. The anticonvulsant L-arginine significantly increases the NO concentration and NOs activity in brain regions [91]. The increased NO concentration and NOs activity in brain regions suppresses convulsions.

Ascorbic acid

Studies suggest that hyperoxic vasoconstriction is mediated by oxidative stress. Moreover, hyperoxia impairs acetylcholine-mediated vasodilation in the setting of intact endothelial function. These effects of hyperoxia are prevented by vitamin C, providing evidence that hyperoxia-derived free radicals impair the activity of endothelium-derived vasoactive factors [92].

Vitamin C may prevent hyperoxic vasoconstriction by several mechanisms involving NO bioavailability, including direct quenching of superoxide anions, and possibly increasing the release of NO from peroxynitrite and nitrosylated compounds [93]. Moreover, it has been demonstrated that prolonged vitamin C supplementation may enhance the production of these vascular mediators [94,95]. Other studies showed that ascorbic acid increases level of prostacyclin, which are a well known vasodilator agent [96,97].

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