

Potential roles of hyperbaric oxygenation in the treatments of brain tumors

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ABSTRACT

Over the past 50 years hyperbaric oxygen (HBO₂) therapy has been used in a wide variety of medical conditions, and one of them is cancer. Many clinical studies have been conducted to evaluate potential therapeutic effects of HBO₂ as a part of cancer treatment. This review briefly summarizes the potential role of HBO₂ therapy in the treatment of malignant tumors and radiation injury of the brain. HBO₂ therapy is used for the enhancement of radiosensitivity in the treatment of some cancers, including malignant brain tumors. Radiotherapy within 15 minutes following HBO₂ exposure, a relatively new treatment regimen, has been studied at several institutes and has demonstrated promising clinical results for malignant gliomas of the brain. HBO₂ therapy also increases sensitivity to some antineoplastic agents;

non-randomized clinical trials using carboplatin-based chemotherapy combined with HBO₂ show a significant advantage in survival for recurrent malignant gliomas. The possibilities of combining HBO₂ therapy with radiotherapy and/or chemotherapy to overcome newly diagnosed and recurrent malignant gliomas deserve extensive clinical trials. HBO₂ therapy also shows promising potential for the treatment and/or prevention of radiation injury of the brain after stereotactic radiosurgery for brain lesions. The possibilities with HBO₂ to enhance the therapeutic effect of irradiation *per se*, and to even increase the radiation dose if there are ways to combat the side effects, should boost new scientific interest into the whole field of oncology looking for new armamentaria to fight cancer.

INTRODUCTION

Hyperbaric oxygen (HBO₂) therapy, which is mainly used for the treatment of hypoxic tissue damage, also has effects that enhance cell or tissue damage; one of these is augmenting the therapeutic effects of radiotherapy and/or chemotherapy [1]. It is well known that hypoxic tumor cells are resistant to some types of chemotherapy and radiotherapy. Tumor oxygenation is a critical determinant of many forms of cancer therapy. HBO₂ therapy improves oxygen supply to hypoxic tumor cells independent of hemoglobin, and offers one approach to overcome tumor cell hypoxia. This treatment has been used in combination with radiotherapy to treat malignant tumors [1-3].

Malignant gliomas, the most common primary brain tumor in adults, have a poor prognosis. Fractionated irradiation has been considered the most effective thera-

peutic approach for the tumors. However, the failure of radiotherapy in malignant gliomas is primarily due to the presence of hypoxic, intrinsically radioresistant, and repair-proficient subpopulations of cells in the tumor.

HBO₂ therapy was first used to improve radiosensitivity for malignant gliomas in the 1970s [1,4]. However, the combination method by which irradiation was administered during HBO₂ exposure was both hazardous to patients and complex [1,4,5]. As a result, HBO₂ therapy has not been routinely adopted with radiotherapy to treat malignant gliomas. In contrast to irradiation during HBO₂ exposure, a new combination method (*i.e.*, irradiation immediately following HBO₂ exposure) was devised in the 1990s and applied at a few institutes in Japan. The efficacy of this treatment method has been demonstrated in patients with newly diagnosed malignant gliomas [6-11]. This regimen is a

simple, easy technique that is safe for patients because of the sequencing of standard irradiation following HBO₂ exposure, rather than its concurrent delivery.

In addition to radiotherapy, hypoxic tumor cells have low growth fractions, leading to relative resistance to chemotherapy [12]. HBO₂ has been shown to potentiate the cytotoxic effects of some chemotherapeutic agents in experimental models [13-15]. However, in the English literature there are no reports suggesting improvement of chemotherapy with HBO₂ therapy in cancer patients. Recently, interesting clinical results in chemotherapy with HBO₂ have been published or presented from an institute in Japan [16,17].

Today, stereotactic radiosurgery is an important method of treatment for vascular malformations, benign and malignant tumors, and functional brain disease. However, radiation-induced brain injury remains a feared complication of this therapy. Radiation injury of the brain has been occasionally treated by HBO₂ therapy, and furthermore, Ohguri *et al.* have applied HBO₂ therapy for protection against this condition after stereotactic radiosurgery for brain lesions [18].

We present here several new adjunctive approaches using HBO₂ therapy in the field of neuro-oncology, specifically HBO₂-induced tumor radiosensitization, enhancement of chemotherapeutic agents and management of radiation injury.

I. RADIOTHERAPY AND HBO₂

A. IRRADIATION DURING HBO₂ EXPOSURE

By using electrode probes, the presence of hypoxic cells in malignant tumors has been demonstrated in human cancer. In particular, malignant gliomas have large numbers of hypoxic cells that respond poorly to radiotherapy. Rampling *et al.*, using an Eppendorf polarographic O₂ electrode, recorded a median oxygen partial pressure (pO₂) value of 7.4 mmHg in 10 patients with glioblastoma and demonstrated the presence of severely hypoxic cells having pO₂ values less than 2.5 mmHg (median 39.5% (9.5-68.5%)) [19]. However, needle electrode studies are invasive and limited to accessible tumor sites. In contrast, Evans *et al.* showed that human gliomas with an increasing World Health Organization grade exhibit tumor hypoxia of increasing severity, as determined by analysis of the 2-nitroimidazole agent EF5 as an *in vivo* hypoxia detector on immunohistochemistry [20]. They noted a correlation between more rapid tumor progression and hypoxia, which is well detected with EF5 binding despite no prediction using Eppendorf measurements. However, Vordermark con-

cluded that the role of hypoxic cells in the biological and clinical behavior of human gliomas detected by direct or indirect pO₂ measurement has not yet been confirmed [21].

Molecular oxygen has long been recognized as one of the most powerful modifiers of cellular radiosensitivity [22,23]. For example, oxygen has been reported to increase, by a factor of approximately 3, the biological effect of ionizing radiation on mammalian cells when radiation is given under well-oxygenated conditions as compared to anoxic conditions. The proportion of cells existing below pO₂ of ~10 mmHg is important because radiosensitivity varies from its minimum to near maximum value over this range (0-10 mmHg). Thus, HBO₂ therapy has been used in combination with radiotherapy to treat cancer patients, with the first pilot study being conducted in the 1950s [1]. There are only two trials of malignant gliomas treated with this therapeutic method [4,24]. In 1977, Chang compared the therapeutic results in 38 and 42 patients with and without HBO₂ therapy, respectively, and showed that the median survival rate at 18 months appeared considerably higher in the HBO₂ group (28%) than in the control (10%), despite no statistical significance [1,4]. However, the different median survival times (MSTs) for 18 and 15 patients who received 60 Gy (2 Gy x 30) with and without HBO₂ therapy were 46 and 25 weeks, respectively, even though the patients in HBO₂ group received initial 30 Gy to the whole brain under aerated conditions and the remaining 30 Gy was delivered to the reduced field of tumor size under HBO₂ exposure. The other 20 and 27 patients in two groups underwent irradiation with a total dose of 36-50 Gy, which is low and not a standard radiation dose for current radiotherapy for malignant gliomas. After examining the above data, the author would have likely demonstrated a significant difference in median survival if all patients had received a total of 60 Gy (2 Gy x 30) with or without HBO₂ therapy. This pilot study suggests that HBO₂ therapy has the potential to enhance radiosensitivity in malignant tumors that include a large percentage of hypoxic cells.

The proportion of radiobiological hypoxic cells, referred to as the hypoxic fraction, in human squamous cell carcinoma was reported to be 19-57% using a clamped assay of the tumor control dose [25]. In general, pO₂ of these radioresistant hypoxic tumor cells is considered to be below 2.5 mmHg [26]. Radiosensitivity of the tumor is well known to be determined by its pO₂, and will increase markedly by delivery of a very small amount of oxygen [22,23]. Recently, Overgaard, in a

TABLE 1. Clinical trials of radiotherapy after HBO₂ decompression on malignant gliomas

| Authors | Tumor localization | Radiation dose/HBO ₂ | Additional therapy | Results |
|----------------------------------|----------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|
| Kohshi <i>et al.</i> 1999 [7] | G-4 (a: 10, b: 11) G-3 (a: 5, b: 3) | a: 57.8 ± 5.7 Gy 15-30 min after HBO ₂ b: 58.7 ± 3.7 Gy | Nitrosourea: 75 mg/m ² Day 1 and 5-6 weeks after radiotherapy) | a: >50 % tumor regression: 73% median survival: 24 months b: >50% tumor regression: 29% median survival: 12 months |
| Beppu <i>et al.</i> 2003 [8] | G-4: 26 G-3: 9 | 60 Gy/2 Gy <15 min after HBO ₂ | Nitrosourea: 80 mg/m ² Interferon-β: 3 million IU/m ² (3 times/week) (Days 1 and 36) | Median time to progression G-4: 38 weeks G-3: 56 weeks Overall: 43 weeks |
| Ogawa <i>et al.</i> 2012 [11] | G-4: 39 G-3: 18 | 60 Gy/2 Gy <15 min after HBO ₂ | Nitrosourea: 80 mg/m ² Procarbazine: 90 mg/m ² Vincristine: 0.5 mg/m ² (3-mo interval, max. 4 courses) | Median survival time G-4: 17.2 months G-3: 113.4 months 2-year overall survival rate G-4: 25.6% G-3: 77.8% |

G-4: glioblastoma multiforme; G-3: anaplastic astrocytoma

review of the published data, identified 4,805 patients with squamous cell carcinoma of the head and neck in 32 randomized clinical trials, applying normobaric oxygen (NBO₂) including carbogen (95% oxygen, 5% carbon dioxide), HBO₂ and hypoxic radiosensitizers [27]. This meta-analysis revealed that overall hypoxic modification was of benefit for radiotherapy in head and neck cancer and that HBO₂ seemed to have the best outcome in disease-specific survival (odds ratio (OR): 0.58, 95% confidence interval (CI): 0.42-0.81) compared with NBO₂ and hypoxic radiosensitizers (OR: 0.83, 95% CI: 0.59-1.17; OR: 0.74, 95% CI: 0.64-0.86, respectively).

For the above glioma and squamous cell tumor treatment regimens, however, irradiation during HBO₂ exposure within a pressure chamber was associated with practical set-up difficulties. It complicated dose delivery calculations because of the thick acrylic viewport, required sedation and myringotomies before each irradiation and, moreover, made it difficult to limit unintended radiation delivery to tissues outside the tumor field of patients in a chamber [1,4,5]. Although about 25% of patients who received HBO₂ therapy had a better quality of survival than the control group, convulsive seizure and extensive radiation necrosis developed in two and three out of 38 patients who received HBO₂ therapy, respectively [4]. Therefore, although HBO₂ appears to be most effective among the various adjuvants to radiotherapy, this regimen of delivering irradiation during HBO₂ exposure has not been adopted as a standard adjunct to radiotherapy for malignant gliomas and other cancers [1].

B. IRRADIATION FOLLOWING HBO₂ EXPOSURE

An interesting study by Wells *et al.* measuring tissue pO₂ demonstrated that, with the exception of arterial blood, pO₂ in normal tissues was maintained at a high level even after HBO₂ exposure [28]. This phenomenon is especially marked in hypoperfused tissues. Because blood flow and oxygen consumption in malignant gliomas are lower than those in the normal brain [29,30], it was hypothesized that highly increased pO₂ in the tumors is maintained for certain periods after HBO₂ exposure. Based on this hypothesis, a consecutive approach (*i.e.*, radiotherapy within 15 minutes following HBO₂ exposure) was used for patients with malignant gliomas [6]. This therapeutic regimen proved simple and safe for patients because neither myringotomy nor sedation was required. As illustrated by Table 1, several clinical studies published from three institutes in Japan showed favorable results using this regimen for malignant gliomas [6-11]. One of them, a small non-randomized trial in 29 patients, demonstrated that HBO₂ was the only significant prognostic factor for survival (relative risk: 0.27, 95% CI: 0.088-0.800) [7]. More recently, Ogawa *et al.* analyzed the long-term results of radiotherapy following HBO₂ exposure in 57 patients with newly diagnosed supratentorial malignant gliomas, reporting overall MSTs in all patients, 39 patients with glioblastoma and 18 patients with Grade 3 gliomas, to be 20.2 months, 17.2 months and 113.4 months, respectively [11]. In several studies of glioblastoma treated with radiotherapy and nitrosourea-based chemotherapy, MST was reported to be seven to 12 months [31-33].

For patients with Grade 3 astrocytoma MST was 30 to 34 months [34,35].

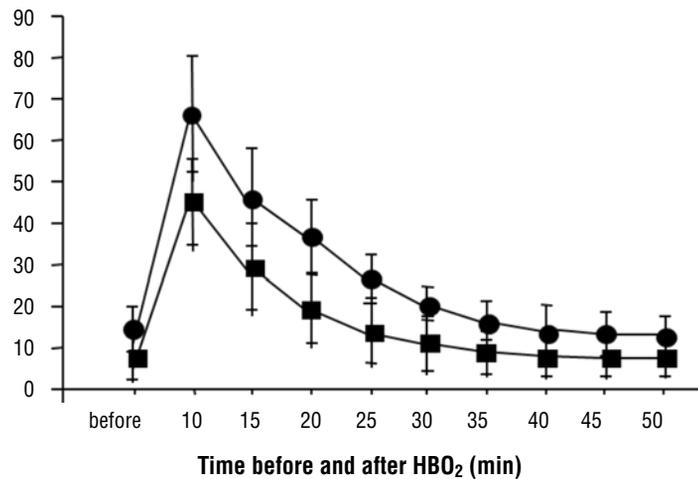
After a randomized trial demonstrated that the addition of concurrent temozolomide to radiotherapy resulted in a survival benefit for patients with glioblastoma [36], the standard adjuvant chemotherapeutic agent shifted from nitrosourea to temozolomide. The median survival was 14.6 months with radiotherapy plus temozolomide and 12.1 months with radiotherapy alone [36]. A recent survey in glioblastoma survival before and during the temozolomide era in the United States showed that the MSTs in 2000-2003 and 2005-2008 were 12.0 and 14.2 months, respectively [37]. Thus, the treatment results in Ogawa's HBO₂ exposure series appear to be favorable when compared to those combining radiotherapy with various chemotherapeutic agents including temozolomide [11].

Another study of radiotherapy following HBO₂ exposure was applied to hypofractionated stereotactic radiotherapy (HSRT) using a gamma unit, which the median marginal and total maximal doses were 22 Gy and 44 Gy, respectively, delivered in eight fractions [38]. Although the calculated biologically equivalent doses were lower than those in other series of HSRT alone, the treatment result combined with HBO₂ showed similar survival patterns to others [39-41]. This pilot study suggests a possible advantage to adding HBO₂ for treatment of recurrent malignant gliomas [38]. Because glioblastoma typically recurs within months, an ideal regimen should be short, well-tolerated and convenient for both patients with malignant gliomas and their caregivers without compromising on efficacy [42,43]. Roa *et al.* studied glioblastoma patients 60 years and older who were treated with a shorter hypofractionated course of radiotherapy (40 Gy in 15 daily fractions over three weeks) [44]. This group, as well as other authors, demonstrated similar survival and palliative benefits compared with a standard six-week course of radiotherapy [44-46]. In this context, these published results indicate that HBO₂ augments radiotherapy, including when it is applied to shorter hypofractionated irradiation in newly diagnosed and/or recurrent malignant gliomas. Increased radiosensitivity of large residual tumors may contribute to an increased response rate and overall survival for patients with low Karnofsky Performance Scale (KPS) and/or large residual tumors. Beppu *et al.* found no significant differences in the response rates with regard to age, KPS or extent of surgical resection for malignant gliomas treated with radiotherapy within 15 minutes

following HBO₂ exposure [8]. These authors concluded that radiotherapy following HBO₂ exposure could be applied to patients with poor prognostic factors, resulting in tumor response identical to that in patients with good prognostic factors.

Furthermore, radiotherapy following HBO₂ exposure has been reported as useful in treating other, non-glioma cancers. Oya *et al.* applied this therapeutic regimen to squamous cell carcinoma of the oral cavity in 101 patients who were locally irradiated with carboplatin chemotherapy, with 51 of them being exposed to HBO₂ (2.5 atmospheres absolute, 60 minutes) before daily irradiation [47]. The overall survival of the HBO₂ group was better than that of the non-HBO₂ group ($p=0.012$), and five-year disease specific survivals of the two groups were 70% and 40%, respectively, with statistical significance ($p=0.004$). In multivariate analysis, HBO₂ was the only significant prognostic factor (OR: 6.37, 95% CI: 2.11-19.24) for survival, in contrast to surgery or chemotherapy.

Experimentally, the enhancement effect of this regimen was confirmed by using the SCCVII tumor model (radiobiological hypoxic fraction: 10%), in which a significantly enhanced effect was continued for 30 minutes after HBO₂ (2.0 atm abs, 60 minutes) exposure [48]. However, 9L tumors, in which the reported hypoxic fractions are lower and near zero [49], were not enhanced during and following HBO₂ exposure [48,50]. Using an MRI technique, tumor pO₂ changes were monitored non-invasively in the SCCVII tumor model after HBO₂ (2.0 atm abs, 60 minutes) exposure [51]. Although pO₂ change of the surrounding muscles decreased rapidly, that of tumors decreased gradually and remained high even 60 minutes after decompression. In contrast, NBO₂ for the same period of HBO₂ showed no significant change of tumor pO₂. As illustrated by Figure 1, similar results were clinically identified by Beppu *et al.* having inserted pO₂ electrodes into the intra- and peritumoral regions of 16 patients with glioblastoma and measuring pO₂ in these tissues before and after HBO₂ exposure [52]. Although the pO₂ values were not elevated under NBO₂ for 15 minutes, increased pO₂ values after HBO₂ therapy (2.8 atm abs, 60 minutes; 20 minutes of decompression with O₂) declined gradually and remained significantly higher than those before HBO₂ exposure until 30 or 35 minutes after decompression. A high pO₂ level greater than 30 mmHg, obtaining maximal radiosensitivity, was maintained until 15 minutes after HBO₂ exposure in both regions.

FIGURE 1. Mean pO₂ value (mmHg)

Comparison between the mean pO₂ value before and after HBO₂ for each region in 16 glioblastoma patients. (●) peritumoral region; (■) intratumoral region. (Reprinted with permission from J Neurooncol 2002 [52])

The timing of irradiation is important to the overall success of radiotherapy following HBO₂ exposure. Irradiation should be performed immediately after decompression in order to improve the therapeutic effects. Further, HBO₂ enhances the effect of radiotherapy in only malignant tumors, including radioresistant hypoxic cells. Consequently, the degree of tumor hypoxia in this regimen should be evaluated before treatment [53,54]. Despite the need for more convincing evidence for this approach to be more widely employed, radiotherapy within 15 minutes following HBO₂ exposure appears to be an attractive treatment option for malignant gliomas.

II. CHEMOTHERAPY AND HBO₂

Analogous to the situation with radioresistant tumors, hypoxia has been shown to increase tumor cell resistance to chemotherapy [12,55,56]. Although some experimental studies have shown that HBO₂ therapy enhances the cytotoxic effects of several chemotherapeutic agents, such as nitrosoureas or platinum coordination complexes [13-15], there have been no reports of therapeutic improvements for clinical subjects. However, Tanaka *et al.* suggested that HBO₂ therapy potentiated therapeutic effects of carboplatin in patients with recurrent malignant gliomas [16]. Eleven patients, who had received conventional radiotherapy and nitrosourea chemotherapy, were enrolled in their pilot study and exposed to one session of HBO₂ (2.0 atm abs, 60 minutes) therapy an hour after intravenous carboplatin (400mg/m²) administration in each six-week cycle. To evaluate the enhanced

effects of carboplatin, a control treatment group that did not receive HBO₂ therapy used for matched-pair analysis. Each control patient was randomly selected with adjustment for age, KPS and histological diagnosis. Despite the small number of patients, there was a difference between survival curves in the two treatment groups (Wilcoxon signed-rank test: $p < 0.05$), but multivariate analysis could not reveal any prognostic factors.

More recently, Tanaka *et al.* enrolled 129 patients with recurrent malignant gliomas, and compared survival between 42 patients with carboplatin plus HBO₂ therapy and 87 patients with carboplatin alone [17]. Mean follow-up evaluation averaged 768.2 days (range, 203-2,973 days) and 591.2 days (range, 43-4,027 days) for the HBO₂ and non-HBO₂ groups, respectively. Survival curves between the two groups were significantly different (Wilcoxon test: $p < 0.05$, log-rank test: $p < 0.01$). Although there were no differences between patient characteristics and treatment parameters in the two groups, significantly prolonged survival was achieved in the HBO₂ group. As illustrated by Table 2, in a Cox model analysis to investigate the relationship between prognostic factors and survival, multivariate analysis showed that the prognostic factors were treatment group (hazard ratio (HR) of HBO₂: 0.59, 95% CI: 0.36 – 0.97), age, tumor grade, removal rate and KPS. As a side effect of carboplatin, mild bone marrow suppression was noted in some patients, but it was not different between the two groups. This clinical result shows a 41% reduction in the relative risk of death for patients

TABLE 2. Prognostic factors and survival of patients with recurrent malignant gliomas

| | Variable | HR (95% CI) |
|------------------|------------|--------------------|
| Age | ~44 | 1.00 (Ref) |
| | 45-59 | 1.57 (0.82 – 3.03) |
| | 60~ | 2.40 (1.31 – 4.38) |
| Histology | G-4 | 1.00 (Ref) |
| | G-3 | 0.78 (0.62 – 0.99) |
| Removal rate | 75% \geq | 1.00 (Ref) |
| | 75% $<$ | 1.89 (1.18 – 3.03) |
| KPS | 80 $<$ | 1.00 (Ref) |
| | 80 \geq | 1.61 (1.01 – 2.58) |
| HBO ₂ | No | 1.00 (Ref) |
| | Yes | 0.59 (0.36 – 0.97) |

HR: hazard ratio; CI: confidence interval; G-4: glioblastoma multiforme; G-3: anaplastic astrocytoma; Ref: reference; KPS: Karnofsky performance status

with recurrent malignant gliomas treated with carboplatin plus HBO₂ therapy, as compared with those who received carboplatin alone. The current study suggests that HBO₂ improves the therapeutic effect of carboplatin for patients with recurrent malignant gliomas. However, the impact on malignant gliomas as an adjuvant to carboplatin chemotherapy has not been reported in experimental study.

Although not a brain tumor model, another experimental study demonstrated that HBO₂ enhances the antineoplastic effects of carboplatin. Kawasoe *et al.* examined the efficacy of carboplatin combined with HBO₂ in osteosarcoma-bearing C3H mice, reporting suppression of both tumor growth and lung metastasis [15]. This group noted a higher survival rate in the carboplatin-plus-HBO₂ group than in the carboplatin-only group. Donnelly *et al.*, using a tumor growth delay assay in a murine tumor model (radiobiological hypoxic fraction: 24%), reported that the effects of carboplatin were increased under normobaric oxygen (NBO₂) breathing for five hours with efaproxiral, a synthetic allosteric modifier of hemoglobin-oxygen binding to increase tumor pO₂, but not under NBO₂ breathing alone [57]. These results suggest that NBO₂ breathing produces minimal or no effects on oxygenation of tumors with hypoxic cells during attempts to alter the cytotoxicity of carboplatin. Brizel *et al.*, using an Eppendorf pO₂ histography, showed that NBO₂ and carbogen caused no significant change in tumor oxygenation, whereas HBO₂ and hyperbaric carbogen led to improvement of oxygenation [58]. Using a non-invasive MRI

technique, Kinoshita *et al.* recorded tissue pO₂ changes in SCCVII tumor (radiobiological hypoxic fraction: 10%) in mice after NBO₂ breathing for 80 minutes and demonstrated no signal change from the tumors [51]. Most cytotoxic agents showing a positive relation between oxygen tension and efficacy in cell culture are less effective under hypoxic conditions [12,55,56], and one in which cytotoxicity is enhanced by oxygenation is carboplatin [15-17]. The mechanisms behind HBO₂ enhancement of clinical and experimental anti-tumor effects of chemotherapeutic agents are unclear. The cytotoxicity of platinum coordination complexes such as carboplatin and cisplatin is mediated by platinum-DNA adducts, which are formed following uptake of the drug into the nucleus of cells [59]. However, the effects of platinum binding to DNA in carboplatin and cisplatin are completely different in the presence of reactive oxygen species (ROS) [60]. The ROS, produced by a system containing Fe-EDTA chelate and ascorbate, are examples of agents capable of converting carboplatin to reactive species able to secondarily bind DNA, interfering with DNA repair [61]. This result presents evidence for an increase of carboplatin conversion to byproducts that are able to react with isolated DNA, in the presence of ROS [60]. Tumor cells under oxidative stress including hyperoxia (HBO₂) produce ROS, which result in mutations [62]. Because HBO₂ therapy improves tumor oxygenation, the effect of carboplatin may be enhanced due to increased ROS after HBO₂ exposure. Another possible mechanism is that HBO₂ increases transendothelial transport of carboplatin because of inhibited P-glycoprotein, ATP-binding cassette transporter [63]. The exact mechanisms, however, are disputed.

III. RADIATION INJURY AND HBO₂

A. HBO₂ AS TREATMENT FOR RADIATION INJURY

Radiation injury of the brain is observed as the most debilitating sequelae of therapeutic radiation, occurring several months to a few years after radiotherapy. The incidence of this condition, which has been associated with treatments using either single or multiple radiation doses [64], is approximately 5% for conventional radiotherapy [65]. In contrast, stereotactic radiosurgery for brain lesions tends to cause a higher rate of radiation injury, with this condition being induced in up to 50% of treated brain metastases [66,67]. Radiation injury is a feared complication for the treatment of benign brain lesions where radiosurgery has become an important option.

Chin *et al.*, who followed radiation necrosis in 17 of 243 patients treated with radiosurgery, demonstrated that the symptomatic injury appeared four months after treatment and symptomatic and radiographic recovery times were 7.5 and 10.5 months, respectively [68]. Histological examination of rat brains found edema 14 days after receiving 200 Gy radiosurgery and development of necrosis at 21 days, and that the necrotic lesion was slightly enlarged at 60 days and had shrunk by 90 days [69]. Another animal study using cat brains receiving 125 Gy radiosurgery showed that an early necrotic lesion with engorged and thrombosed vessels was recognized at 3.5 weeks, progressive cavitation with necrotic debris from 12 to 29 weeks, and a glial scar without hypervascularity at 63 weeks [70]. These clinical and experimental studies suggest that the effects of brain radiation injury after radiosurgery are usually temporary and at least partial recovery within several months is expected. However, where radiation-induced necrosis of brain tissue occurs, it is not reversible. Histological characteristics of this condition in the early stage are coagulation necrosis with thickened vessel walls, thrombosed vessels, vascular proliferation and micro-hemorrhage [69,70]. These findings are similar to those in soft tissues including the skin, bladder and intestine [1,71]. The pathogenic mechanism of brain radiation injury is presumed to be primarily vascular, with necrosis and glial loss secondary to ischemia. HBO₂ therapy, which has been proven to be effective for radiation injury at other sites, is a promising therapeutic intervention for this condition of the brain [1].

Although many studies have shown that HBO₂ therapy effectively treats radiation injury in several organs, there are only a few case reports of this condition of the brain in the literature to date [72-76]. Chuba *et al.* reported that 10 young patients who had not responded to steroid therapy for this condition received HBO₂ therapy and all of them demonstrated initially stabilized or improved symptoms and/or imaging findings [72]. Tandon *et al.*, who treated a patient with serious radiation injury, managed its progression by steroid and anticoagulant treatments combined with HBO₂ [73]. Their reported case had gradual clinical improvement after the initiation of HBO₂ therapy, although the patient's condition had worsened despite steroid and anticoagulant therapies. In an abstract from a series of 29 patients with radiation injury receiving 20-60 sessions of HBO₂ therapy, 27 showed neurological improvement or stabilization and decreased steroid requirement, and only two patients had tumor progression clinically and radiographically [77]. HBO₂ therapy also

appears to be effective for brain radiation injury, especially in early or progressive stages. However, the therapeutic effects have not been conclusive [74].

B. HBO₂ AS PROPHYLACTIC AGAINST RADIATION INJURY

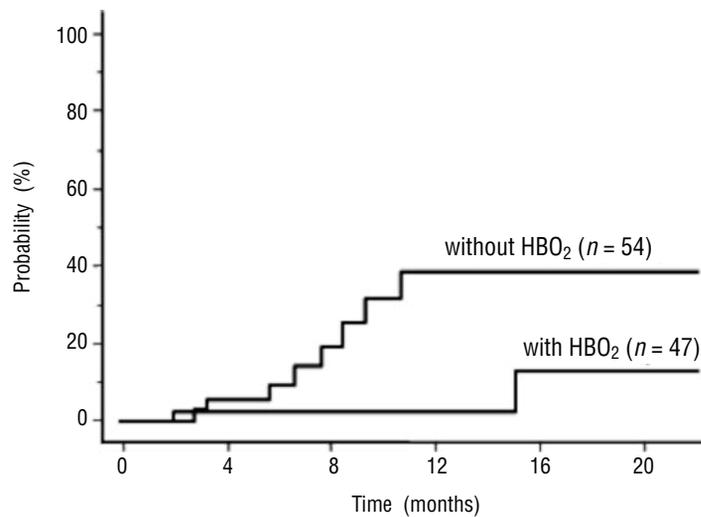
The above reports suggest a beneficial effect of HBO₂ therapy for pre-existing brain radiation injury. However, there have been no published clinical results considering HBO₂ therapy for the prevention of this condition in the central nervous system. In the spinal cord, two experimental studies could not show the prophylactic effects of HBO₂ therapy for radiation injury [78,79].

A pilot trial by Ohguri *et al.* was conducted to assess the preventive effects of HBO₂ therapy for radiation injury after stereotactic radiosurgery in patients with metastatic brain tumor [18]. Thirty-two patients with 47 brain lesions were prophylactic treated with HBO₂ therapy, and another 46 patients with 54 lesions did not undergo this treatment. HBO₂ therapy (2.5 atm abs, 60 minutes) was started within a week after radiosurgery, and 20 sessions of this treatment were performed for one month (five sessions per week). They divided radiation injury into two categories; one being radiation-induced necrosis (or tumor necrosis) at or near the tumor, and the other being white matter injury (WMI), defined as peritumoral hyperintensity areas on T2-weighted images of magnetic resonance imaging or radiation-induced brain edema [70]. Because of reversible effects of the WMI, this condition is considered to be an early or progressive stage of radiation injury. In their series, there were no differences between the two treatment groups in terms of tumor volume and radiation dose. However, all 21 patients who received fractionated standard radiotherapy prior or subsequent to radiosurgery were included in the HBO₂ group, but not in the non-HBO₂ group. Although the patients treated with radiosurgery plus standard radiotherapy were at high risk for radiation injury, the rate of radiosurgery-induced WMI was less frequent in the HBO₂ group (two lesions, 4%) than in the non-HBO₂ group (nine lesions, 17%) ($p=0.05$). Multivariate analysis by logistic regression to evaluate the effects of certain factors on WMI is shown in Table 3. None of the factors reached statistical significance, although trends toward significance were seen for HBO₂ therapy on WMI ($p=0.07$). As illustrated by Figure 2 and Table 3, one-year actuarial probabilities of radiation injury were 2% and 36% in the two groups ($p=0.02$). This preliminary clinical trial suggests that prophylactic HBO₂ therapy limits the progression of radiation injury of the brain after radiosurgery.

TABLE 3. Univariate analysis by the Kaplan-Meier approach with log-rank testing for evaluation of certain factors on white matter injury

| Variable | No. of brain metastases | WHITE MATTER INJURY | |
|-----------------------------------|-------------------------|--------------------------|----------------|
| | | Probability (%) (1 year) | <i>p</i> value |
| Patient age: $\geq 66/\leq 65$ | 56/45 | 21/16 | 0.44 |
| Tumor volume (cc): $>5/\leq 5$ | 34/67 | 17/19 | 0.70 |
| Marginal dose (Gy): $>20/\leq 20$ | 42/59 | 19/20 | 0.86 |
| Maximal dose (Gy): $>25/\leq 25$ | 66/35 | 20/17 | 0.68 |
| MX/MR: $\geq 1.5/<1.5$ | 16/85 | 11/20 | 0.88 |
| HBO ₂ : No/Yes | 54/47 | 36/2 | 0.02 |

MX: maximal dose within treatment volume; MR: marginal dose.
(modified table published in Int J Radiat Oncol Biol Phys 2007 [18])

FIGURE 2. Actuarial risk of developing white matter injury for the HBO₂ group and the non-HBO₂ group

The 1-year actuarial probability was 2% and 36% between the HBO₂ group and the non-HBO₂ group, respectively ($p = 0.02$).

(Reprinted with permission from Int J Radiat Oncol Biol Phys 2007 [18])

From clinical and experimental studies [68-70], endothelial cell degeneration of small vessels of the brain is observed within one month after radiosurgery. Using surgical wounds or experimental models, some investigators have indicated that HBO₂ is a greater stimulant of vascular endothelial growth factor (VEGF) production than hypoxia and mobilizes the endothelial stem/progenitor cells (SPCs) from the bone marrow [80-83]. Neovascularization occurs by two processes; one is regional new blood vessel growth (angiogenesis) and the other is formation of *de novo* vessels (vasculogenesis). HBO₂ has progressive effects on both these

processes. It has therefore been proposed that HBO₂ promotes neovascularization in the radiation-injured brain. Furthermore, intracellular adhesion molecule 1 (ICAM 1) is an inducible cell surface glycoprotein with diverse biological functions, including an important role in inflammation [84,85]. Its expression is increased in vascular endothelium within hours after irradiation [86]. Specifically, Prabhakarbandian *et al.* demonstrated that ICAM 1 expression is increased in human microvascular endothelial cells irradiated with a single 10-Gy dose of radiation [87]. One study showed that HBO₂ inhibits ICAM 1 expression in an *in vitro* model of

human endothelial cell ischemic injury [88]. It is possible that HBO₂ reduces endothelial inflammation mediated in part by ICAM 1 expression following radiation. However, further investigations are needed to define the precise nature of the mechanisms. Histologically, early radiation injury characterizing brain edema and vascular changes are already recognized 2 or 3.5 weeks post-radiosurgery in the experimental studies despite correlating radiation dose [69, 70]. Moreover, molecular response on the cultured human endothelium of cerebral blood vessels appears at 24 hours after a single gamma radiation dose of 50 Gy [89]. Ohguri *et al.* initiated HBO₂ therapy within one week after radiosurgery and continued for one month to protect against radiation injury [18]. This trial suggests that the results for the WMI were strongly associated with the timing of prophylactic HBO₂ therapy to control

early radiation injury of the brain. However, further evaluations of the HBO₂ dosing protocol, such as the number of treatments, treatment pressure and timing, using clinical trials and experimental analysis, are needed to confirm its definite benefit.

CONCLUSION

Based on clinical trials evaluating HBO₂ therapy in a range of neuro-oncology treatment settings, the accumulated data suggest the distinct possibility that HBO₂ therapy enhances the therapeutic effects of radiotherapy, potentiates carboplatin-based chemotherapy and repairs radiation injury or protects from it after stereotactic radiosurgery for brain lesions.

Conflict of interest

The authors report no conflict of interest with this submission.

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