Cerebral Blood Flow Increase During Prolonged Hyperbaric Oxygen Exposure May Not Be Necessary for Subsequent Convulsion

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Prolonged exposure to hyperbaric oxygen (HBO) can result in central nervous system (CNS) toxicity. The manifestations ranged from anxiety, nausea, altered vision, and altered hearing to muscular twitching, unconsciousness, and convulsions. It has been reported that cerebral blood flow (CBF) increases well before the onset of the first seizure, and the abnormal increase in CBF is an important precipitating factor of the seizures [1]. Further studies have shown that nitric oxide (NO), especially that produced by neuronal nitric oxide synthase (nNOS), is responsible for the increases in CBF [2–4]. Meanwhile many studies showed that reactive oxygen species (ROS) can also affect cerebrovascular activity [5, 6], and indeed in the course of HBO exposure, large amounts of ROS are formed [7]. In this study, we investigated the effects of two antioxidants, *N*-acetylcysteine (NAC) and vitamin E (Vit E), on the CBF during HBO exposure to preliminarily probe if ROS might also play a role in CBF changes during HBO exposure.

Male Sprague–Dawley rats were inserted two needle electrodes through the skull to record electroencephalogram (EEG). A fiber optic laser Doppler flowmeter probe fixed by a cylindrical eraser which was cemented to the skull was attached to the surface of dura for CBF measurement. The EEG and CBF were monitored throughout 5 atmosphere absolute (ATA) HBO exposure until the appearance of electrical discharge in EEG and tonic–clonic motions of the head and forelimbs. Latency to seizures was

calculated from the moment of pressure reaching 5 ATA to the moment of EEG discharge. The average values at every 1 min interval of CBF were calculated and expressed as percentages of the average value of the 1-min interval prior to the pressure reaching 5 ATA, which was used as the baseline level. Thirty minutes before HBO exposure, rats were injected intraperitoneally with saline or 200 mg/kg NAC, respectively. Vitamin E was orally administered with the dose of 600 mg/kg. Besides, a nNOS inhibitor, 7-nitroindazole (7-NI), which had been verified to have definite effects to CBF alterations and convulsion induced by HBO exposure, was selected as a positive control at a dose of 30 mg/kg (ip). All results were expressed as mean $\pm$ SD and analyzed with repeated ANOVA followed by Fisher's PLSD. A value of $P < 0.05$ was accepted as significant for statistical tests.

Our results showed HBO exposure could induce CBF increased gradually, and peaked before the onset of seizure at almost 50% above the basal level. 7-NI can downregulate the increase in CBF and markedly extend the latency period. These results were in agreement with previous studies [8]. Meanwhile, the results showed NAC and Vit E could also effectively inhibit the CBF increase that precedes seizures (Figures 1 and 2A). As NAC and Vit E could scavenge ROS, such as O_2^- , the present results indicate at least that ROS may also have very important effect on the increase in CBF induced by prolonged HBO exposure.

Figure 1 Representative changes of cerebral blood flow (CBF) and electroencephalogram (EEG) in control, 7-nitroindazole (7-NI), *N*-acetylcysteine (NAC), and Vitamin E groups.

Figure 2 Statistical results of cerebral blood flow (CBF) and convulsion latency. In **(A)**, each spot represents the CBF average values of a 1 min interval. Results are expressed as a percentage of these averages prior to the chamber pressure reaching to 5 atmosphere absolute (ATA), which was used as the baseline level. In **(B)**, latencies were calculated based on the electroencephalogram (EEG) results. 7-Nitroindazole (7-NI) dramatically delayed the onset of seizures, while *N*-acetylcysteine (NAC) and Vitamin E had no any effects on convulsion latency at selected doses. * $P < 0.01$ versus control group, $n = 8$.

An unexpected finding of this study was that the results showed although NAC and Vit E could inhibit increases in CBF, but they had no protective effects of extending the latency of convulsion caused by HBO exposure at the doses evaluated (Figure 2B). Such results indicate that the abnormal rise in CBF prior to seizure may not be a necessary and sufficient condition for the succedent convulsion.

Among the drugs selected in this study, the biggest difference was that after administration of 7-NI, NO production was markedly inhibited, but with NAC or Vit E, NO was still generated in large quantities. Therefore, it could be speculated that NO, especially produced by nNOS, is extraordinarily crucial to the seizure, and the primary mechanism may be through the neurotoxicity which involved several downstream signaling pathways, instead of its vasodilating and overly increasing CBF action. It has been reported that exposure to HBO above 2.5 ATA can lead to a markedly increase in brain NO levels [9]. Besides, numerous studies have shown that neurons are sensitive to NO-induced excitotoxicity because NO can rapidly inhibit mitochondrial respiration and induce both depolarization and glutamate release which together activate the NMDA receptor. In fact, high levels of NO could cause acute glutamate release from neurons within seconds, which is attributed either to inhibition of mitochondrial respiration followed by reversal of glutamate uptake or to a direct (calcium-independent) activation of vesicular exocytosis by modifying protein thiols [10].

Our studies conclusively show that the abnormal increases in CBF during prolonged HBO exposure can be suppressed through inhibiting ROS production, but the seizures were not postponed, which indicate the abnormal rise in CBF prior to seizure may not be a necessary and sufficient condition for the succedent convulsion. Our data also indicate that NO derived from nNOS plays a very important role in CNS oxygen toxicity. It may participate in CNS oxygen toxicity through complicated molecular mechanisms requiring to be fully defined.

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