The effect of hyperbaric oxygenation on bone in spontaneously hypertensive rats

Tatsuo Matsuda[1], Yukiharu Hasegawa[1], Yuji Kataoka[1], Hisashi Iwata[1], Takayuki Miura[1] and Hideyo Takahashi[2]

We investigated whether hyperbaric oxygenation treatment can enhance osteogenesis in spontaneously hypertensive rats (SHRs). 8-week-old SHRs were exposed to hyperbaric oxygen for 6 weeks and were killed at 17 weeks of age. Wistar-Kyoto normal rats and untreated SHRs of the same age were used as controls. Radiographic measurements of cortical thickness and length of femora showed that these parameters increased in oxygen-treated SHRs; they became almost normal. Ash weight and ash, Ca and P content of L5 vertebras showed similar results. We conclude that high partial pressure oxygen has an effect on osteogenesis in SHRs.

Departments of [1]Orthopedics and [2]Hyperbaric Medicine, Nagoya University School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya, 466 Japan. Tel +81-52 741 2111. Fax -52 741 4512
Submitted 92-01-25. Accepted 92-08-13

Hyperbaric oxygenation therapy (HBO) enhances oxygen transport in ischemic tissues and thereby accelerates tissue regeneration. Bone revival can be also expedited by utilizing HBO, which has been reported to accelerate fracture healing and osteogenesis enhancement (Coulson et al. 1966, Penttinen et al. 1972). However, the effect of HBO on osteoporosis or osteopenia has never been investigated. Spontaneously hypertensive rats (SHRs), which are often used in cardiovascular experiments, are known to show skeletal disorders, including growth disturbance and osteopenia (Izawa et al. 1985, Hirano et al. 1988). We have studied whether HBO treatment in SHRs can prevent bone disorders, growth disturbance, and osteopenia.

Material and methods

20 male SHRs and 10 male normotensive Wistar-Kyoto rats (WKYs) were obtained from Nippon Charles-River Co. at 4 weeks of age. The spontaneously hypertensive rats were divided into 2 groups as follows: HBO-SHRs (10) and SHRs (10). HBO-SHRs were exposed to HBO for 60 minutes under 2.8 atmospheres absolute pressure (ATA) of pure oxygen, during 8–13 weeks of age for 6 weeks (5 days a week, a total of 30 occasions). The rats were housed in cages and were fed ad libitum a standard laboratory diet (Nippon Crea Inc.) throughout the experiment. All the rats were killed by ether inhalation at 17 weeks of age. The soft tissues were removed, and femurs and vertebras were fixed in 70% ethanol. Lateral radiographic projections of the femurs were obtained using a soft x-ray (Softex Co., Ltd), according to Okumura et al. (1984), and cortical thickness indices (CTI; Figure 1) of femurs at the middle of the whole bone were measured with a microdensitometer (PDS-15, Konishiroku Co.). The lengths of the femurs from the top of the major trochanter to the distal end were measured on the radiograms with a Digitizer (KL 4300, Graphite Co., Ltd). The wet weights of L5 vertebras were measured and the vertebras subsequently ashed in porcelain crucibles at 660 °C for 20 hours to obtain the ash weight. The calcium content was measured with atomic absorbance analysis and phosphate

Figure 1. Cortical thickness index (CTI) of femora.

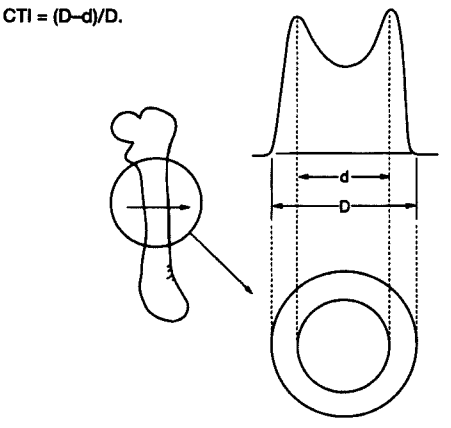

Table 1. CTI, length of femora and ash weight of L5 vertebrae. Mean *SD*

Group	n	CTI	Femoral length (mm)	n	Ash weight (mg)
WKYs	18	0.29 *0.03*[a]	34.3 *0.70*	9	64.1 *4.8*
SHRs	16	0.25 *0.02*	33.8 *0.59*	8	60.2 *7.6*
HBO-SHRs	16	0.28 *0.02*[a]	35.0 *0.47*[b]	8	67.5 *5.2*[c]

[a] $P < 0.01$ (vs. SHRs), [b] $P < 0.001$ (vs. SHRs), [c] $P < 0.05$ (vs. SHRs).

Table 2. Ash, Ca, and P content/wet bone weight (percent) of L5 vertebrae. Mean *SD*

Group	n	Ash	Ca	P
WKYs	9	52 *1.7*	22 *1.2*	9.8 *0.4*
SHRs	8	49 *1.6*[a]	21 *0.6*	9.5 *0.3*
HBO-SHRs	8	52 *1.3*[c]	22 *0.6*[b]	10 *0.2*[c]

[a] $P < 0.01$ (vs. WKYs),
[b] $P < 0.05$ (vs. SHRs),
[c] $P < 0.01$ (vs. SHRs).

content with Goldenberg's method. Ash, Ca and P were expressed as a percentage of the wet weight. All the results were expressed as mean (*SD*), and statistical analysis was performed by the Student's *t*-test.

5 rats were excluded; 2 in HBO-SHRs, 2 in SHRs and 1 in WKYs died of unknown causes before 17 weeks of age. The 2 HBO-SHRs showed no obvious findings of oxygen toxicity at autopsy. We reviewed the other rats which survived until the end of this experiment. There were no significant differences in the body weight among the groups.

Results

Cortical thickness index (CTI). The mean CTI of femurs was higher in HBO-SHRs than in SHRs and the same as in WKYs.

Length of femur. The mean length of the femurs in HBO-SHRs was larger than in SHRs and the same as in WKYs.

Ash weight. The mean ash weight of L5 vertebras was higher in HBO-SHRs than in SHRs and the same as in WKYs (Table 1).

Ash Ca and P content. The mean ash Ca and P percentages of L5 vertebras in HBO-SHRs were higher than those in SHRs. There were no differences between HBO-SHRs and WKYs (Table 2).

Discussion

Several reports on the effects of HBO upon osteogenesis have been published (Coulson et al. 1966, Yabron and Cruess 1968, Penttinen et al. 1972, Mainous 1977, Nilsson 1988). Mainous (1977) treated mandibular osteoradionecrosis with HBO and concluded that when the partial pressure of oxygen increased, collagen formation and fibroblastic proliferation, capillary budding, osteoblastic and osteoclastic activity, callus formation and mineralization were all increased. On the contrary, some reports have suggested that anerobic conditions enhance osteogenesis (Brighton and Krebs 1972, Heppenstall et al. 1975). Boskey (1981) described that the energy required for calcification is produced under aerobic conditions, i.e., ATP is produced from the TCA cycle in mitochondria and can be detected at the epiphysis and in cancellous bone; also calcium release from mitochondria requires anerobic conditions. This implies that both aerobic and anerobic conditions are necessary for bone formation. We postulate that the production of energy for calcification is enhanced during exposure to HBO, but the interval is essential. Penttinen et al. (1972) treated femoral fractures of rats using HBO (2.5 ATA 2 hours twice daily) to find that callus formation and hypertrophy of cartilage at the metaphysis increased more than in controls. He suggested that intermittent exposure to HBO might enhance osteogenesis. Concerning the growth of bone and oxygen tension, Persson (1968) reported that there was a stimulation of growth in rabbit tibia during the exposure to 100% oxygen at 1 ATA and a decrease in growth when the oxygen tension was reduced below normal. He concluded that the cartilage cells in the zone of degeneration and calcification were very sensitive, and dependent on oxygen.

The purpose of this investigation is to determine the possibility of such osteogenic enhancement to osteoporosis or osteopenia and bone growth disturbance. There are few reports showing such data. We used SHRs as animals because they were easily available and developed bone disorder. SHRs were isolated from WKYs by Okamoto and Aoki (1963), and have been used for experiments in essential hypertension.

Izawa et al. (1985) reported that the bone volume in SHRs is lower than that in WKYs 12 weeks of age. Hirano et al. (1988) demonstrated longitudinal growth disturbance and osteonecrosis of the femoral head. Lucas et al. (1986) described that abnormality of Ca absorption and vitamin D metabolism is involved in these bone disorders. However, our investigation showed that bone volume and length of femora in HBO-SHRs are close to those in normal rats at the age of 17 weeks, indicating that oxygenation might activate osteogenesis as Mainous and Persson mentioned. We had no speculation about the HBO effect on normal rat bone. Kawamura et al. (1978) stated that HBO had no effect on the normal tissue but had a beneficial effect on the hypoxic tissue in dogs. Shaw et al. (1967) showed that the excessive exposure to high concentrations of oxygen cause cultured tissue destruction. HBO is known to be more effective on hypoxic tissue with ischemia. We agreed with this principle.

We previously investigated necrosis of the femoral head in SHRs and described the low blood flow, accompanied by an increase in the capillary vessels' resistance to femoral head necrosis, which might cause local hypoxia, and that was the reason why HBO was favored (Kataoka et al. 1992). Considering the result of this bone investigation, it is also suggested that such low blood flow and hypoxia occur in the metaphysis and spongiosa. This may cause the disorders and can be treated well with HBO.

The method for determination of the relationship between oxygen and bone is not easy but of interest. Further studies are planned.

Acknowledgements

We would like to thank Mr.Y Miwa, Mr. J Suzuki and Mr. J Miyakawa for technical assistance.

References

Boskey A L. Current concepts of the physiology and biochemistry of calcification. Clin Orthop 1981; 157: 225–57.

Brighton C T, Krebs A G. Oxygen tension of healing fractures in the rabbit. J Bone Joint Surg (Am) 1972; 54 (2): 323–32.

Coulson D B, Ferguson A B Jr, Diehl R C Jr. Effect of hyperbaric oxygen on the healing femur of the rat. Surg Forum 1966; 17: 449–50.

Heppenstall R B, Grislis G, Hunt T K. Tissue gas tensions and oxygen consumption in healing bone defects. Clin Orthop 1975; 106: 357–65.

Hirano T, Iwasaki K, Yamane Y. Osteonecrosis of the femoral head of growing, spontaneously hypertensive rats. Acta Orthop Scand 1988; 59 (5): 530–5.

Izawa Y, Sagara K, Kadota T, Makita T. Bone disorders in spontaneously hypertensive rats. Calcif Tissue Int 1985; 37 (6): 605–7.

Kataoka Y, Hasegawa Y, Iwata H, Matsuda T, Genda E, Miura T, Takahashi H. Effect of hyperbaric oxygenation on femoral head osteonecrosis in spontaneously hypertensive rats. Acta Orthop Scand 1992; 63 (5): 527–30.

Kawamura M, Sakakibara K, Yusa T. Effect of increased oxygen on peripheral circulation in acute, temporary limb hypoxia. J Cardiovasc Surg (Torino) 1978; 19 (2): 161–8.

Lucas P A, Brown R C, Drueke T, Lacour B, Metz J A, McCarron D A. Abnormal vitamin D metabolism, intestinal calcium transport, and bone calcium status in the spontaneously hypertensive rat compared with its genetic control. J Clin Invest 1986; 78 (1): 221–7.

Mainous E G. Hyperbaric oxygen in maxillofacial osteomyelitis, osteoradionecrosis, and osteogenesis enhancement. In: Hyperbaric Oxygen Therapy. Undersea Medical Society Inc, Bethesda 1977: 191–216.

Nilsson L P. The effect of hyperbaric oxygen treatment on bone regeneration. An experimental study in the rabbit using the bone harvest chamber. Intern J Oral Maxillofac Impl 1988; 3: 43–8.

Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. (in Japanese). Jpn Circ J 1963; 27: 232–93.

Okumura H, Yamamuro T, Kasai R, Ichisaka A, Hayashi T, Matsushita M. Microdensitometrical analysis of the bones of experimental animals (in Japanese). In: Handbook of Bone Histomorphometry. Nishimura Shoten Ltd, Niigata 1984; 3: 99–109.

Penttinen R, Niinikoski J, Kulonen E. Hyperbaric oxygenation and fracture healing. A biochemical study with rats. Acta Chir Scand 1972; 138 (1): 39–44.

Persson B M. Growth in length of bones in change of oxygen and carbon dioxide tensions. Acta Orthop Scand (Suppl 117) 1968: 1–99.

Shaw J L, Bassett C A. The effects of varying oxygen concentrations on osteogenesis and embryonic cartilage in vitro. J Bone Joint Surg (Am) 1967; 49 (1): 73–80.

Yabron I G, Cruess R L. The effect of hyperbaric oxygen on fracture healing in rats. J Trauma 1968; 8 (2): 186–202.