Study on animals' femur blood flow alteration under inadequate decompression with ^{133}Xe isotope washout method

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Abstract Animals' regional femur blood (F) alteration was studied under an inadequate decompression by using the inhaled isotope ^{133}Xe washout method. Results showed that the average F on left and right side of minipigs' femur decreased from 15.4±1.8 and 16.9±2.0 ml/(100 g·min) (before exposure) to 10±1.8 and 11.1±1.6 ml/(100mg·min) (after exposure for 1.5 h under the pressure of 0.5 MPa and then decompression to normal with the rate of 0.03~0.04 MPa/min), respectively; the blood flow of hematopoietic marrow tissues (f_1) on both sides also decreased from 19.3±2.0 and 22.1±1.9 ml/(100 mg·min) to 13.9±1.4 and 13.8±1.0 ml/(100 mg·min). The exact same alteration also happened in the experiment on New Zealand rabbits. It indicates that inadequate decompression in hyperbaric exposure can give rise to decrease animals' bone blood flow and insufficient bone blood supply serves as one of the reasons for causing decompressive osteonecrosis.

Keywords Bone blood flow, ^{133}Xe washout method, Decompressive osteonecrosis, Minipigs, New Zealand rabbits

1 Introduction

Divers and workers for working in hyperbaric environment frequently suffer from decompressive osteonecrosis because of improper decompression and other reasons. The incidence of the disease is very closely related to the time and degree of hyperbaric exposure. According to surveys conducted by some countries on divers or those who work in hyperbaric environment or in deep tunnels for 30~40 years, 20%~60% of the subjects suffer from bone injury.[1] Although reasons for osteonecrosis are still not completely clear for the time being, the major reason given by most theories is that various blockers, such as bubble, thrombus and fat embolism formed during decompression, bring out regional blood shortage in bone tissues and then result in bone nutrition obstruction. This study makes use of ^{133}Xe isotope washout method to measure minipigs' and New Zealand rabbits' femur blood flow under inadequate decompression, aiming to obtain blood dynamics data which are helpful to illustrate the mechanism of decompressive osteonecrosis and to observe the relationship between symptoms of decompression sickness and blood flow alteration.

2 Materials and methods

2.1 Hyperbaric exposure and decompression

2.1.1 Animals

15 minipigs at the age of 2 (9 female, 6 male), weighing 11.5-25.0 kg and bought from Guizhou Experiment Animals Institute; 10 New Zealand rabbits, bought from Shanghai Seed Multiplication Farm of Experiment Animals. The heart rate and the mental status, examined before experiment, were normal. 25 measurements of bone blood flow among the 25 animals were performed to observe the normal value of bone blood flow before hyperbaric exposure. The measurement of bone blood flow after inadequate decompression was performed on 7 minipigs and 5 rabbits.

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2.1.2 Decompression experment

The animals were placed in batches into a $0.05m^3$ hyperbaric cabinet, in which the pressure was increased to 0.5 MPa(~5atm) at the rate of 0.25 MPa/min, 1.5 h later the pressure was uniformly decreased to the normal at the rate of 0.03-0.04 MPa/min. When the animals were taken out of the cabinet, their behaviors, states and symptoms of decompression sickness were observed and their heart rate was measured. Doppler ultrasonic bubble measurer was placed on the precordia to measure the bubble sound, after which bone blood flow was measured in isotope laboratory.

2.2 Measurement of bone blood flow

2.2.1 Measurement equipment

^{133}Xe. isotope washout method was used to measure regional femur blood flow. The animals were fixed standing on a special wooden frame, with face guard on for breathing. They inhaled ^{133}Xe gas mixture in airtight circuit system for 2 min (the radioactivity of ^{133}Xe was about 1.1×10^{-7} Bq/L). Two NaI (Tl) scintillation detectors of 20 mm in diameter were pressed in proximal femur on left and right sides, respectively. The crystal surface of a probe equipped with a cylinder-shaped Pb collimator, was 20 mm far from the skin. Femur radioactive counts were recorded for 30 min after ^{133}Xe inhale finished. The data were automatically recorded, stored by multi-functional blood flow measurer connected to a computer, which thereafter counted out the value of bone blood flow with the program compiled on the basis of bone blood flow counting model.

2.2.2 Bone blood flow counting model

The inhaled ^{133}Xe washout can be described by following formulas:

$$C(t) = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} \qquad (1)$$

where $C(t)$ is the ^{133}Xe radioactive count (cpm) at t moment after ^{133}Xe inhale finishes; A_1, A_2 mean the ^{133}Xe radioactive count (cpm) at the moment ^{133}Xe inhale finishes ($t = 0$); k_1, k_2 mean the ^{133}Xe discharge rate constant from all the bone tissue banks. $k=\ln 2/T(h)$. $T(h)$ is the ^{133}Xe semi discharge term from all bone tissue banks (min).

According to Fick theory, f_i, the blood flow of all bone tissue banks can be described with the following formula:

$$f_i = \lambda_i k_i \qquad (i = 1, 2) \qquad (2)$$

where λ_i is the distributive coefficient of ^{133}Xe in blood and the i-th bone blood tissue bank. i=1,2 represent, respectively, the ^{133}Xe washout from hematopoietic marrow tissues (the sharp down curve) and from nonhematopoietic marrow tissues (the gradual down curve) such as compact bone, net sponge bone and endosteal fat. The average bone blood flow F is the weighted sum of blood flow of all tissue banks:

$$F = \Sigma w_i f_i = \Sigma w_i \lambda_i k_i \qquad (3)$$

where w_i is the weighted factor of all bone tissue banks in total bone mass.

3 Results

3.1 Normal bone blood flow value

Averages of femur blood flow of the 15 minipigs measured before hyperbaric exposure are listed in Table 1. The averages F of bone blood flow are 15.4±1.8 and 16.9±2.0 ml/(100 g.min) on left and right side, respectively. The F values on left or right side, of male or female minipigs show little difference($P>0.20$). The averages f_1 of blood flow for hematopoietic marrow, are 19.3±2.0 on the left and 22.1±1.9 ml/(100 g.min) on the right. This portion of marrow tissue forms the major component of bone blood flow. The averages f_2 of blood flow for nonhematopoietic marrow are 7.9±1.0 on the left and 9.7±1.2 ml/(100 g.min) on the right. The measured results on 10 New Zealand rabbits are listed in Table 2, the F values, bone blood flow, on both sides being 14.5±1.7 and 14.1±1.9 ml/(100 g.min) respectively.

3.2 Bone blood flow after inadequate decompression

3.2.1 Bone blood flow alteration

Table 3 gives the measured data of 7 minipigs' bone blood flow before hyperbaric exposure and after inadequate decompression. The comparison of the average F, f_1 and f_2 values is shown in Fig.1. It can be noticed that, after inadequate decompression, both the bone blood flow average F and the blood flow of hematopoietic marrow f_1 are noticeably lower than those values ($P < 0.05$) before hyperbaric exposure. The F values, before exposure, 15.4±1.8 and 16.9±2.0ml/(100 g.min) on the left and right respectively, decrease to 10.3±1.8 and 11.1±1.6 ml/(100 g.min). The f_1 values before exposure, 19.3±1.0 and 22.1±1.9 ml/(100 g.min), decrease to 13.9±1.4 and 13.8±1.0 ml/(100 g.min). The f_2 values show no so noticeable difference compared with those before exposure (P >0.05). The measured results of bone blood flow before hyperbaric exposure and after inadequate decompression on New Zealand rabbits are listed in Table 4, and show the same alteration rule as that of minipigs.

Table 1 The proximal femur blood flow of minipigs (ml/(100 g.min), n=15)

No	Sex	Mass /kg	Left side F	Left side f_1	Left side f_2	Right side F	Right side f_1	Right side f_2
1	F	24.0	11.2	15.3	3.0	9.8	13.7	4.7
2	F	12.5	11.0	14.1	6.2	16.9	19.4	5.3
3	F	12.5	13.1	17.5	5.0	18.4	24.3	10.1
4	M	16.0	11.9	15.6	8.6	12.1	17.9	5.1
5	M	25.0	15.2	21.5	6.4	17.4	24.0	6.1
6	M	16.5	9.4	13.7	1.5	12.4	17.3	3.2
7	F	12.0	21.7	26.1	11.1	24.7	20.3	11.0
8	F	14.0	15.1	17.5	7.1	13.1	15.8	8.9
9	F	14.5	25.4	32.6	14.5	19.7	25.0	10.8
10	M	19.0	13.9	18.4	7.5	18.1	15.6	6.6
11	F	19.0	13.2	16.8	10.7	14.9	21.2	14.0
12	F	19.0	13.1	7.0	6.1	14.6	2.7	11.9
13	M	16.0	17.4	20.2	13.8	21.9	24.2	18.8
14	M	16.5	11.9	14.2	4.5	13.9	17.6	5.9
15	F	11.5	21.3	26.8	16.2	18.2	23.4	8.8
$\bar{x}$		16.7	15.4	19.3	7.9	16.9	22.1	9.7
s			1.8	2.0	1.0	2.0	1.9	1.2

Table 2 The proximal femur blood flow of normal rabbits (ml/(100 g.min), n=10)

No	Sex	Mass /kg	Left side F	Left side f_1	Left side f_2	Right side F	Right side f_1	Right side f_2
01	F	3.0	17.6	24.5	10.7	15.2	22.1	7.8
02	M	2.7	13.3	21.9	4.9	16.4	22.9	6.4
03	M	2.1	14.6	19.7	8.3	12.8	18.9	7.7
04	F	2.3	12.6	15.7	9.0	11.6	15.3	7.8
05	F	2.7	15.2	18.1	8.8	14.1	16.5	8.1
06	F	2.6	12.3	13.2	8.9	14.6	14.1	12.2
07	F	3.0	16.2	18.1	14.0	17.7	18.3	9.7
08	F	2.9	15.4	16.6	13.6	14.2	14.7	13.2
09	M	2.8	13.2	21.5	6.4	12.0	22.1	4.1
10	F	3.0	14.8	21.6	7.4	12.7	17.5	6.1
$\bar{x}$		2.7	14.5	19.1	9.2	14.1	18.2	8.3
s			1.7	3.4	2.9	1.9	3.2	2.8

Table 3 Femur blood flow of minipigs after rapid decompression (ml/(100 g.min), n=7)

No	Dysbarism symptom	Left side F	Left side f_1	Left side f_2	Right side F	Right side f_1	Right side f_2
1	middle	7.5	13.6	60	6.3	9.7	4.3
2	middle	8.3	12.0	3.9	10.5	14.8	7.3
4	serious	6.1	11.4	4.5	6.9	13.3	2.2
7	middle	6.3	10.1	5.6	8.2	11.8	6.6
13	serious	18.7	19.3	7.7	16.7	16.9	14.1
14	light	11.4	12.4	6.4	14.7	16.2	10.3
15	light	13.7	18.4	10.7	14.3	14.1	14.5
$\bar{x}$		10.3	13.9	7.8	11.1	13.8	8.5
s		1.8	1.4	1.8	1.6	1.0	1.8

Table 4 Femur blood flow of rabbits before and after rapid decompression (ml/(100g.min), n=5)

No	Dysbarism symptom	Before		After	
		Left	Right	Left	Right
02	Serious	11.7	9.8	8.9	11.3
03	Middle	15.5	10.9	14.2	15.4
04	Light	15.5	18.5	15.3	10.1
05	Light	17.7	16.2	12.8	15.2
06	Serious	20.8	20.1	9.1	7.9
x		16.2	15.1	12.1	12.0
S		2.4	3.8	2.4	2.6

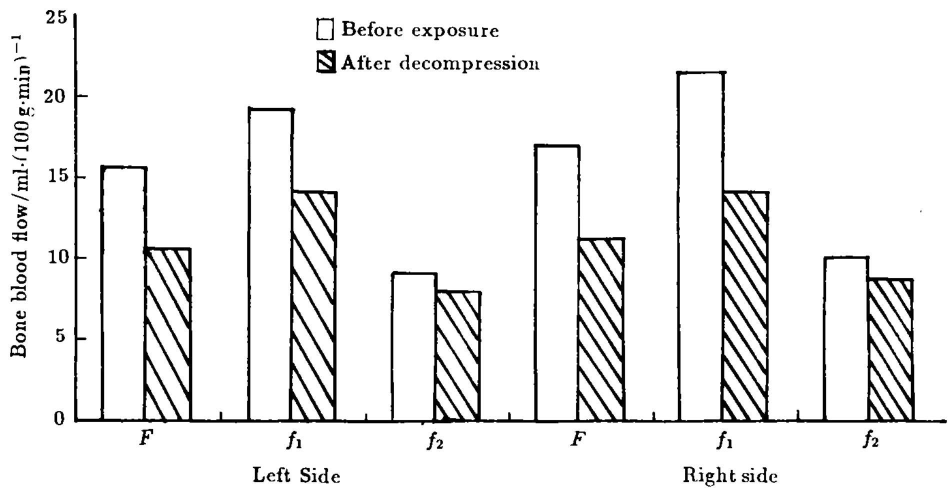

Fig.1 Comparison of bone blood flow in minipigs after decompression and before exposure

3.2.2 The relationship between bone blood flow alteration and symptoms of decompression sickness

5 minipigs among the 7 showed symptoms of decompression sickness at different levels after inadequate decompression. The sickness of 1 minipig was slight and the only symptom was the weak limbs, while the sickness of the other 4 was more serious with the symptoms of agitation, breath quickened, out of breath and weakness accompanied by struggling and choke. 2 minipigs (No.4, 13) received in-time remedy of pressure increase, which resulted in the survival (No.13) and the death (No.4) 6 h after they were taken out of the cabinet. The other 2 minipigs among the 7 showed no noticeable symptoms of decompression sickness and behaved as usual. Doppler ultrasonic measurer showed the bubble sound of all animals were over level Ⅲ. The average heart rate was 110±9.1 (ls) bpm before pressure increase and kept unchanging at 104±8.1 bmp after decompression.

Table 3 lists the relationship between bone blood flow alteration and symptoms of decompression sickness of minipigs. 4 of the 5 cases which had symptoms of decompression sickness showed bone blood flow decrease at different levels. The other 1 case (No.13) showed no noticeable alteration on the left and a slight decrease on the right. One of the 2 cases which didn't gave symptoms of decompression sickness (No.15) decreased in bone blood flow and the other (No.14) didn't show noticeable alteration in bone blood flow.

4 Discussion and Summary

The method of using ^{133}Xe isotope washout to measure bone blood flow was firstly reported by Lahtinen *et al.*[2,3] This method has such advantages as no trauma and repetitious measurement. Furthermore, it can be used to distinguish the bone blood flow of hematopoietic marrow and that of other bone tissues. In hyperbaric medicine, Pooley *et al.* measured marrow blood circulation during simulated diving period and after decompression by injecting ^{133}Xe physiological saline solution into bone of home rabbits. The measurement showed the ^{133}Xe washout rate from marrow increased (i.e. blood flow increased) during hyperbaric exposure. After decompression, the washout rate decreased or stopped completely.[4] The present study replaces intravenous injection or injection into bone with ^{133}Xe inhale to measure bone blood flow alteration in minipigs after inadequate decompression. The result is that inadequate decompression does give rise to the decrease in bone blood flow of minipigs, which is basically in conformity with Pooley's conclusion.

It is considered for the time being that the decrease in bone blood flow after inadequate decompression are mainly due to marrow pressure alteration, too much fat in marrow and thrombus caused by air embolism or fat embolism. Hills *et al.* indicate that decrease in marrow pressure can give rise to increase in bone blood flow, and vice versa.[5] Therefore, any factors that possibly increase marrow pressure will result in decrease in bone blood flow and then inadequate bone blood supply. If marrow pressure increases for a long time, osteonecrosis will inevitably be brought out. Walder *et al.* indicate that fat in marrow cavity is the determinant of osteonecrosis since decrease in bone blood flow caused by increase in marrow pressure is closely related to the fact that volume of fat in marrow will expands when decompression happens.[6] It is shown by data of the present article that decrease in bone blood flow of experiment animals is mainly caused by decrease in blood flow of hematopoietic marrow while blood flow of nonhematopoietic marrow has no noticeable alteration, which supports the idea of Walder. On the other hand, there's no apparent relationship between decrease in bone blood flow and seriousness degree of decompression sickness. Decrease in bone blood flow at different levels appears on those pigs which have no symptoms of decompression sickness.

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