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Comparison of Intravascular Bonghan Ducts from Rats and Mice

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Abstract

New method to obtain Intravascular Bonghan ducts of rats and mice were developed. By extracting blood from caudal veins and arteries that were hold by forceps we took broken pieces of BHDs which were examined using microscopes. The advantage of this method is to gain the pure BHD without fibrin, and its disadvantage is the smallness of the sample.

Key words : Intravascular Bonghan ducts, rats and mice

쥐와 생쥐의 혈관내 봉한관을 채취하는 새로운 방법을 개발하였다. 복대정맥과 동맥에서 양 끝을 겸자로 묶고 혈액을 뽑아냄으로써 부서진 봉한관 조각들을 찾아 광학 현미경으로 관찰하는 방법이다. 장점은 피브린이 없이 순수 봉한관을 얻는 것이고, 단점은 그 길이가 너무 짧은 점이다.

Introduction

Therapeutic effects of acupuncture has been widely accepted as an alternative medicine,[1] and the acupuncture points and

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meridians were shown to have peculiar physiological characteristics : In particular they have low electrical resistance and impedance compared to non-acupoints,[2] which have been applied for electro-therapeutic treatments.[3] However, the search for anatomical structure corresponding to the acupoints have not been successful.[4] Nevertheless, Bonghan Kim reported in a series of articles that he discovered the anatomico-histological system in the living body representing the acupoints and meridians.[5]

Bonghan ducts(BHD) which connect the acupoints are thin threadlike ducts composed of multiple tubular structures. They are distributed in the superficial layer of the skin and they are just the network of the meridians. More surprisingly, the BHDs link the acupoints to the internal organs, and they form an integrated circulatory system entirely different either from the nervous system or blood and lymphatic vessels. The BHD carries electrical and mechanical signals originated at the acupoints to the corresponding internal organs. Through the BHD flows biochemical fluids (Bonghan Liquid) containing a large amount of DNA which plays an essential role in cell-regeneration and repair.[5]

One of the most remarkable aspects of Bonghan theory is that the Bonghan ducts are distributed even within the artery, vein and lymphatic vessel in an isolated manner as well as in the superficial layer of the skin. These intravascular BHDs have not been confirmed by independent researchers for a long time. Bonghan Kim stated that he used staining technique to trace the BHD without a detail description of procedures and the dye substance, which kept people from reproducing his results.

Only very recently a method which does not use a staining dye was applied to take samples of the BHD inside blood vessels of rabbits and rats.[6, 7] In this method dextrose solution is injected into the femoral vein to dilute the blood until the blood vessel is transparent, and strings of fibrin with coagulated blood are observed in the caudal veins, and arteries. Pieces of broken BHDs are obtained from the strings of fibrin using processes to separate them from the fibrin either by an electrical method,[8] or urokinase solution. The BHD shows a substructure of smaller tubules by the light microscopy (Fig.1), and these tubules can be separated by application of an electric field as shown in Fig.2. Studies by Mallor's triple and Verhoff's elastic stain revealed thin collagen fibers intermingled with connective tissues, and abundance of elastic fibers, respectively[9](Fig.3).

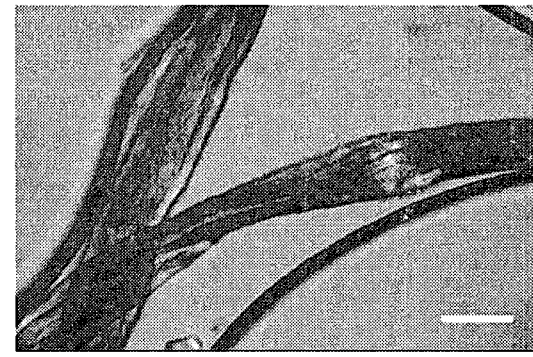

Fig.1. The substructure of smaller tubules inside the Bonghan duct

Fig. 2. A Bonghan duct is disunited to the separated tubules by application of an electric field

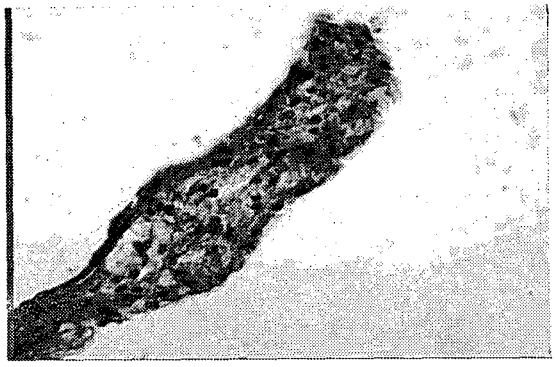

Fig.3. Histological studies of the Bonghan duct by allor's triple and Verhoff's elastic stain revealed thin collagen fibers intermingled with conne. ctive tissues, and abundance of elastic fibers.

The above injection method of dextrose solution has a shortcoming of not taking the BHD samples directly from the blood vessels, but indirectly from the fibrin strings which include randomly the broken pieces. Thus it would be desirable to obtain pure BHDs directly from the blood vessels. In this article we present an improved method to get the BHDs from the caudal vena cava and artery of rats and mice. Both sides of the caudal vein or artery are blocked by forceps, and blood are rapidly sucked out through a needle with a manual syringe such that pieces of BHDs are taken out with the blood. Examing the sucked out blood under a microscope we get broken pieces of BHDs which are about 20㎛ order in diameter and 1mm order in length.

It is the first report on the intravascular BHD of mouse which we found that the diameter and shape of mouse BHD are similar to those of rat BHD despite the large size difference between the diameters of corresponding blood vessels. This might suggest that the diameters of BHDs are more or less determined by the size of granules in the Bonghan liquid which flows through the BHD.

Obviously further improvements are required to fully reveal the whole system of intravascular Bonghan ducts. Notwithstanding the incompleteness of the technique our method yielded pure BHD samples from a mouse and a rat reliably and reproducibly such that further analysis of the samples could be possible, which will reveal detail structures, biochemical compositions, and other important physiological properties. The reliable reproducibility is the most important advantage of the new method to confirm the existence of hitherto unknown threadlike structure inside blood vessels.

Method and Procedure

Sprague-Dawley rats and I.C.R mice were

obtained from the Laboratory Animal Center of Seoul National University. They were housed in a constant temperature-controlled environment(23°C) with relative humidity 60%. All animals were fixed at 12 hr. light-dark cycle, and had a libitum access to food and water. Procedures involving animals were in full compliance with current international laws and policies(NIH guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985).

A Rat or a mouse is anesthetized with barbital sodium(450mg/kg) administrated intra-peritoneally. All surgical procedures were performed under general anesthesia. Under deep anesthesia, the frontal side of the animal is incised, and stomach, intestines, and perivascular fats were removed such that vascular systems are exposed for easy approach.

Two sides of the caudal vein and artery were tightly blocked using forceps. Blood was sucked out rapidly from the abdominal artery by a needle with a manual syringe(Fig.4).

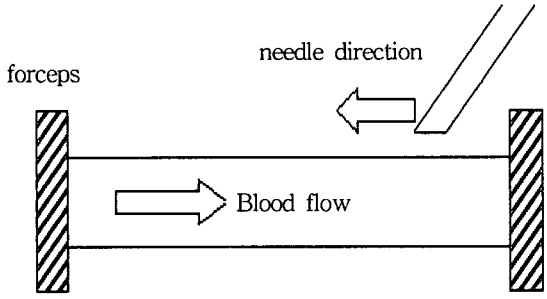

Fig.4. Both ends of the caudal vena cava or the abdominal artery were tightly blocked by forceps, and blood was sucked out rapidly into a syringe through a needle.

The gauges of the needles were 17, 22, 26 and 30 for rats, and 22, 26, and 30 for mice. After sucking from the blocked region of abdominal artery, similar blood extraction was done from the caudal vena cava. The blood in the syringe was coagulated usually in about 10 minutes, and we put the blood between two slide glasses in order to spread it thin. Using the light microscope(Axiovert 100, Zeiss Germany) we searched Bonghan ducts which were easily identified in the thinly spread blood. The BHDs were taken from the slide and kept in formalin.

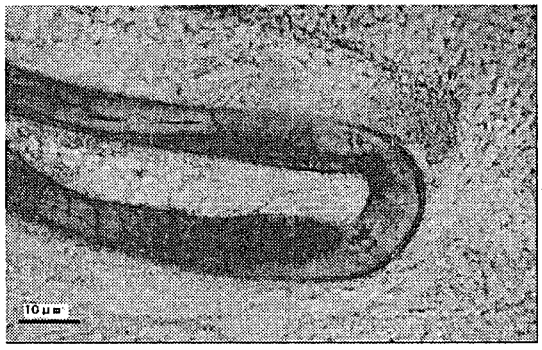

Fig.5. A typical sample of Bonghan ducts from the caudal vena cava of a rat

The blood vessels between the two forceps were cut after blood extraction, and they were put and compressed between two slide glasses in order to search for possible remnants of BHD pieces inside the vessels. They were taken and kept inside formalin.

Results

We have examined male rats(Sprague-Dawley) age of about 30 weeks, and male

mice(I.C.R) age of 5-7 weeks. The BHD samples obtained are listed in the Table I and II, for rats and mice, respectively.

The subject number in the table is the month-date of the year 2003, when the surgery was performed. The blood vessel where the sample was obtained are abbreviations :

HT=heart, CV=caudal vena cava, HV= hepato vein, HPV=hepatlo portal vein, CA= abdominal artery.

The largest diameter of the BHD from the rats is 45㎛ which was taken from the caudal vena cava. The average and the standard deviation are 26.8±6.4㎛. For the sample collection we put a criteria to accept only the samples whose diameters are larger than 20㎛. We considered that the smaller ones may not be BHD, but broken debris from Bonghan corpuscle.

The largest diameter of the BHD from the mice is 75㎛ which was taken from the abdominal artery. The average with the standard deviation is 33.1±15.0㎛, and we collected samples whose diameter is larger than 20㎛ like the rat case.

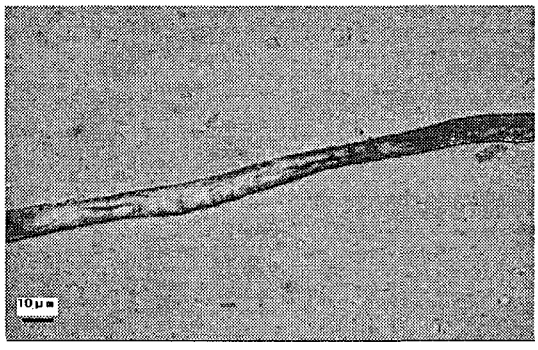

Fig.6. A typical sample of Bonghan ducts from the abdominal artery of a mouse

It is rather surprising that the diameter of the BHD from mice is larger than that from rat. This could be understood, however, if we look more closely the blood vessels where the samples are taken. The average diameter of the BHD from the caudal vena cava are 26.6 ± 6.6㎛, 26.6 ± 5.5㎛, for rats and mice, respectively. They are of the same size, and they are also about the same size with those of rabbit as reported by Bonghan Kim.[5] We have still not understood why the samples of the abdominal artery is much larger in diameter than those of the caudal vena cava. The average diameter of the BHD from the abdominal artery of mice is 41.6±26.7㎛. Since the samples we obtained are only few pieces from the broken BHD, thus the data may not represent faithfully the real size distribution.

Discussion

As shown in the Tables I and II, the lengths of the BHD samples are very short. The reason why the BHD are broken into small pieces by the current method is not yet understood. It will be a major break through if one can develop a technique to get longer BHDs so that further analysis such as H & E staining, transmission electron microscope analysis and others can be performed. At present, with such small pieces, it is very difficult to make a sample preparation for analysis.

The size distribution of the diameters of the BHDs is rather large. The reason for this, we surmise, is that there are more than

one BHDs in a vessel as mentioned Bonghan Kim's paper. Furthermore, one thread of BHD is not uniform in its diameter according to its contents which might vary in the physiological state of the animals. The broken small pieces could have come either from different threads or various parts of one thread. For the pieces with diameter less than 20㎛ we speculate that they are from the Bonghan corpuscle which is a globular or oval structure and contains inter-woven smaller BHDs and capillaries. Thus small-diameter samples we obtained could be these BHDs and capillaries from the Bonghan corpuscles. Unfortunately we have not yet observed the Bonghan corpuscles directly except its broken remnants.

The physiological significance of the BHD is, according to Bonghan Kim, that it is a new

Table I. Data of intravascular Bonghan ducts from Rat

Subject number	Blood Vessel	Bonghan duct	
		length (mm)	diameter (㎛)
02-20	HT	0.86	26.0
	HT	1.60	23.4
02-21	CV	0.13	32.4
	CV	0.79	19.8
02-22	CV	0.23	27.0
	CV	0.50	21.6
02-24	CV	0.83	21.6
	CV	3.75	25.0
02-26	CV	5.10	22.5
02-27	HV	1.76	20.7
	CV	0.56	24.3
03-24	CA	0.30	31.5
04-14	HV	3.20	37.5
04-18	HV	1.02	22.5
05-02	HPV	2.20	27.0
	HPV	1.30	36.0
05-09	HPV	0.45	20.0
	CV	0.71	36.0
	CV	0.86	22.5
05-16	CV	0.37	27.0
	CV	1.30	25.2
	CV	0.60	22.5
	CV	1.13	45.0
	HPV	1.01	21.0
	HPV	0.79	32.4
AVE±STD		1.3±1.2	26.8±6.4

HT = Heart, CV = Caudal Vena Cava,
HV = Hepato Vein
HPV = Hepato Portal Vein,
CA = Abdominal Artery

Table II. Data of intravascular Bonghan ducts from Mouse

Subject number	Blood Vessel	Bonghan duct	
		length (mm)	diameter (㎛)
03-11	CV	2.00	28.0
	CV	4.00	35.0
03-13	CV	4.05	20.7
03-17	CA	0.37	56.0
03-18	CA	1.24	25.0
	CA	0.23	50.0
	CA	0.49	45.0
03-19	CA	0.32	19.8
03-20	CA	1.84	20.7
	CA	0.26	75.0
	CA	0.27	59.4
03-21	CA	0.24	23.4
	CV	0.21	30.0
03-29	CV	0.62	26.3
	CV	0.75	25.2
04-28	CV	0.37	19.8
	CV	0.50	25.0
	CV	2.76	21.6
	CV	0.60	32.4
	CV	0.34	36.0
	CV	0.75	19.8
AVE±STD		1.1±1.2	33.1±15.0

CV = Caudal Vena Cava,
CA= Abdominal Artery

circulatory network through which granules containing DNA flow. These granules can generate cells of the damaged tissues where the granules reach through the BHD network. This function suggests that the BHDs form the circulating system for primordial universal stem cells that are totipotent,[10] which is an important hypothesis to be examined in future.

Acknowledgement

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