

Basic Investigations

Distribution and Permeability of Capillaries at the Skin of the Conception Vessel and the Governor Vessel in Healthy Rabbits

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Objective: To investigate the distribution and permeability of blood vessels on the Conception Vessel and the Governor Vessel in the physiological state.

Methods: Evans blue (EB) solution was injected into the marginal ear vein of healthy rabbits. Three hours after injection, the rabbits were sacrificed and the skin on the Conception Vessel and the Governor Vessel and the corresponding bilateral non-channels was collected. EB was extracted with 7:3 acetone: physiological saline, and the absorbance of EB at each skin tissue was measured with a spectrophotometer.

Results: The A value of EB absorbance at the Conception Vessel on the abdominal skin was lower than that of the corresponding bilateral non-channels with a statistically significant difference ($P < 0.01$). The A value of EB absorbance at the Governor Vessel on the back was higher than that of the corresponding bilateral non-channels ($P < 0.05$). There was no statistical difference in the A value of EB absorbance between the bilateral non-channels of the abdomen and the back ($P > 0.05$).

Conclusion: There were differences in capillary distribution and permeability between the Conception Vessel, the Governor Vessel and the corresponding bilateral non-meridians.

Keywords: *the Conception Vessel; the Governor Vessel; EB content at skin*

Channels and collaterals play very important roles in vital activities of the human body, and acupuncture can affect the transmitting function of channels and collaterals. In the past few decades, modern scientific research on channels and collaterals focused on three aspects:¹ 1) to confirm the widespread existence of channel phenomena, such as transmission of sensation along the channels; 2) to detect and demonstrate the course of channels with various instruments and indicators; and 3) to carry out systematic studies on corresponding relationships between viscera and acupoints.

A great number of achievements have been realized in the specific study of channel structure. Several studies preliminarily demonstrated that there are abundant blood vessels, lymphatic vessels, nerve endings and mast cells in the tissues of different layers of acupoints. Kerdall found that there are many capillaries, venules, small lymphatic vessels, mast cells, nerve bundles, nerve plexuses and free nerve endings in acupoints of both animals and humans.² The study by Shanghai University of Traditional Chinese Medicine also showed that, in the anatomies of 309 acupoints, there were 262 acupoints (84.36%) with arterio-venous trunks within 0.5 cm. 24 (7.26%) acupoints were on the truncus arteriosus directly, and 286 (92.6%) acupoints were related to arteries or veins.³ These findings demonstrate that acupoints have a close relationship with blood vessels.

Although channels and acupoints contain abundant blood vessels, there is still not sufficient evidence to prove any

corresponding functional expression, or whether vascular permeability on channels possesses acupoint specificity at present. Using reactive dye as a marker is the earliest and most universal method for measuring microvascular permeability. On the basis of previous work, this study measured the absorbance of 0.5% Evans Blue solution (EB) in skin tissue after intravenous injection into the marginal ear vein of healthy rabbits, in order to investigate the distribution and permeability of microvessels in the skin on the Conception Vessel and Governor Vessel meridians and the corresponding bilateral non-channel acupoints.

MATERIALS AND METHODS

Animals

Sixteen healthy New Zealand female white rabbits with bright coat color, aged 2 months, weighing 1.8 ± 0.1 kg, were supplied by the Experimental Animal Research Center of Tianjin University of Traditional Chinese Medicine. The rabbits underwent adaptive feeding for one week. They were housed at $25 \pm 2^\circ\text{C}$ and relative humidity $\geq 40\%$, with appropriate lighting and ventilation. Food and water were freely available. The feeding and use of the animals were in accordance with the relevant

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animal welfare requirements of International Animal Ethics Committee Guidelines.

Experimental Reagents and Equipments

0.9% saline injection (Shandong Qidu Pharmaceutical Co., Ltd., No.1D07102005, Zibo, Shandong, China); Acetone (The First Chemical Reagent Factory of Tianjin, batch No. 20080425, Tianjin, China); 0.5% EB solution (Evans blue made by Sigma Company (Sigma-Aldrich Co., St. Louis, MO, USA), batch No. 035k1524, prepared with 0.9% saline injection); Mono-use infusion set (Tianjin Hanahao Medical Materials Co., Ltd., Tianjin, China); Centrifuge (Shanghai Surgical Instruments Factory, model 80-1, Shanghai, China); 722 Grating Spectrophotometer (The Third Analysis Instrument Factory of Shanghai, model 722RS, Shanghai, China); Precision Balance (Mettler-Toledo, model AL204, Zurich, , Switzerland).

Treatment of Experimental Animals

The 16 healthy rabbits were randomly divided into two groups of 8 (group A and group B). In group A, the absorbance of EB in the skin tissue of the Conception Vessel on the abdomen was detected. First, the hair in the area was carefully trimmed, avoiding damage to the skin tissue. The rabbits were then fixed in a supine position. Thirty minutes later, 0.5% EB solution was injected into the marginal ear vein for at 8 drops/min for 3 h, for a total dose of 72 mL. Following this, the rabbits were immediately sacrificed by injecting air into the marginal ear vein. The skin tissue was collected for further study. In group B, the absorbance of EB in the skin tissue of the Governor Vessel on the dorsum was detected. The hair was trimmed at the location of study and the rabbits were fixed for 30 min in a prone position. All other treatment methods were as same as for group A.

Sampling and Detection

Group A: The entire abdominal skin was collected. The line linking the mid-point of the xiphisternal synchondrosis horizontal line and the mid-point of the horizontal line between the two highest points of iliac crest marked the Conception Vessel. The line 3 cm lateral to the Conception Vessel marked the Stomach Channel of Foot-*Yangming*, which overlapped with two nipples of the rabbit. The lines 1 cm lateral to the Conception Vessel were considered as the bilateral non-channel lines.⁴

Group B: The entire dorsal region of skin was collected. The line linking the mid-point of the horizontal line between the two inferior angles of the scapulae and the mid-point of the horizontal line between the two highest points of iliac crest marked the Governor Vessel. The line 3 cm lateral to the Governor Vessel marked the second lateral line of the Bladder Meridian of Foot-*Taiyang*. The lines 3.5 cm lateral to the Governor Vessel were considered as the bilateral non-channel lines.⁴

The skin specimens were refrigerated at -20°C for 30

min. The Conception Vessel and the bilateral non-channel lines were equally divided into 8 sections. The Governor Vessel and the bilateral non-channel lines were also equally divided into 8 sections. The top and bottom bisectors were removed. Seven pieces of skin tissue of equal area were taken at the intersections of the remaining seven bisectors and channels, and the lateral intersections of the remaining seven bisectors and non-meridians with a 0.6 cm diameter punch biopsy. The seven points on each line were respectively named: a, b, c, d, e, f, g from the top to the bottom.

The samples were placed in a 5 mL centrifuge tube and shredded with ophthalmic scissors. Four mL of 7:3 acetone: physiological saline was added and maintained for 48 h, after which the samples were centrifuged at 500 rpm for 10 min. After standing for 10 min, the supernatant was collected for detection of absorbance A values with a 722 grating spectrophotometer at 600 nm wavelength.

Preparation of Evans Blue Standard Curve

EB solutions of different concentrations (0.391, 0.781, 1.562, 3.125, 6.25, 12.5 and 25 ug/mL) were prepared. The absorbance A values were detected with the 722 grating spectrophotometer at 600 nm wavelength. Then the regression line equation, $y=0.026 + 0.028x$, $r=0.999$, was obtained by taking the concentrations as the X-axis and the absorbance A values as the Y-axis. Therefore, the absorbance A value represented the content of Evans blue in the skin tissue.

Comparison Method of the Absorbance of EB on the Channels and non-Channels

The average absorbance A value of the seven detected points on each channel represented the absorbance of EB on that meridian. The average absorbance A values on the Conception Vessel and its corresponding bilateral non-channel lines, and the average value of the absorbance A values on the Governor Vessel and its bilateral corresponding non-meridian lines were compared.

Statistical Analysis

Statistical analysis was performed with the SPSS statistical software system (SPSS Inc. version 11.5). All data were expressed as mean \pm standard deviation. Inter-group comparisons were performed with paired *t*-test, and $P<0.05$ was considered as statistically significant.

RESULTS

General Observation

Three hours after intravenous injection of 0.5% EB, in some rabbits, a thin blue-stained line could be seen at the middle of the abdomen, which resembled the Conception Vessel. Furthermore, the thin blue-stained line was different than the blue-stain of adjacent regions, and the blood vessel of the skin layer was distinctly different

(Figure 1). A thin blue-stain line could also be seen at the middle of the back, which resembled the Governor Vessel. It was different than the blue-stain of adjacent

regions. However, after the skin was removed, a similar phenomenon was not found in the muscle layer (Figure 1).

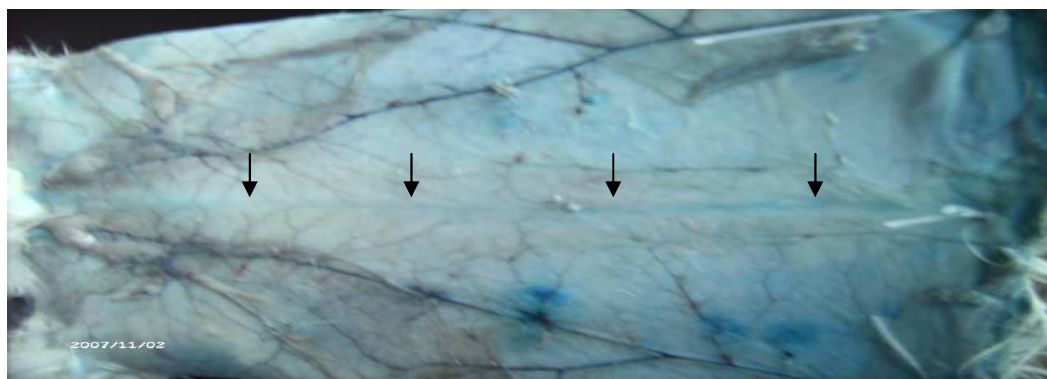


Figure 1. The thin blue-stained line located in the middle of the abdominal skin of a healthy rabbit.

Comparison of EB Absorbance between the Conception Vessel and the Corresponding Non-channels

The A value of EB absorbance on the Conception Vessel was lower than that on the left non-channel ($P=0.001$) and the right non-channel ($P=0.002$), with no significant difference between the left non-channel and the right non-channel ($P>0.05$). These results indicate that under physiological conditions, the distributions of EB on the Conception Vessel and the corresponding non-channels were significantly different.

Table 1. EB absorbance of the Conception Vessel and the corresponding non-channels ($\bar{x} \pm s$)

Lines	<i>n</i>	The absorbance A
The Conception Vessel	8	0.0456±0.0024
The left non-channel	8	0.0493±0.0021 [#]
The right non-channel	8	0.0503±0.0026 [#]

Note: Compared with the Conception Vessel, [#] $P<0.01$.

EB Absorbance of the Governor Vessel and the Corresponding Non-channels

The A value of EB absorbance on the Governor Vessel was higher than that on the left non-meridian ($P=0.031$) and the right non-meridian ($P=0.022$), with no significant difference between the left non-channel and the right non-channel ($P>0.05$). The result indicated that under physiological conditions, the distributions of EB on the Governor Vessel and the corresponding non-channels were different.

Table 2. EB absorbance of the Governor Vessel and the corresponding non-channels ($\bar{x} \pm s$)

Lines	<i>n</i>	The absorbance A
The Governor Vessel	8	0.0513±0.0066
The left non-channel	8	0.0483±0.0053
The right non-channel	8	0.0494±0.0063

Note: Compared with the Governor Vessel, [#] $P<0.05$.

DISCUSSION

Morphological Research on Channels and Collaterals

According to Traditional Chinese Medicine theory, channels and collaterals can reflect the physiological functions and pathological changes of *Zang-fu* organs, and have the effect of transporting *qi* and blood through the whole body. Therefore, channels and collaterals have a close relation with blood and blood vessels. Some researchers have found arteries, veins, and their branches on each traveling path of the 12 channels.³ However, because the Conception Vessel and the Governor Vessel belong to the eight extraordinary vessels, and also are located on the midlines of the abdomen and the back of the human body, the morphological research on the conception Vessel and the Governor Vessel is limited. According to anatomy, the Conception Vessel is located on the midline of the abdomen which overlaps with the linea alba abdominis, with more fascia tissue and fewer blood vessels, while the Governor Vessel is located on the midline of the back, with no concentrated distribution of large blood vessels in the skin of the back.

Biophysical Research on the Conception Vessel and the Governor Vessel

With the development of science and technology, many biophysical technologies have been applied in the research on channels and collaterals. Research on heat radiation on channels have found that skin temperature of the Governor Vessel is higher than the corresponding bilateral control points,⁵ and blood perfusion volumes at three skin depths (0.25 mm, 1 mm and 2 mm below the cuticula) on the Governor Vessel are also higher than at the corresponding bilateral control points.^{6,7} Therefore, the Governor Vessel possesses the characteristics of higher skin temperature and high blood perfusion volume. Infrared radiant track along channels course (IRRTC) studies in healthy subjects have demonstrated that the appearance rate of IRRTC on the Governor Vessel was 77.9%, in which 30.9% appeared along the Governor Vessel; while the appearance rate of IRRTC on the Conception Vessel was only over 25%.⁸

Skin temperature is a reflection of local blood circulation and metabolism. However, there are no large longitudinal blood vessels at the skin of the back, which implies that the higher skin temperature and IRRTC appearance along the Governor Vessel are not associated with large blood vessels. The higher skin temperature here may instead come from a greater distribution of capillaries and high micro-circulation perfusion on the Governor Vessel.

Analysis of the Experimental Results

EB is an Azo compound dye, which rapidly binds to plasma albumin when it is injected into blood and forms a macromolecular compound which is delivered to every organ and tissue through the circulatory system. When the microvascular permeability of tissue is increased, EB dye will leak out with plasma-albumin from blood vessels.^{9,10} Therefore, EB is one of the most commonly-used reactive dyes for detecting tissue and microvascular permeability.¹¹ When it is injected into a rabbit's vein, the EB dye is evenly distributed throughout the entire blood volume of the rabbit. A higher EB absorbance will be detected at the organs or tissues with more blood vessels and higher capillary permeability.

At the abdominal region, the Conception Vessel overlaps with the linea alba abdominis, where there are fewer capillaries, and the content of EB detected on the Conception Vessel was lower than its corresponding bilateral non-channels. While the distribution of blood vessels is relatively even at the skin of the back, the value of EB absorbance on the Governor Vessel was higher than its corresponding bilateral non-channels. Combined with the modern biophysical findings about the Governor Vessel, this indicates that compared with the corresponding bilateral non-channels, the skin along the Governor Vessel has more capillaries and higher microvascular permeability.

The Conception Vessel is located on the midline of the abdomen, and it is known as "the sea of the *yin* channels," governing all the *yin* channels of the body. The Governor Vessel is located on the midline of the back, and it is known as "the sea of the *yang* channels," governing all the *yang* channels of the body. The experimental results revealed that the content of EB was low on the skin of the Conception Vessel and was high on the skin of Governor Vessel, which are consistent with the *yin* and *yang* properties of the Conception Vessel and the Governor Vessel in traditional Chinese medicine theory. In a study on cyclic nucleotide content changes at the Bladder Channel of Foot-*Taiyang* and Back-*shu* points, researchers found that there were certain differences in the contents of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) and their ratio between the *Yin* channels (the Kidney Channel and the Conception Vessel) and the *Yang* channels (the Stomach Channel and the Bladder Channel). Contents of cAMP and cGMP on the Kidney Channel and the Conception Vessel were higher

than that on the Stomach Channel and the Bladder Channel.¹² Therefore, the researchers believe that the different distributions of the cyclic nucleotides might be related to the *yin* and *yang* properties of channels.

The distribution of blood capillaries and the permeability of microvessels are closely related to local material exchange and metabolism. It is indicated from this study that under physiological conditions, the distribution of blood capillaries and permeability of microvessels on the Conception Vessel and the Governor Vessel have significant differences compared with the corresponding non-channels. This difference may be related to the specific function of channels or may be evidence for reasonable explanation of the *yin* and *yang* properties of the Conception Vessel and the Governor Vessel in traditional Chinese medicine theory.

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