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Does the Acupuncture Point Shaoshang (Lu11) in Humans Match with an Increased Density of Connexines?

Ines Pilz-Klement and Christian-Albrecht May*

Department of Anatomy, Medizinische Fakultät Carl Gustav Carus, TU Dresden, Dresden, Germany.

***Corresponding author:** Christian Albrecht May, Anatomisches Institut, Fetscherstr. 7401307 Dresden, Germany, Tel: +49 351 458 6105; Fax: +49 351 458 6303; E-mail: Albrecht.May@tu-dresden.de

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Abstract

Acupuncture points are clinically described by their localization and sensation measuring skin resistance. As a morphological correlate, gap junctions and connexines (Cx) were postulated but up to now only few animal studies tried to identify these structures histologically. Immunohistochemical staining of acupuncture point Lu11 of 4 human donors (76-81 years of age) were stained with antibodies against Cx 26, 30, 32 and 43. A densitometric evaluation was performed using analySIS Software 3.2 (Olympus). In the region of the acupuncture point Lu11 the density of Cx 26 and Cx 43 was increased. This increase was not restricted to the epidermal layers but also included the dermis and subcutis. Our findings give evidence that there are persistent regions of increased Cx density in the human skin. They provide a morphological base for fast alterations in electrical skin resistance dependent on the functional stage of the Cxs by forming either gap junctions or hemi-channels.

Keywords: Acupuncture point; Connexin; Density; Human

Introduction

Early work of the 1950es by Nakatani [1] suggested that acupuncture points (APs) might be characterized by a low skin resistance. This hypothesis was transferred to AP location devices widely used in clinical practice today. However, a recent review dealing with this topic found that most of the studies trying to substantiate this hypothesis are of poor quality and show controversial results [2]. Using a more elaborated system to measure electrical skin resistance, only 37.2% examinations of six APs showed a difference to the surrounding skin area (two-third showing a lower, one third a higher resistance [3]). Even if these data seem to disillusion the correlation of APs and physio-morphological parameters, they show that a change in skin resistance can be generated. We were wondering if, regardless of the actual resistance, morphological parameters could be found that define the region of an AP in the skin.

Screening the literature, gap junctions are the most favoured candidates. Unfortunately, they can only be identified by electron microscopy and there is only one study (using mice and rabbits) claiming an increase of gap junctions in APs [4]. Since gap junctions contain of connexines (Cxs), a more recent study (using rats) examined the expression of Cx 43, and confirmed an increase in the single AP Ma36 [5]. There are numerous Cxs present in the human epidermis, Cx 26 and Cx 43 being the most ubiquitous [6].

Materials and Methods

In our series of experiments, the AP Shaoshang (Lu11) was studied using different fixation protocols of 4 human donors (76-81 years of age) of the Department of Anatomy, TU Dresden, Germany. Lu11 was chosen for its secure identification without individual distances or postmortal relocation. It can be identified as the intersection point of two tangents confining the thumbnail radial and proximal. Skin samples (cubes with a lateral length of 8 mm) were collected 12-27 h postmortem, containing the AP in its middle, and either fixed in formalin or deep frozen unfixed. As a control, skin samples were taken near the middle finger using the same two-tangential method; there, no AP is described. Serial 20 µm sections were performed through the complete sample. Every 4th section was incubated for 24 h at 4°C with one of the following antibodies: Cx 43 (1:200; Sigma-Aldrich, Prod.-Nr.: C6219), Cx 26 (1:100; Zymed laboratories, Cat.Nr: 71-0500), Cx 30 (1:400; Zytomed Systems, Cat.Nr.: 203-1125), Cx 32 (1:100; Zytomed Systems, Cat. Nr: 203-1133). The sections were then washed and incubated with an appropriate fluorescent antibody: goat anti rabbit Alexa 488 (1:200; MoBiTec, Cat.-Nr: A-11008) or goat anti-rabbit Cy3 (1:1000; Dianova, Code-Nr: 111-165-144). The samples were viewed with a fluorescence microscope (Olympus BX 60) and evaluated using the analySIS Software 3.2 (Olympus). The size of the area with increased Cxs in the middle of the sample (presumably the AP) was measured within single sections (largest distance in a series of sections) and calculated by counting the number of sections with increased positive staining. At least 10 measuring points (600µm^2 in size) were chosen within the defined AP and outside of it on the same section. The difference of corresponding tissues was calculated in %: a staining increase within the AP resulted in numbers above 100%, a decrease in numbers below 100%. The quantification was separately conducted for the inner epidermis (stratum basale and spinosum) and the dermis.

Results

All tested Cx antibodies showed a specific bright staining reaction, mainly located in the stratum basale and stratum spinosum of the epidermis (Figure 1), but also in the dermis (sweat glands, blood vessels, fibrocytes) and subcutis. Quantitative measurements revealed an abundant and constant presence of connexin 43 in the middle finger skin samples, but a specific increase of connexines 26 and 43 within an epidermal area of 1.1 - 2.5 mm of the thumb skin samples,

identified as the region of the AP. The increase showed variations between the different Cxs and between the individual donors (Table 1), but was present in all specimens investigated. The depth of increased Cx density into the dermis and subcutis was 0.2-0.4 mm.

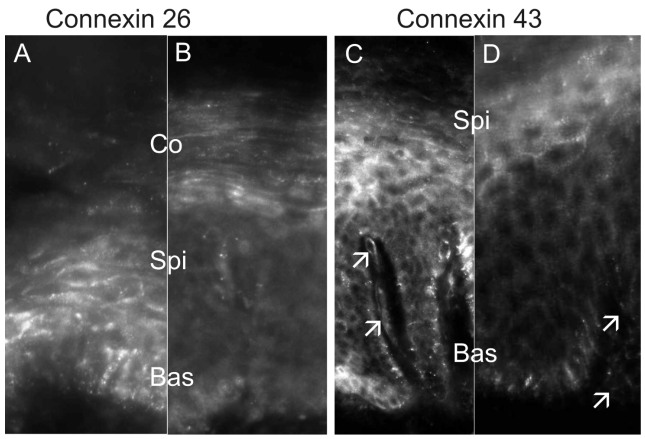

Figure 1: Examples of the epidermis within acupuncture point Lu11 (A and C) and next to it (B and D) on the same section, stained with antibodies against connexin 26 and connexin 43. Sagital sections of the skin of a 80 years old human donor. Co = stratum corneum; Spi = Stratum spinosum; Bas=Stratum basale; original magnification x400. Note the increase of connexin 26 in the Stratum spinosum and basale. The increase of connexin 43 in this sample is not as prominent, but there is more intensity in the vascular wall within the dermis (arrows).

	Cx 26	Cx 43
Donor 1, 76 years of age	187	149
Donor 2, 80 years of age	317	199
Donor 3, 81 years of age	139	484
Donor 4, 79 years of age	214	140

Table 1: Summary of the difference of connexin (Cx) density in the inner epidermis between the acupuncture point (AP) Lu11 and its surrounding region in %, separated for different donors and Cxs. Note that all samples show an increase in Cx density within the AP region for both Cxs listed. Measuring formula: intensity of AP x 100 / intensity of surrounding region; an increase is defined by numbers over 100, a decrease by numbers below 100.

Discussion

The AP Lu11 has a clear anatomical localization, in contrast to numerous other APs which are much more difficult to localize [7-9]. This anatomical identification was, however, crucial to match the AP with the described changes of Cx density. The size of increased Cx density in Lu11 was smaller than expected from clinical experiences of other APs (4 mm [9]), but this may vary between different APs. No data exists about this topic so far, since the morphological identification of APs was not possible; Cx staining might now help to establish these references and investigate if changes occur after stimulation or under specific pathological conditions.

The study was conducted on aged human donors. A decrease of Cx density is known to occur during development up to adulthood in the brain [10,11] and epidermis [12]. There is no report about Cx changes in normal senescent vs. adult tissue. We therefore assume that our findings are representative for elder humans.

The increase of Cxs is per se not identical with an elevation of gap junctions, but the possibility for gap junction formation is increased. As an alternative, Cxs could also form mechanosensitive hemi-channels [13]. The actual realization and function of Cxs in acupuncture points needs further evaluation. This includes also their different modifications, e.g. the phosphorylation state [14,15]. The different alternatives for Cxs to interact with their environment, however, could explain the different electrical skin resistance of APs at various moments [3,16]. From our study we cannot exclude that there might be additional regions of Cx increase that do not colocalize with APs. Single points with reduced electrical skin resistance that did not match with APs were observed from their early description on [1], but further studies are necessary to clarify if they show the same morphological characteristics.

Not only the epidermal layers, but also the underlying dermis and subcutis contained a higher density of Cxs. Since the connective tissue is thought to be involved in the effect of acupuncture [17-19], our finding, which confirms observations in the rat [5], strengthens the theory of pre-existing specific regions, which were postulated and named as APs or biological active points [20], rather than an arbitrary mechanical stimulation of the connective tissue. General features for the identification of APs other than epidermal skin resistance were discussed frequently, but none could be confirmed for all APs (e.g. macroscopic aspects like perforation sides through fascias [21]). The findings described in this study show a significant difference of Cx density in the presumed region of the AP in postmortem, unstimulated tissue. The findings are limited by the low number of donors (4 older humans with consistent results) and by testing only one AP. Further studies are necessary to show if the microscopic findings in Lu11 are representative or rather represent regional characteristics.

In addition, it remains to be determined which factor is responsible for the increased expression of Cxs in the specific AP regions, and whether the changes in the underlying connective tissue are in some relation to the APs connecting meridians.

In conclusion, our findings give evidence that there are persistent regions of increased Cx density in the human skin. In the present study they matched with the localization of the AP Lu11. These regions provide a morphological base for fast alterations in electrical skin resistance dependent on the functional stage: if the Cxs form gap junctions, the resistance decreases, if they form hemi-channels, the resistance might not change or even increase. Therefore, our study supports the theory of APs as structurally defined entities by simultaneously explaining the diversity of clinical identification.

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