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Electrophysiological changes of autonomic cells in left ventricular outflow tract in guinea pigs with iron deficiency anemia complicated with chronic heart failure

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ABSTRACT

Objective: To investigate the electrophysiological changes of autonomic cells in left ventricular outflow tract in guinea pigs with iron deficiency anemia complicated with chronic heart failure.

Methods: Guinea pigs model of iron deficiency anemia complicated with chronic heart failure in 10 guinea pigs of the experimental group was made by feeding a low iron diet, pure water and subcutaneous injection of isoproterenol. The control group consisting of 11 guinea pigs was given normal food, normal water and injected with normal saline. The left ventricular outflow tract model specimen was also prepared. The standard microelectrode technique was used to observe electrophysiological changes of autonomic cells in the outflow tract of left ventricular heart failure complicated with iron deficiency anemia in guinea pig model. The indicators of observation were maximal diastolic potential, action potential amplitude, 0 phase maximal depolarization velocity, 4 phase automatic depolarization velocity, repolarization 50% and 90%, and spontaneous discharge frequency.

Results: Compared with the control group, 4 phase automatic depolarization velocity, spontaneous discharge frequency and 0 phase maximal depolarization velocity decreased significantly ($P < 0.01$) and action potential amplitude reduced ($P < 0.01$) in model group. Moreover, repolarization 50% and 90% increased ($P < 0.01$).

Conclusions: There are electrophysiological abnormalities of the left ventricular outflow tract in guinea pigs with iron deficiency anemia complicated with heart failure.

1. Introduction

Clinically, iron deficiency anemia complicated with chronic heart failure is a relatively common disease [1]. More than half of the patients died of sudden cardiac death caused by various malignant ventricular arrhythmias [2]. It is found that the ventricular arrhythmias originating from the outflow tract are closely related to the slow response autonomic cells existing in the ventricular outflow tract tissue [3,4]. In order to study the electrophysiological changes of autonomic cells in left ventricular outflow tract in patients with iron deficiency anemia complicated with chronic heart failure, this experiment was designed.

2. Materials and methods

2.1. Experimental animals

Guinea pigs, weighing 250–350 g, both male and female, were purchased from the Beijing Gold Muyang Experimental Animal Breeding Co. Ltd. (animal license: SCXK (Beijing) 2010-0001).

2.2. Grouping

The guinea pigs were randomly divided into two groups: experimental group (iron deficiency anemia complicated with chronic heart failure group, $n = 18$) and control group ($n = 12$).

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The experimental group was given the homemade low iron diet in which formula did not add salt and the material in the formula was soaked with 1% EDTA-2Na to remove iron ions in feed [5], and drank pure water along with subcutaneous injection of isoproterenol [6]. The control group was given normal food, normal water and injected with normal saline, the dose and time were the same as the experimental group. After 6 weeks, 10 cases survived in the experimental group, 11 cases survived in the control group. Animal breeding, care and all experiments were performed in adherence to Hebei North University animal experiment center guidelines and approved by Animal Ethics Committee.

2.3. Determination of modeling results in the experimental group

After 6 weeks, the right common carotid artery of guinea pigs in experimental group was intubated, and the RM6240 signal acquisition system (Chengdu instrument factory) was used to collect the following indexes: heart rate, blood pressure, left ventricular systolic pressure, left ventricular diastolic pressure, left ventricular end diastolic pressure, left ventricular systolic maximum velocity and left ventricular diastolic maximum velocity in order to determine whether the model of heart failure was made successful. After the above indexes were collected, blood from the carotid artery was bled in a 1 mL test tube, and the hemoglobin content, red blood cell count and serum iron content were measured.

2.4. Preparation of left ventricular outflow tract specimens and measurement of left ventricular outflow tract specimen's action potential

After cardiac function was measured and carotid artery blood sampling were taken, the heart was removed quickly and placed in the modified Locke solution saturated with O_2. Left ventricular outflow tract tissue specimens were made [3,4]. The prepared specimens were fixed on the silicone rubber in the perfusion chamber with Locke fluid by a stainless steel needle at constant temperature (36 ± 0.5) °C, constant speed (5 mL/min) perfusion, perfusion continuously filling pure O_2 fluid, pH 7.4. Standard intracellular microelectrode technique was used to record the spontaneous slow action potential of the outflow tract which was collected by RM6280 multi-channel physiological signal acquisition and processing system (Chengdu instrument factory).

2.5. Statistical analysis

Software SPSS version 16.0 was used for statistical analysis. Mean ± SD was used to express the measurement data. Software SPSS version 16.0 was used to carry out the test of homogeneity of variances. Data of homogeneity of variance ($P > 0.10$) in multiple groups were compared using one-way ANOVA, and student's t-test was used for the comparison of two groups. $P < 0.05$ was considered statistically significant difference.

3. Results

3.1. The changes of cardiac function and hemodynamic indexes

The cardiac function indexes of two groups were shown in Table 1. Compared with the control group, the blood pressure of the experimental group significantly reduced ($P < 0.01$), the absolute values of left ventricular systolic pressure, left ventricular diastolic pressure, left ventricular systolic maximum velocity and left ventricular diastolic maximum velocity significantly decreased ($P < 0.01$) while left ventricular end diastolic pressure significantly increased ($P < 0.01$).

3.2. Comparison of blood indexes between the control group and experimental group

Compared with the control group, the hemoglobin content, red blood cell count and serum iron content in the experimental group significantly decreased ($P < 0.01$, Table 2).

Table 1

Comparison of cardiac function indexes between two groups of guinea pigs (mean ± SD).

Groups	n	Heart rate (time/min)	Blood pressure (mmHg)	Left ventricular systolic pressure (mmHg)	Left ventricular diastolic pressure (mmHg)	Left ventricular end diastolic pressure (mmHg)	Left ventricular systolic maximum velocity (mmHg/s)	Left ventricular diastolic maximum velocity (mmHg/s)
Control group	11	244.7 ± 23.8	52.63 ± 5.98	67.23 ± 6.65	−5.35 ± 1.63	1.18 ± 0.67	2992.91 ± 525.76	−2911.58 ± 496.28
Experimental group	10	247.3 ± 21.2	42.75 ± 6.11**	53.99 ± 7.01**	−2.78 ± 1.76**	4.02 ± 1.15**	1985.56 ± 472.46**	−1906.76 ± 427.59**

Compared with control group, **: $P < 0.01$.

Table 2

Comparison of blood indexes between the control group and experimental group (mean ± SD).

Groups	n	Hemoglobin content (g/L)	Red blood cell count ($\times 10^{12}$/L)	Fe (mg/L)
Control group	11	141.98 ± 16.04	6.03 ± 0.61	442.36 ± 52.31
Experimental group	10	84.62 ± 18.51**	4.18 ± 0.69**	367.40 ± 54.37**

Compared with control group, **: $P < 0.01$.

Table 3
Electrophysiological changes of autonomic cells in left ventricular outflow tract in guinea pigs with iron deficiency anemia complicated with heart failure (mean ± SD).

Groups	VDD (mV/s)	RPF (bpm)	Maximal diastolic potential (mV)	Vmax (V/s)	Action potential amplitude (mV)	Repolarization 50% (ms)	Repolarization 90% (ms)
Control group	35.25 ± 3.76	128.54 ± 5.17	58.21 ± 5.36	11.85 ± 1.49	63.68 ± 4.17	126.13 ± 5.86	184.89 ± 6.46
Experimental group	29.35 ± 2.34**	119.31 ± 6.06**	57.62 ± 4.98	9.26 ± 1.31**	56.07 ± 3.23**	131.39 ± 4.92**	191.53 ± 6.51**

Compared with control group, **: $P < 0.01$.

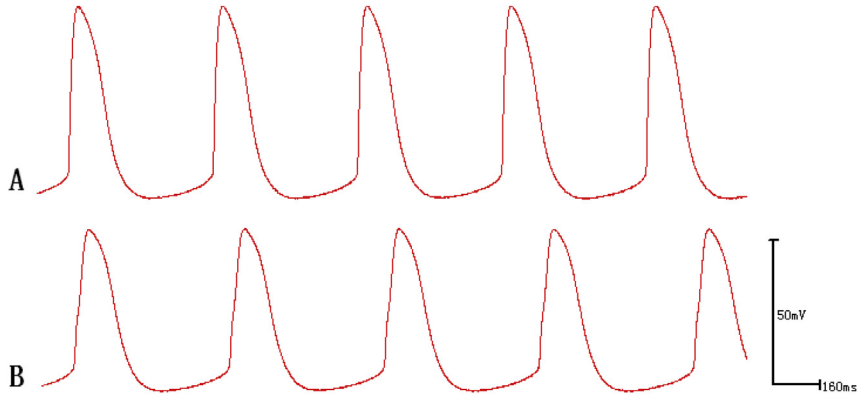

Figure 1. Electrophysiological changes of autonomic cells in left ventricular outflow tract in guinea pigs with iron deficiency anemia complicated with heart failure: A: Control group; B: Experimental group.

3.3. Comparison of electrophysiological parameters of left ventricular outflow tract between the control group and experimental group

The electrophysiological parameters of left ventricular outflow tract in two groups were demonstrated in Table 3 and Figure 1. Compared with the control group, 4 phase automatic depolarization velocity (VDD) and 0 phase maximal depolarization velocity (Vmax) of the experimental group significantly slowed ($P < 0.01$), and spontaneous discharge frequency (RPF) reduced ($P < 0.01$) while repolarization 50% and repolarization 90% increased ($P < 0.01$).

4. Discussion

The experimental use of a low iron diet, pure water intake, and limited intake of exogenous iron caused iron deficiency anemia, and subcutaneous injection of large doses of isoproterenol caused myocardial cell necrosis, diffuse fibrosis and ventricular dilation, leading to heart failure. The survival of 10 guinea pigs of the experimental group detected for iron deficiency anemia and heart failure indexes conforms to the diagnosis standard of iron deficiency anemia in patients with chronic heart failure.

Clinically, most of the idiopathic ventricular tachycardias and ventricular premature beats of patients with iron deficiency anemia and chronic heart failure originate from the ventricular outflow tract, which means that the electrophysiological characteristics of ventricular outflow tract in these patients may change and it is related to the mechanism of arrhythmia originating from the outflow tract. From the point of view of biological evolution, the ventricular outflow tract of mammals is evolved from the arterial sphere and the arterial sphere of fish and amphibians is an independent excitation and contraction unit located behind the ventricle. In the early stage of human embryo, the occurrence of cardiovascular also passes through the arterial ball phase. Previous studies showed that there were slow reaction automatic cells in some parts of the outflow tract of guinea pigs, rats and rabbits. These cells which have the same automatic excitement as the arterial sphere is another potential pacemaker of the heart with its electrophysiological characteristics similar to the sinoatrial node p cells [7].

The slow response action potential of automatic cells of left ventricular outflow tract in the experimental group and control group was determined. The results showed that the experimental group, VDD, RPF and Vmax significantly decreased, action potential amplitude reduced, and repolarization 50% and repolarization 90% increased. The mechanism may include the following points: Anemia combined with heart failure causes myocardial remodeling, changes the perimeter of autonomic cells of the outflow tract and decreases I_f and I_{KS} channel density which leads to I_f and I_{KS} reduction as well as decreased VDD and RPF. It was found that the $I_{Ca\text{-}L}$ and $I_{Ca\text{-}T}$ play an important role in 4 phase depolarization autonomic cells of left ventricular outflow tract [7]. In the development of iron deficiency anemia and heart failure, the density of $I_{Ca\text{-}L}$ reduced, resulting in the decrease of Vmax and action potential amplitude. In the diseases such as heart failure, cardiac hypertrophy and arrhythmia, the expression of I_{to} channel protein reduced, resulting in the prolongation of repolarization [8] which was also caused by the decrease of I_{KS}.

The results demonstrated that there was electrophysiological abnormalities in the left ventricular outflow tract tissue in guinea pigs with iron deficiency anemia complicated with heart failure. The results still need to be confirmed by large samples. Besides, the detailed mechanism of electrophysiological change and its relation with ventricular arrhythmia originating from the outflow tract are to be further studied.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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