Experimental Studies

Sodium Hydroxide Pinpoint Pressing Permeation Method for the Animal Modeling of Sick Sinus Syndrome


SUMMARY

Sodium hydroxide pinpoint pressing permeation (SHPPP) was investigated in order to build a rat model of sick sinus syndrome (SSS), which is easy to operate and control the degree of damage, with fewer complications and applicable for large and small animals.

Thirty healthy Wistar rats (15 males and 15 females, weighing 250-350 g) were randomly divided into 3 groups, namely a formaldehyde thoracotomy wet compressing group (FTWC), formaldehyde pinpoint pressing permeation group (FPPP) group, and SHPPP group. The number of surviving rats, heart rate (HR), sinoatrial node recovery time (SNRT), corrected SNRT (CSNRT), and sinoatrial conduction time (SACT) were recorded 3 days, one week, and two weeks after modeling.

The achievement ratio of modeling was 10% in the FTWC group, 40% in the FPPP group, and 70% in the SHPPP group, and the differences were statistically significant ($\chi^2 = 7.250, P = 0.007$). Meanwhile, the HR was reduced by about 37% in these 3 groups 3 days after modeling, while the reduction was maintained only in SHPPP ($P > 0.05$) and the HR was re-elevated in the FTWC and FPPP groups 2 weeks after modeling ($P < 0.05$). Additionally, the SNRT, CSNRT, and SACT were significantly prolonged compared with pre-modeling in all 3 groups ($P < 0.01$).

SHPPP was the best method with which to build an SSS model with stable and lasting low HR and high success rate of modeling, which might be helpful for further studies on the SSS mechanisms and drugs. (Int Heart J 2015; 56: 439-443)

Key words: Formaldehyde thoracotomy wet compressing, Formaldehyde pinpoint pressing permeation

The sinoatrial node is the dominant pacemaker in the heart and it initiates each normal heart beat. Sick sinus syndrome (SSS) is a common and refractory arrhythmia which is defined as an intrinsic sinus node dysfunction. Cerebral hypoperfusion is one common symptom of SSS, and about 50 percent of patients with SSS have near-fainting spells or syncope. Statistics show that approximately 400,000 patients are diagnosed with SSS and treated with a pacemaker per year. Intrinsic causes of SSS include degenerative fibrosis of the sinoatrial node, remodeling of the sinoatrial node, and ion channel dysfunction. Inherited dysfunction of ion channels within the sinoatrial node has been reported to play a significant part in age-related SSS.

The etiology of SSS is far from completely understood. Building an animal damage model is the most common and important method in the experimental study of diseases. Thus, a stable and credible animal damage model of SSS is urgently needed. Some models have been established for the study of SSS, such as cryocoagulation of the sinus node area, and ligating the artery supplying the sinus node with blood. However, it is difficult to pinpoint the sinus node area with these methods and they require large incisal openings, which might lead to a low survival rate. Recently, sinus node tissue wetted with chemicals or reagents has been used to construct an SSS model, because it is easy to control the degree of damage to the sinus node, has fewer complications, and is applicable to large and small animals. The formaldehyde pinpoint pressing permeation (FPPP) method has been certified to have a lower death rate, higher achievement ratio, better stabilization effect, and less damage compared with the traditional method.

Sodium hydroxide has also been used in multiple studies of animal models. In this study, the sodium hydroxide pinpoint pressing permeation (SHPPP) method was used to construct an SSS damage model in rats. The success rate, heart rate (HR), and electrophysiology index of sinus node were determined and recorded and then compared with the FPPP.
method and traditional thoracotomy method.

**Methods**

**Animals and grouping:** All animal experiments conformed to the national legislation and local guidelines and were approved by the Animal Ethics Committee of the First Affiliated Hospital of Heilongjiang University of Chinese Medicine. Thirty healthy Wistar rats (15 males and 15 females, weighing 250-350 g) purchased from Province Arima Songbei Huayu Animal Centre (Harbin, Heilongjiang, China) were used. All rats were housed 4 per cage with free access to water and food. The room was controlled under a suitable temperature and humidity and with a 12 hour light/dark cycle. The rats were randomly divided into 3 groups; a formaldehyde thoracotomy wet compressing group (FTWC), FPPP group, and SHPPP group, with 10 rats in each group.

**Animal model establishment:** The rats in the formaldehyde thoracotomy group were anaeasthetized with 20% urethane (HengYuan Biological Technology Co., Ltd, Shanghai, China). After thoracotomy, the sinus regions of the rats were wetted with 40% formaldehyde (Jinsui Bio-Technology Co., Ltd, Shanghai, China) using a 3 mm cotton ball for 3-5 minutes. Sinoatrial node acute injury was considered as the symbol of the success of the model, including sinus arrest, nodal escape, and a heart rate (HR) decrease of more than 30% compared to before intravenous anesthesia. Epicardial electrocardiography was continuously monitored during the operation. In the FPPP and SHPPP group rats, a homemade mapping catheter (Figure 1) was inserted close to the right edge of the second intercostal sternum. Epicardial electrocardiography was mapped by connecting the tail end of the catheter wire with V1 of the electrocardiogram. The direction of the catheter was then adjusted and fixed when the sinoatrial node electrogram (deep negative P wave and relatively small QRS occurring) appeared in the electrocardiogram. Bolus injection of formaldehyde or sodium hydroxide with a micro syringe was conducted for local infiltration until acute injury of the sinoatrial node occurred as previously described. The V1 electrocardiogram was recorded at a paper speed of 50 mm/s and the voltage was 0.05 mV/mm. Pinpoint pressing permeation is shown in Figure 2.

**Determination of cardiac electrophysiology:** The 4 limbs of each rat were subcutaneously connected to a heart motor (FCP-7101, Fukuda Denshi, Japan) to show the surface electrocardiogram throughout the entire operation. For all 3 groups, the HR was calculated from the average of 10 continuous A-A intervals and recorded before modeling and 3 days, one week, and two weeks after modeling. Sinoatrial node recovery time (SNRT) was determined using the hierarchical ascending speed suppression (S1S1) method. The basal heart rate was measured first. The rats were then stimulated with a voltage of 1.5-2.0 V, pulse width of 10 ms, and frequency of 20%, 40%, 60% and 80% higher than the primitive sinus rate, with a stimulus duration of 20-30 seconds and a stimulus interval of 3 minutes. Corrected SNRT (CSNRT) was calculated as SNRT - SCL (average sinus cycle length). Sinusoidal conduction time (SACT) was also calculated using Narula’s method.

The electrophysiology indexes of the sinoatrial node were evaluated using epicardial atrial pacing with a homemade epicardial allocations electrode.

**Statistical analysis:** Qualitative data are expressed as the frequency, and continuous variables as the mean ± standard deviation (SD). The modeling success rates between these 3 groups were analyzed by the chi-square test. Heart rates and electrophysiology (SNRT, cSNRT and SACT) were analyzed by one-way analysis of variance (ANOVA) followed by the LSD test for differences among different times. All statistical compari-

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**Figure 1.** Homemade mapping catheter. Materials: needle body, acupuncture pin, plastic trocar and intravenous catheter, intravenous needle and tubing, 1 mL syringe. Method: A was connected to V1 of electrocardiogram, and B was connected to sinus note. The formaldehyde or sodium hydroxide was then drawn into the 1 mL syringe, whose other end was connected to the intravenous tubing. Formaldehyde or sodium hydroxide was subsequently injected into the sinus node area, becoming the homemade pinpointing and injecting electrode.

**Figure 2.** Pinpoint pressing permeation. Acupuncture pin was bent into a 135° angle at the top of the needle handle, keeping operators away from touching the acupuncture pins when they hold the top part of the bamboo, which might interfere with the allocating results. The handle tops were connected to two allocating conducting wires. Second, the acupuncture pins below the bend were tightly taped with the bamboo, which made the needle bodies below the bamboo as the epicardial allocations electrode. The 2 taped pin and bamboo were taped together with a 1 cm interval between 2 needle bodies. This is the self-made epicardial allocations electrode, with the other extremity being linked to the V1 of the electrocardiogram.
sions were performed by SPSS version 16.0 statistical software (SPSS Inc, Chicago, IL, USA). A P value less than 0.05 was considered to be a statistically significant difference.

**RESULTS**

**Rate mortality and modeling success rates:** The numbers of model rats were recorded 3 days, one week, and two weeks after modeling for the 3 groups. As shown in Table I, the number of surviving rats was larger in the pinpoint pressing permeation groups than in the thoracotomy group (P < 0.05). Though the survival rates between the FPPP and SHPPP groups were similar at 3 days, no rats died from 3 days to 2 weeks in the hydroxide intervention group, while the number of surviving rats decreased from 8 to 4 in the formaldehyde intervention group. The achievement ratio of modeling was 10% in the FTWC group, 40% in the FPPP group, and 70% in the SHPPP group and the difference was statistically significant ($\chi^2 = 7.250, P = 0.007$). Thus, the SSS model by the SHPPP method was the most stable and successful.

**Changes in HR:** The HR was reduced by about 37% in these 3 groups 3 days after modeling compared with the HR before modeling (P < 0.01) (Table II). After two weeks, the RH was re-elevated and was 85% of the HR before modeling in the formaldehyde thoracotomy group and formaldehyde intervention group. However, there was no statistically significant difference in the HR in the SHPPP group from 3 days to two weeks after modeling (P > 0.05). Thus, the sinoatrial nodes were damaged in all groups after modeling while stable damage only occurred in the SHPPP group.

**Changes in electrophysiology:** The SNRT, cSNRT, and SACT were significantly prolonged 3 days, 1 week, and 2 weeks after modeling compared with pre-modeling in all 3 groups (P < 0.01) (Table III). Meanwhile, there were no statistically significant differences in these indexes among the 3 groups (P > 0.05).

**DISCUSSION**

Studies on animals are important methods for contemporary medical research, and are also critical tools for the study of medicine. Though clinical research provides sufficient clinical evidence for the treatment of medicine on diseases, the action mechanisms of drugs still needed to be supported by animal models.\(^{18,19}\) Animal models are considered as the tie and bridge between clinical and basic studies, and are an effective method to enhance the awareness of disease, screen effective Chinese medicines, and study the mechanisms of drugs.\(^{18,20}\)

There are also some sample animal models used for the study of SSS, such as cryocoagulation and radiofrequency catheter ablation, genetically engineered models, FTWC model, and FPPP model.\(^{4,11,21}\) In this study, an SHPPP model which is more suitable for the study of SSS was successfully established.

The survival rate was higher in the FPPP and SHPPP groups than in the FTWC group. The reason may be that formaldehyde or sodium hydroxide tightly contacted and permeated into the sinus node area through the micro-injector and could also prevent formaldehyde or sodium hydroxide from outflowing to other tissues.\(^{18}\) Our homemade electrode is tiny and easy to use, which might decrease the likelihood of damaging the surrounding tissues of the sinus node and bleeding. Thus, the pinpoint pressing permeation method was better than the traditional thoracotomy method. Although the survival rate in the FPPP group was similar to that of the SHPPP group 3 days after modeling, the survival rate was significantly higher in the SHPPP group than the FPPP group 2 weeks after modeling. This result indicated that the SHPPP method of SSS damage model was more stable and suitable.

The HR was also examined in these 3 groups. After modeling, the HR was decreased in all groups and a significant decline was only maintained in the SHPPP group. The human sinus node occupies an approximate 10 mm subepicardial region on the sulcus terminalis at the superior cavo–atrial junction.\(^{22}\) A decrease in HR is a typical feature of sinus node dysfunction. An electrophysiological study is a common and important

### Table I. Survival Rates in the Three Groups During the Modeling

<table>
<thead>
<tr>
<th>Group</th>
<th>Before modeling (n)</th>
<th>3 days after modeling (n)</th>
<th>1 week after modeling (n)</th>
<th>2 weeks after modeling (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTWC</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>FPPP</td>
<td>10</td>
<td>8</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>SHPPP</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td>3.222</td>
<td>4.755</td>
<td>7.250</td>
<td></td>
</tr>
<tr>
<td>$P$</td>
<td>0.073</td>
<td>0.028</td>
<td>0.007</td>
<td></td>
</tr>
</tbody>
</table>

FTWC indicates formaldehyde thoracotomy wet compressing group; FPPP, formaldehyde pinpoint pressing permeation; SHPPP, sodium hydroxide pinpoint pressing permeation. Chi-square test was used for the differences and P < 0.05 was considered as statistically significant difference.

### Table II. Changes in HR Before and After Modeling

<table>
<thead>
<tr>
<th>Group</th>
<th>Before modeling</th>
<th>3 days after modeling</th>
<th>1 week after modeling</th>
<th>2 weeks after modeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTWC</td>
<td>409.08 ± 24.13$^1$</td>
<td>262.39 ± 25.73$^1$</td>
<td>278.98 ± 25.87$^1$</td>
<td>346.16 ± 22.70$^1$</td>
</tr>
<tr>
<td>FPPP</td>
<td>412.88 ± 21.19$^1$</td>
<td>257.87 ± 26.70$^1$</td>
<td>282.49 ± 23.56$^1$</td>
<td>329.25 ± 13.89$^1$</td>
</tr>
<tr>
<td>SHPPP</td>
<td>407.25 ± 22.68$^1$</td>
<td>282.04 ± 25.68$^1$</td>
<td>273.32 ± 28.76$^1$</td>
<td>274.86 ± 24.91$^1$</td>
</tr>
</tbody>
</table>

Values are shown as mean ± SD in beats/minute. FTWC indicates formaldehyde thoracotomy wet compressing group; FPPP, formaldehyde pinpoint pressing permeation; and SHPPP, sodium hydroxide pinpoint pressing permeation. One-way analysis of variance (ANOVA) followed by LSD test was used for the differences. $^1$, $^2$, $^3$, values with different marks showed significant difference (P < 0.05).
technique for the evaluation of sinus node function and the major electrophysiological indexes such as SNRT, cSNRT, and SACT. Meanwhile, there are several methods for measuring the electrophysiological indexes, such as transesophageal atrial pacing, direct epicardial atrial pacing, atrial pacing by percutaneous atrial pacing, and external jugular vein intubation. 3,26-30 Here, a homemade mapping catheter (Figure 1) was inserted close to the right edge of the second intercostal sternum. Epicardial electrocardiography was mapped by connecting the tail end of the catheter wire to the V1 of the electrocardiogram. The SNRT, cSNRT, and SACT were significantly prolonged compared with pre-modeling in all 3 groups (P < 0.01) (Table III), which indicated a damaged sinus node in all groups. There were no significant differences in these indexes among the 3 groups (P > 0.05).

Table III. Changes in Electrophysiological Index of Sinoatrial Node Before and After Modeling

<table>
<thead>
<tr>
<th>Index</th>
<th>Group</th>
<th>Before modeling</th>
<th>3 days after modeling</th>
<th>1 week after modeling</th>
<th>2 weeks after modeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNRT (ms)</td>
<td>FTWC</td>
<td>162.33 ± 20.07†</td>
<td>282.57 ± 24.96†</td>
<td>239.73 ± 16.55†</td>
<td>220.56 ± 25.78†</td>
</tr>
<tr>
<td></td>
<td>FPPP</td>
<td>161.78 ± 19.72†</td>
<td>277.67 ± 22.27†</td>
<td>245.60 ± 23.39†</td>
<td>222.67 ± 14.24†</td>
</tr>
<tr>
<td></td>
<td>SHPPP</td>
<td>160.54 ± 21.38†</td>
<td>272.27 ± 29.06†</td>
<td>253.33 ± 25.99†</td>
<td>234.31 ± 23.22†</td>
</tr>
<tr>
<td>cSNRT (ms)</td>
<td>FTWC</td>
<td>15.40 ± 2.37†</td>
<td>43.90 ± 5.77†</td>
<td>34.66 ± 5.81†</td>
<td>37.23 ± 5.62†</td>
</tr>
<tr>
<td></td>
<td>FPPP</td>
<td>16.10 ± 3.50†</td>
<td>44.99 ± 5.60†</td>
<td>33.20 ± 6.28†</td>
<td>40.44 ± 7.50†</td>
</tr>
<tr>
<td></td>
<td>SHPPP</td>
<td>13.30 ± 2.90†</td>
<td>39.53 ± 5.09†</td>
<td>33.79 ± 5.99†</td>
<td>36.02 ± 5.35†</td>
</tr>
<tr>
<td>SACT (ms)</td>
<td>FTWC</td>
<td>15.07 ± 2.93†</td>
<td>23.12 ± 6.77†</td>
<td>26.20 ± 3.81†</td>
<td>20.80 ± 5.62†</td>
</tr>
<tr>
<td></td>
<td>FPPP</td>
<td>15.40 ± 2.37†</td>
<td>22.73 ± 4.14 †</td>
<td>21.07 ± 3.43†</td>
<td>19.87 ± 3.63†</td>
</tr>
<tr>
<td></td>
<td>SHPPP</td>
<td>14.93 ± 3.90†</td>
<td>22.40 ± 3.72†</td>
<td>21.53 ± 3.96†</td>
<td>21.60 ± 4.56†</td>
</tr>
</tbody>
</table>

FTWC indicates formaldehyde thoracotomy wet compressing group; FPPP, formaldehyde pinpoint pressing permeation; and SHPPP, sodium hydroxide pinpoint pressing permeation. One-way analysis of variance (ANOVA) followed by LSD test was used for the differences. † and ‡, values with different letters showed significant difference (P < 0.05).

Conclusion: In summary, we have shown that SHPPP was the best method with which to construct an SSS model with a stable and lasting low HR and with a high success rate of modeling. Thus, this SHPPP model with its simple procedure could be used for further studies of the SSS mechanisms and drugs. However, the effects of high sodium hydroxide concentrations on the model were not examined and need to be studied in the future. We will continue to study the influence of ion channels on the sinus node, in order to provide a more convenient model for the study of the treatment of sinus disease and SSS medication.

DISCLOSURE

Conflict of interest: none declared.

REFERENCES

8. Jones SA, Boyett MR, Lancaster MK. Declining into failure: the dynamic properties of the sinoatrial node are closely related with the Na⁺ channel. Kurata, et al confirmed that the dynamic properties of the sinoatrial node are closely related with the Na⁺ channel. The Na⁺ channel in mammals contains an α subunit and one or more β subunits and the α subunit is the major functional channel subunit, which is encoded by SCN5A. Additionally, SCN5A gene mutations could impair the driving ability of the SAN. Therefore, we speculated that SHPPP modeling might damage the sinus node function by influencing the Na⁺ channel, which impaired the driving ability of SAN.