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Detection of Acute Myocardial Infarction in a Pig Model Using the SAN-Atrial-AVN-His (SAAH) Electrocardiogram (ECG), Model PHS-A10, an Automated and Integrated Signals Recognition System

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Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: The aim of this study was to compare the use of the standard 12-lead electrocardiogram (ECG) with the SAN-Atrial-AVN-His (SAAH) ECG (Model PHS-A10), a new automated and integrated signals recognition system that detects micro-waveforms within the P, QRS, and T-wave, in a pig model of acute myocardial infarction (MI).

Material/Methods: Six medium-sized domestic Chinese pigs underwent general anesthesia, and an angioplasty balloon was placed and dilated for 120 minutes in the first diagonal coronary artery arising from the left anterior descending (LAD) coronary artery. A standard ECG and a SAAH ECG (Model PHS-A10) were used to evaluate: 1) the number of wavelets in ST-T segment in lead V5; 2) the duration of the repolarization initial (Ri), or duration of the wavelets starting from the J-point to the endpoint of the wavelets in the ST interval; 3) the duration of the repolarization terminal (Rt), of the wavelets, starting from the endpoint of the wavelets in the ST interval to the cross-point of the T-wave and baseline; 4) the ratio Ri: Rt.

Results: Following coronary artery occlusion, duration of Ri and Ri/Rt increased, and Rt decreased, which was detected by the SAAH ECG (Model PHS-A10) within 12 seconds, compared with standard ECG that detected ST segment depression at 24 seconds following coronary artery occlusion.

Conclusions: The findings from this preliminary study in a pig model of acute MI support the need for clinical studies to evaluate the SAAH ECG (Model PHS-A10) for the early detection of acute MI.

MeSH Keywords: Acute Coronary Syndrome • Anterior Wall Myocardial Infarction • Electrocardiography

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Background

The standard 12-lead electrocardiogram (ECG) is the most widely used method to detect acute and chronic cardiac ischemic changes, changes in cardiac rhythm, and cardiac structural changes in clinical practice because it is convenient, low in cost, and non-invasive [1, 2]. However, the current standard ECG has certain limitations, including limited sensitivity in the detection of the early stages of myocardial ischemia and myocardial infarction (MI), especially in patients with stable angina and silent MI [1,2]. Studies have shown that nearly 50% of all patients with an acute MI have a normal ECG, and approximately 5–10% of patients with acute coronary syndrome (ACS) have normal, or nearly normal, ECG findings [3,4]. Furthermore, current automated methods to evaluate ECG findings may lack accuracy, as the interpretation of the ECG relies on the input of the expertise of the cardiologist [5,6].

Because of the limitations of the standard ECG, the use of the exercise ECG was proposed to increase the sensitivity in the detection of early MI [7]. However, for disabled patients and those who have contraindications for exercise testing, exercise ECG is not recommended as a diagnostic option [7–10]. Furthermore, the basis for the detection of acute ischemia and infarction has changed from the appreciation of changes at the organ level to the cellular level, to the level of the action potential, and recently, to the molecular biological level [10].

Although the standard ECG has been used and developed for so many years, the basic information that can be obtained from P, QRS, and T-wave analysis have changed very little [11]. The standard ECG can only record the P, QRS and T-wave, but micro-waveforms within the P, QRS, and T-wave can be missed. With the development of new signals recognition technology, the previously missed micro-waveforms within the P, QRS, and T-wave by the standard ECG can now be detected. However, the meaning of these micro-waveforms within the P, QRS, and T-wave remain to be explored.

The SAN-Atrial-AVN-His (SAAH) ECG (Model PHS-A10) is a new automated and integrated signals recognition system that detects micro-waveforms within the P, QRS, and T-wave, and was first introduced as a new non-invasive method to detect cardiac electrical signals by Liu et al. in 2015 [12,13]. The SAAH ECG (Model PHS-A10) is a real-time cardiac scanning and recording system that detects the natural signals from the discrete segments of the heart [12,13]. The SAAH ECG (Model PHS-A10) has evolved from the standard ECG and includes all the components of the standard ECG, but displays newly detailed micro-waveforms from the sino-atrial, atrial, and atrioventricular nodes, bundle branches, and Purkinje fibers, disproving a commonly held view that these waveforms could not be detected. Therefore, the SAAH ECG (Model PHS-A10) may have clinical potential as a non-invasive tool for the detection of cardiac electrical signals.

The aim of this study was to compare the use of the standard 12-lead ECG with the SAAH ECG (Model PHS-A10), a new automated and integrated signals recognition system that detects micro-waveforms within the P, QRS, and T-wave, in a pig model of acute myocardial infarction (MI).

Material and Methods

Laboratory animals

Six female Chinese domestic medium-sized pigs (weight, 39–67 kg; age, 10–15 months) were supplied by the Experimental Animal Center, Xiangya Medical School of Zhongnan University, China. The experimental protocol was approved by the Animal Care and Use Committee of the Central South University. The experimental procedures were performed in accordance with the 1996 Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH) (Publication No. 85-23).

The SAN-Atrial-AVN-His (SAAH) ECG (Model PHS-A10)

The SAAH ECG (Model PHS-A10) (Physiosign Inc., Los Angeles, CA, USA) was used with a sampling frequency 1000–5000 Hz, central frequency 75 Hz, scanning speed at 25 mm/s, the amplitude at 20 mm/mV or 40mm/mV. The SAAH ECG uses special signal processing technology to distinguish mixed signals from 0–150 Hz. The SAAH ECG extracts the ultra micro-waveform signals using frequency division linear technology and then synchronously scans and records with the standard ECG [14]. Therefore, the SAAH ECG can record not only the P, QRS, and T-waves, as in the standard ECG, but also the micro-wavelets, that occur before the P wave, PR segment, ST segment and upstroke of the T-wave, according to the standard 12-lead ECG connection methods.

The X-ray machine used was purchased from the General Electric Company (GEC, CA, USA). All of the guide wires including balloon guide wires, artery sheaths, catheters, and pressure pumps were purchased from the Terumo Corporation (Japan).

Indices used for SAAH ECG evaluation

As shown in Figure 1, in which the use of the SAAH ECG is demonstrated humans, the following indices were evaluated [12–14]:

1. the number of wavelets in ST-T segment in lead V5;

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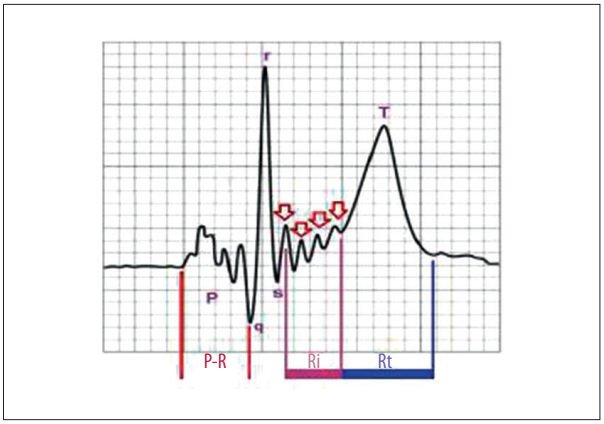

Figure 1. Demonstration of the new waveforms detected by the SAN-Atrial-AVN-His (SAAH) electrocardiogram (ECG) (Model PHS-A10) in the PR interval and ST-T interval in a human patient. All detected indices have been marked in the figure.

2. the duration of the repolarization initial (Ri), or duration of the wavelets starting from the J-point to the endpoint of the wavelets in the ST interval;
3. the duration of the repolarization terminal (Rt), of the wavelets, starting from the endpoint of the wavelets in the ST interval to the cross-point of the T-wave and baseline;
4. the ratio Ri: Rt.

The number of wavelets in the ST-T interval were calculated only if they demonstrated a full sine waveform. There were 'distraction factors,' or complications, that could potentially interfere with the analysis, including respiration and the condition of the skin, which could affect the recordings from the SAAH ECG and the standard ECG. Qualified recordings were defined as those with stable baseline, little noise, and a clear form of wavelets. Recordings that had artifacts, a drifted baseline and deformed wavelets were abandoned. The final value of each measured index was the averaged values obtained from three continuous cardiac cycles. Wavelets in the P, QRS, and T-wave were measured from the V5 lead, and recorded in the same position as the standard 12-lead ECG in humans, using a needle to puncture into the pig's skin.

Experimental animal procedures

Pigs were anesthetized as previously described [15]. Briefly, the pigs fasted for 12 hours, which was followed by a liquid diet for 4 hours before being anesthetized with ketamine (2 mg/kg), medetomidine (0.06 mg/kg) and azaperone (0.06 mg/kg) by intramuscular injection. The pigs were then injected with the following anesthesia: propofol (8 mg/kg/min), remifentanil (0.15 μg/kg/min) and cisatracurium (0.1 mg/kg/h), through an ear vein.

The animals were continuously monitored by standard 12-lead ECG or SAAH ECG (Model PHS-A10) and were provided with a continuous oxygen supply (3 L/min). The pig was laid on its back, with its right inguinal region, front chest and limbs shaved. Disposable electrodes were attached to the front chest and limbs of the animal in the same way as in a standard ECG examination. The right inguinal region was disinfected, and the right femoral artery was punctured with the insertion of a 6F radial arterial introducer sheath (Terumo, Japan). Unfractionated heparin (40000 U) was used to prevent thrombus formation. A Judkins guide catheter was advanced into the ostium of the left and right coronary arteries, which were selectively visualized by coronary artery angiography (CAG) (Figure 2A). After CAG, a percutaneous transluminal coronary angioplasty (PTCA) balloon guide wire was inserted into the root of the left coronary artery to assist the placement of a 20 mm or 25 mm angioplasty balloon in the mid-left anterior descending (LAD) artery distal to the first diagonal branch. The angioplasty balloon was inflated at 4 or 6 atm to block the LAD. The CAG indicated that the blood flowing downstream of the angioplasty balloon was prevented (Figure 2B). The LAD was occluded for 120 min. During the surgery, the pig underwent continuous standard ECG monitoring and intra-arterial pressure monitoring. Also, both SAAH ECG (Model PHS-A10) and standard ECG recordings were made throughout the process.

Histological evaluation of myocardial ischemia and infarction in the pig model

Four weeks after the surgery and the ECG monitoring, the pigs were euthanized. The pig hearts were removed at post-mortem. Sections were taken from the areas of myocardium supplied by the occluded coronary arteries. Sections were fixed in formalin and paraffin-embedded, and then sectioned for histopathology. Histochemical staining of the sections was done with hematoxylin and eosin (H&E) was used with light microscopy to determine the incidence and extent of MI.

Statistical analysis

Data were reported as the mean ± standard errors of the mean (SEMs). The differences between the means at different time points before and after balloon occlusion were evaluated using a linear mixed effects model that included a random effect for each animal. SPSS 17.0 software was used. Differences between means and non-repeated measures were evaluated using the analysis of variance (ANOVA) and the Student's t-test (paired data, unpaired data, and multiple data sets). Differences with $P<0.05$ were considered to be statistically significant.

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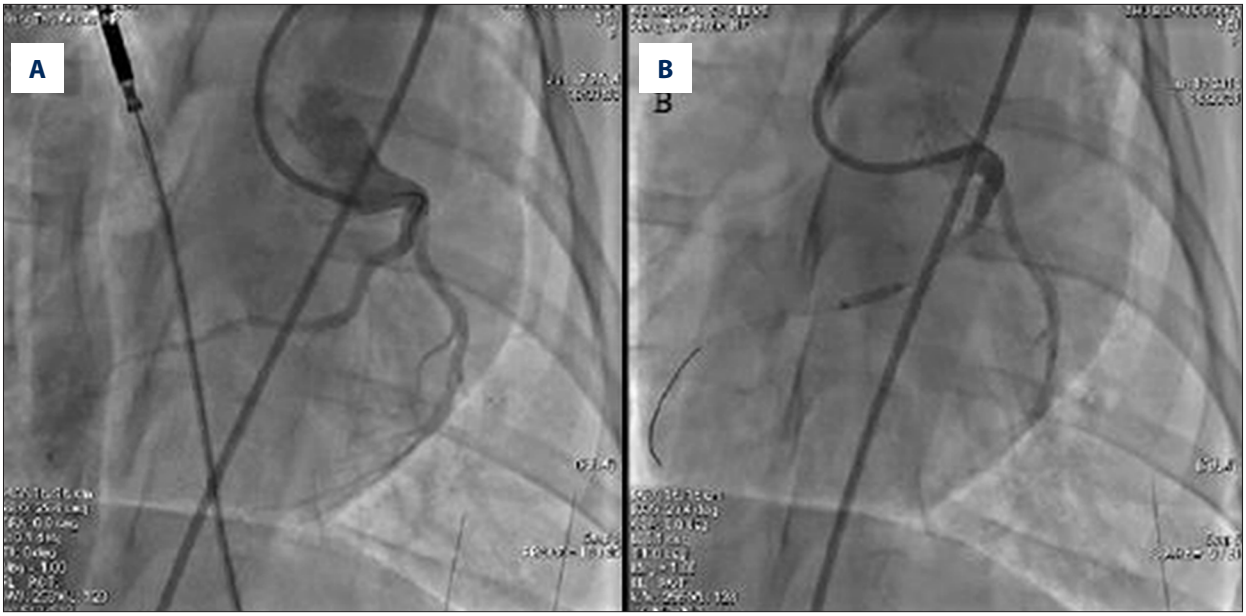

Figure 2. Left and right coronary artery angiography (CAG) in the pig model. (**A**) Left and right coronary arteries were selectively visualized by coronary artery angiography (CAG). (**B**) CAG indicated that the downstream blood flow of the angioplasty balloon was cut off.

Table 1. Immediate change in PHS-A10 EKG after balloon occlusion (n=6).

Time	Wavelets (n)	Ri (ms)	Rt (ms)	Ri/Rt
Baseline	4.8±0.12	129.57±3.96	130.23±2.92	0.99±0.03
12 s after occlusion	5.02±0.23	129.05±1.87	105.45±3.04*	1.22±0.04a
24 s after occlusion	4.6±0.457	131±2.16	102±2.37*	1.27±0.087*
39 s after occlusion	5.7±0.36	150.10±2.12*,**	87.75±4.13*,**	1.71±0.09*,**
2-hour after balloon occlusion	4.73±0.58	135.81±2.41	77.83±2.64*	1.66±0.05*
P value		<0.001	<0.001	<0.001

* Means that compared with baseline situation, $P<0.001$; ** means that compared with 12 s after occlusion, $P<0.001$.

Results

Immediate changes detected by the SAN-Atrial-AVN-His (SAAH) ECG (Model PHS-A10) following myocardial infarction (MI)

Compared with the baseline, the duration of the repolarization terminal (Rt) was decreased after occlusion for 12 s ($P<0.05$) and further decreased after 39 s. After occlusion for 39 s, the duration of repolarization interval (Ri) and Ri/Rt were increased when compared with the baseline ($P<0.05$) (Table 1 and Figure 3A). As shown in Figure 3B, synchronously recorded standard ECG did not show any positive change (usually ST elevated 0.01 mV) 24 s after balloon occlusion. Therefore, the SAAH ECG detected earlier manifestation of AMI when compared with the standard ECG.

Changes detected by the SAAH ECG 2 hours following acute MI

As demonstrated in Figure 4, four weeks after the balloon occlusion (2-hour balloon occlusion), MI was confirmed by H&E staining in sections of the heart from every pig that received balloon occlusion. The infarction zones include the apex, anterior and septal walls of the left ventricle.

As shown in Table 1, the duration of Rt was significantly shortened by ds after balloon occlusion ($P<0.001$). Ri/Rt became more than 1 after 2 hours of balloon occlusion, from near 1, before balloon occlusion. There was a significant difference in Ri/Rt, 2 hours after balloon occlusion ($P<0.001$). But the duration of Ri appeared to have no major difference 2 hours after balloon occlusion (P>0.05).

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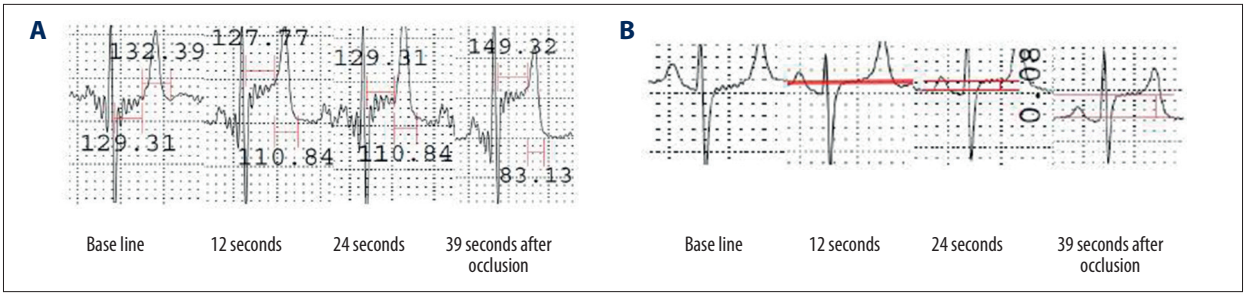

Figure 3. Representative changes SAN-Atrial-AVN-His (SAAH) electrocardiogram (ECG) (Model PHS-A10) synchronously recorded with the standard ECG during the different time points after the blood flow was blocked by the balloon in the coronary artery in the pig model.

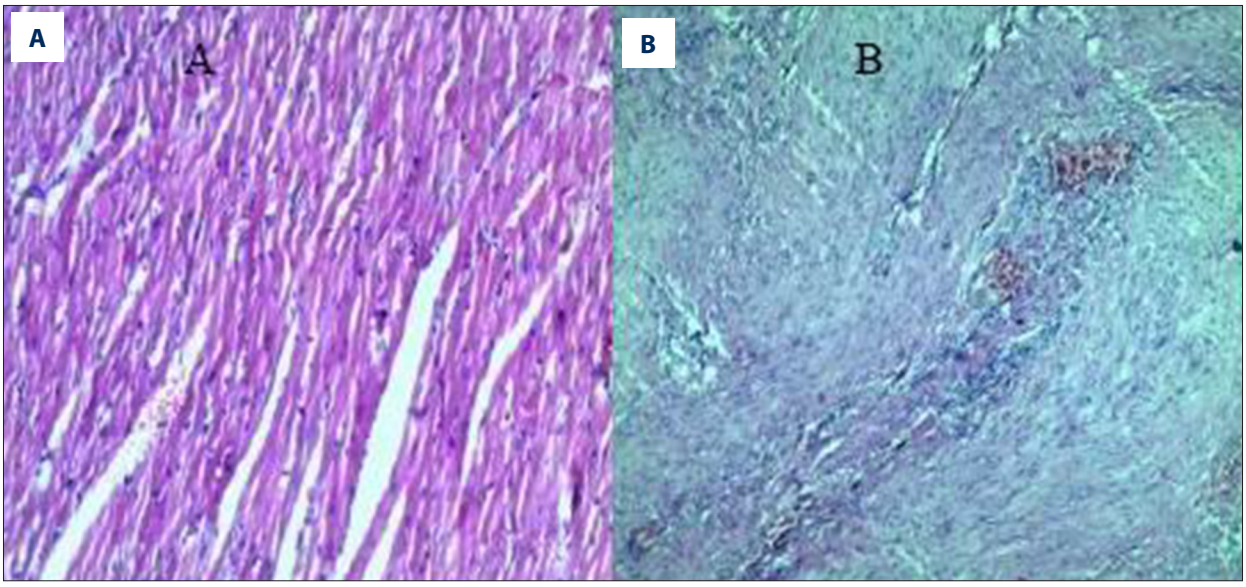

Figure 4. Photomicrograph of the hematoxylin and eosin (H&E) stained section of pig myocardium showing a myocardial infarction (MI) that was produced four weeks after the two-hour balloon occlusion of the coronary artery.

Discussion

Sustained and severe MI will cause myocardial infarction. Therefore, the early detection and treatment of MI are of clinical significance to avoid myocardial infarction (MI) or improve its prognosis [16]. Currently, ECG and serum troponin levels are used to detect MI, but previous studies showed that there was a lack of sensitivity for the first ECG and serum troponin test in patients with non-ST elevation acute coronary syndrome (NSTE-ACS) [17].

Since SAN-Atrial-AVN-His (SAAH) ECG (Model PHS-A10) reveals the underlying signals, which are not shown by the standard 12-lead ECG, it is regarded as a promising non-invasive method for cardiac electrical signal detection. Studies have shown that the SAAH ECG can record between three to five new wavelets in the PR interval and four to seven new wavelets in ST-T interval. The wavelets in PR interval are thought to reflect the conduction signal from the atria to the ventricles, and the wavelets in ST-T interval are thought to be related to the second and third phases of the repolarization process, which involve the complicated ion channels and currents of the ventricles [18–20]. The present study investigated the value of the SAAH ECG in a pig model as a noninvasive measure in the detection of MI in acute and infarction period. When the myocardial infarction occurred (2 hours after the balloon occlusion), Ri came back to the baseline, but Rt continued to be shortened, and Ri/Rt continued to increase.

The current diagnostic criterion of coronary artery disease (CAD) is based on the assessment of the coronary artery by CAG. Since CAG is invasive, it is not listed as a routine examination for the diagnosis of CAD. Also, CAG could not detect the unusual microcirculation of the coronary artery, which could also cause abnormal cardiac electrical activity [21]. In normal condition, the repolarization process involved complicated ion channels and currents, starting from the epicardium and extending towards the endocardium. In the same layer of myocytes, the

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repolarization takes place simultaneously. The whole process tends to be smooth and fast. On the contrary, ischemia may lead to a change in the ion channel activity. This electrical activity of myocardium deviates from the normal path, resulting in decreased resting potential, slow conduction of the electrical signal, and partial depolarization status. Additionally, the action potential of the myocytes in the ischemic area would be lengthened, which would increase the repolarization dispersion from normal myocytes without ischemia [22,23].

In the ECG, the ST-T interval is related to the repolarization potential of the ventricular myocytes. The increased repolarization dispersion between ischemic myocardium and normal myocardium might help in explaining the increased number of wavelets in the ST-T segment, longer duration of Ri, increased Ri/Rt, and shorter duration of Rt. This increased repolarization dispersion between ischemic myocardium and normal myocardium confirms the hypothesis that these parameters in ST-T interval are related to the 2nd and 3rd phases of repolarization process of the ventricle. Since the SAAH ECG collects more detailed information than standard ECG, the SAAH ECG may be a promising tool for the diagnosis of MI via its accurate digitized measurement.

In the pig model of acute MI, there were marked alterations after 2-hour balloon occlusion compared with the baseline level, as shown by the SAAH ECG. We found that two hours after balloon occlusion, Ri returned to the baseline, but Rt continued to be shorter and Ri/Rt continued to increase. In the pig model, MI usually occurs between 1.5 to 3 hours after blocking the mid-LAD artery distal to the first diagonal branch by a percutaneous transluminal coronary angioplasty (PTCA) balloon [24,25]. The histological evidence showed that the acute MI that occurred after 2 hours of balloon occlusion. We could only speculate that this change may reflect the boundary current around the infarction area because the central area consisted of dead cardiomyocytes.

This study had several limitations. The question remains as to what is the meaning of the 12 second earlier detection of MI by the SAAH ECG compared with the standard 12-lead ECG. The heart of a pig is different from that of a human and is more prone to myocardial infarction [26–31]. In a pig model, cardiomyocytes undergo cell death (infarction) usually between 1.5 to 3 hours after blocking the blood flow to the coronary artery; but in humans, cardiomyocytes undergo cell death by 12 hours following coronary artery occlusion [32]. However, the pigs in our experiment were very young and were female, and future comparative studies may be performed using elderly male pigs. Also, humans live much longer than pigs, a 12-second blockage of coronary artery blood flow is likely to result in different pathophysiological changes in humans. It is clear that future large-scale controlled clinical studies are required to compare the clinical use of the SAAH ECG with standard 12-lead ECG in patients with ischemic heart disease, including CAD, and acute MI.

Conclusions

This preliminary study in a pig model of acute myocardial infarction (MI) has shown that the SAN-Atrial-AVN-His (SAAH) ECG (Model PHS-A10) detects acute myocardial infarction (MI) than the standard 12-lead ECG. The SAAH ECG may have clinical potential in the early diagnosis of ischemic heart disease, and this potential should be evaluated in future controlled clinical trials.

Conflict of interest

None.

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