**Preliminary Report**

**PISA: A Noninvasive Method in Detection and Quantification of Acid-induced Myocardial Infarction in Dogs**

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**Summary:** Phase invariant signature algorithm (PISA), a new noninvasive technique, was used in the detection and quantification of acid-induced myocardial damage in anesthetized dogs. The diagnostic capabilities of this method were compared with those of conventional electrocardiogram and biochemical markers, MB-creatine phosphokinase (MB-CPK) and lactic dehydrogenase (LDH). Myocardial damage of varying degree was induced by injecting diluted sulphuric acid (0.01 to 0.10 ml) into the free wall of the left ventricle. Conventional ECG, wideband ECG for PISA analysis, blood samples for LDH, and MB-CPK were taken before and after 15, 30, 60, and 90 min of acid injection. The heart was removed at the end of 90 min for estimation of myocardial damage. PISA Index increased within 10-15 min of acid injection and remained elevated for the duration of the experiment (90 min). The increase in the PISA index was directly related to the extent of myocardial damage and the amount of acid injected. Although the conventional electrocardiogram detected large myocardial damage, it was unable to detect small myocardial damage. Also, most of initial changes in conventional ECG with large myocardial damage disappeared within 90 min, while the PISA index was still elevated to the maximum level. The MB-CPK was not detected before or after myocardial damage. There was no significant change in the LDH at any time after myocardial infarction. These results suggest that the PISA technique is superior to the conventional ECG and the biochemical markers and would be a valuable diagnostic tool in the detection and quantification of incipient as well as advanced myocardial infarction.

**Key words:** noninvasive PISA system, myocardial infarction, MB-CPK, lactic dehydrogenase, conventional ECG, wideband ECG, detection and quantification of myocardial infarction

**Introduction**

Early and accurate identification of ischemic heart disease is an important clinical challenge. Because of certain limitations the diagnostic techniques currently available are incapable of detecting ischemic heart diseases in their early stages. They also present with some difficulties in quantification. Radionuclide imaging has been used extensively in the detection and quantification of myocardial infarction (Holman, 1976; Holman et al., 1978; Parisi et al., 1976). However, quantification by this method is difficult and needs elaborate equipment (Holman et al., 1978; Parisi et al., 1976). Many biochemical markers have been used in the detection and quantification of myocardial infarction. The MB isoenzyme of creatine phosphokinase (MB-CPK) which has been widely used in recent years does not give an accurate measurement of infarct size. Also, detectable amounts appear too late for early diagnosis (Ahumada et al., 1976; Bleifeld et al., 1976; Shell et al., 1973). The popularity of ST-segment mapping for estimating infarct size is attributed to its non-invasive approach and simply applied rules in interpretation. The reliability of information obtained by this process in estimating changing size of infarction is controversial (Holland and Brooks, 1977; Henning et al., 1978; Surawicz, 1972). ST-segment mapping provides a qualitative index of size of infarction and can signal directional changes in size of evolving infarct, but it cannot quantitate the degree of necrosis (Maroko et al., 1972). Currently, no technique can accurately measure the infarct size during the early period, 4-8 h, after onset of symptoms. Creatinine kinase curves require 10-12 h before accurate sizing is possible (Sobel and Shell, 1972).
A diagnostic technique which is noninvasive, and therefore widely applicable and of low risk, and which can detect and quantify at a very early stage of the disease, would be a tremendous advantage. Present studies using the statistical processing and pattern recognition aspects of wideband electrocardiogram have led to the development of a new algorithm called the phase-invariant signature algorithm (PISA) for detection and quantification of early as well as advanced cardiac disorders that introduce phase-locked perturbations in the ECG (Prasad and Gupta, 1978; 1979; 1980; Prasad et al., 1978a,b; Roberts et al., 1975). Our previous studies (Prasad and Gupta, 1980; Prasad et al., 1978b) in dogs have shown that the PISA index increased with coronary ligation and returned to control value after some time, which might be due to the existence of an extensive collateral circulation. However, it was difficult to demonstrate this theory.

The present investigation was, therefore, undertaken to study the effect of localized acid-induced cardiac damage in dogs on the PISA index, conventional ECG, biochemical markers (MB-CPK and LDH₁), and histologic changes. The diagnostic capabilities of these techniques were compared and are reported in this paper.

Methods

The Methods section has been divided into two parts, (a) the basic concept of PISA method, and (b) the data acquisition and signal processing.

PISA Concept

The basics of PISA concept have been reported in detail elsewhere (Prasad and Gupta, 1980; Prasad et al., 1978b). A summary of the concept is given here.

The ECG is a measurement of electrical activity of the cardiac system, which is a cyclic process and can be regarded as a function of time \( t \) from \( 0 - T \) or equivalently as a function of phase \( \phi \) from \( 0 - 2\pi \) radians. The period \( T \) may vary from beat to beat, but the cyclic process will always go through the same phase over \( 0 - 2\pi \) radians. Thus in the phase-domain, the ECG can be regarded as a periodic or phase-invariant process. The hypothesis made in this PISA technique is that the ECG of a diseased heart is composed of three main components:

\[
s(\phi) = \text{a hypothetical ECG signal generated by a healthy heart, under noise-free conditions;}
\]

\[
n(\phi) = \text{the background measurement noise component uniformly distributed over (0, 2\pi) which occurs during the transmission and measurement of ECG and which is not phase-locked to the cardiac cycle;}
\]

\[
p(\phi) = \text{the phase-locked wideband perturbation due to a disorder in the heart.}
\]

The measured ECG, \( m(\phi) \), is therefore a function \( \{f(.)\} \) of \( s(\phi), n(\phi), \) and \( p(\phi) \), and is expressed as:

\[
m(\phi) = f[s(\phi); n(\phi), p(\phi)],
\]

where \( \phi \) is the cardiac phase, \( \phi \epsilon [0, 2\pi] \).

The detection of cardiac disorders using the PISA technique is therefore the detection of \( p(\phi) \) in the presence of \( s(\phi) \) and \( n(\phi) \). The signal \( \{s(\phi)\} \) is a stationary process and, therefore, is distinguishable from perturbations \( \{p(\phi)\} \) and noise \( \{n(\phi)\} \). By employing a phase-locked statistical demodulation technique, \( p(\phi) \) can be identified. Wideband ECG is recorded on an FM tape. Fifty cycles of ECG are digitized and converted from the time-domain to the phase-domain. Using the standard signal averaging technique the stationary component \( \{s(\phi)\} \) is obtained by averaging out the random components \( \{n(\phi), p(\phi)\} \) from the measurement \( \{m(\phi)\} \). The power in the random component is then averaged over many heart beats and plotted as a function of cardiac phase \( \phi \). The power distribution of noise component \( \{n(\phi)\} \) which is independent of phase, is uniformly distributed over the phase \( \phi \epsilon (0, 2\pi) \). The power distribution of random component of the perturbation \( \{p(\phi)\} \) which is phase-locked to the cardiac cycle, appears as peaks or spikes, occurring at a particular phase of the cardiac cycle.

Data Acquisition and Signal Processing

Experiments were carried out in eight dogs weighing 14–18 kg under pentobarbital anesthesia (35 mg/kg, i.v.). The trachea was intubated, and the dogs were ventilated with room air using a Harvard respiratory pump with a volume of 20 ml/kg and a respiratory rate of 15–20 min. The heart was exposed through left fifth intercostal space thoracotomy, and the pericardium was removed.

Various grades of myocardial damage were produced by injecting 0.01–0.10 ml of distilled sulfuric acid (1:1) in the left ventricular wall. Only one dose was used in each dog. Conventional ECG, wideband ECG for PISA analysis, and blood samples for LDH₁ and MB-CPK were taken before and after 15, 30, 60, and 90 min of acid injection. The heart was removed at the end of 90 min for morphologic and histologic studies.

Conventional ECG and wideband ECG for PISA analysis were recorded on an FM tape. The computer analysis of the records was made to determine the PISA signature and PISA index.

Total CPK was determined on an Abbott ABA-100 using...
Warthington Statzyme CPR-N-1 reagent and the methods of Oliver (1955) and Rosalki (1967). CPK Isoenzymes were separated with agarose gel electrophoresis using a Corning ACI system. Fluorometric quantification of isoenzyme bands was made with the use of a Helena Fluro-vis scanner. Total LDH determination was made on an Abbott ABA-100 using Warthington Statzyme LDH (L-P) reagent and the methods of Wacker et al. (1956) and Amador et al. (1963). The LDH isoenzymes were separated with agarose gel electrophoresis using a Corning ACI system. Fluorometric quantification of isoenzyme bands was made by using a Helena fluoro-vis scanning densitometer.

Histologic studies were carried out by the method described earlier by Prasad and Gupta (1980). A large piece of muscle including necrosed, ischemic, and normal tissue, was dissected from the left ventricle and fixed in Bouin's fluid. The tissue was kept in fixative for 1 week and then washed with a mixture of 50% alcohol in water. The tissue was then dehydrated and embedded in paraffin at 57°C. Seven-micron thick sections were cut and stained with hematoxylin/eosin for histological studies.

**Results**

**Myocardial Damage and PISA Index**

Sulphuric acid (50% diluted) was injected into the left ventricular myocardium in various volumes (0.01–0.10 ml) and determination of PISA index and recording of conventional electrocardiogram were simultaneously made at various intervals in eight dogs. The myocardium was removed at the end of the experiment for histologic and naked eye examination. Sulfuric acid produced a volume-dependent damage of the myocardium (Figs. 4 and 5). The extent of myocardial damage with various volumes of acid injected into
FIG. 4 Histological changes in myocardium in (a) dog no. 97 and (b) dog no. 73. Dog no. 97 received 0.03 ml, and dog no. 73 received 0.10 ml, of sulfuric acid. Note the myocardial damage.

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TIME MSEC

FIG. 3 (a) Conventional ECG, (b) ensemble average of wideband ECG, (c) corresponding PISA signature from a relatively large myocardial infarct in a dog where there was an obvious change in the conventional electrocardiogram.

The myocardium is summarized in Fig. 5. The PISA index was directly related to the extent of myocardial damage. The results from 3 such dogs are shown in Fig. 6. The PISA index increased within 10 min and remained elevated to the end of the experiment.

When two methods, i.e., conventional electrocardiogram and the PISA technique, were compared for their capability in detecting cardiac damage, it was observed that in the presence of definite myocardial damage, even though there was no observable change in the conventional electrocardiogram, there was a significant increase in the PISA index. For example, the conventional electrocardiogram of dog #61 (Fig. 7) did not show any observable change although there was definite myocardial damage (Fig. 5) and a marked increase in the PISA index (Fig. 6). Conventional electrocardiogram of dog #80 shows a marked elevation of ST segment within 10 min which progressively subsided, and there was only T-wave inversion at the end of 90 min (Fig. 8). The PISA index in this dog increased significantly within 10–15 min and remained elevated despite improvement in the ST-segment changes (Fig. 6). These results indicate that the PISA method was not only able to detect a small myocardial infarct but was also able to quantify when the conventional ECG was unable to do so.

Correlation between PISA Index and Amount of Acid Injected

The results of the acid-induced changes in the PISA index are summarized in Fig. 9. The PISA index increased within 10–15 min after acid injection and remained elevated to the end of the experiment. There was a direct relationship between the amount of acid injected and the increase in the PISA index. It is apparent from the graph that two similar doses produced similar changes in the PISA index.
Fig. 5  Extent of myocardial damage observed with naked eye in 8 dogs with varying amounts of acid injection. The extent of damage was related to the amount of acid injected.
Abbreviations: IM, intramural damage; NIM, nonintramural (epicardial or endocardial damage included).

Fig. 6  Sequential changes in the PISA index in 3 dogs. Also the relation between PISA index and the extent of myocardial damage is shown. Note that PISA index increased within 10 min and remained elevated for the duration of the study. Also note that the increase in the PISA index is related to the extent of myocardial damage. Symbols: O, dog no. 80; O, dog no. 91; A, dog no. 61.

Fig. 7  Sequential changes in the conventional electrocardiogram after 0.01 ml of sulfuric acid injection into the myocardium. 0, Control ECG; arrow MI (Acid), myocardial damage was produced by injecting 0.01 ml of diluted sulfuric acid. The numbers above each tracing show the time in minutes after acid injection. Note that there is no observable change in the ECG.
Acid-induced Myocardial Damage and Serum Enzymes

Blood samples were collected before and at intervals after intramyocardial injection of acid for estimation of creatine phosphokinase (CPK) and lactic dehydrogenase (LDH) and their isoenzymes. The results are summarized in Table I. It is apparent that the total CPK increased after acid injection. The MM-CPK was the largest component of the total CPK. The MB-CPK was not detectable either before or any time after acid-induced myocardial damage. Total LDH increased at 60 and 90 min after acid injection. There was no significant change in the LDH₁. These results indicate that although there was an increase in the total CPK and LDH heart specific MB-CPK did not appear and LDH₁ did not change after acid-induced myocardial damage.

Discussion

The purpose of this investigation was to use the PISA method, and compare it with the conventional ECG and biochemical markers (MB-CPK and LDH₁) in the detection of intramyocardial damage.

FIG. 8 Sequential changes in the conventional ECG after 0.1 ml of intramyocardial sulfuric acid injection. Arrow indicates where acid was injected to produce myocardial damage (M.I.). The other notations are the same as Fig. 7. Observe the elevation of the ST segment 10 min after acid injection. Also observe that the ST segment slowly returned to normal level, and at the end of 90 min only the T wave was inverted.

FIG. 9 Effects of intramyocardial injection of acid on PISA index in six dogs. Note the dose-dependent changes in the PISA index. Symbols and corresponding doses of acid (ml): ○, dog no. 0061, 0.01 ml acid; ∙, dog no. 0097, 0.03 ml of acid; ×, dog no. 0002, 0.05 ml of acid; Δ, dog no. 0080, 0.10 ml of acid; □, dog no. 0073, 0.10 ml of acid.

TABLE I Serum enzyme level before and at various time intervals (tin) after intramyocardial injection of diluted sulfuric acid in 8 dogs

<table>
<thead>
<tr>
<th>Serum enzymes</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>IU/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total CPK</td>
<td>218±23</td>
<td>285±20</td>
<td>319±37</td>
<td>442±53</td>
<td>420±57</td>
</tr>
<tr>
<td>MMCPK%</td>
<td>80–100</td>
<td>80–100</td>
<td>80–100</td>
<td>80–100</td>
<td>80–100</td>
</tr>
<tr>
<td>BBCPK%</td>
<td>trace to 20</td>
<td>trace to 20</td>
<td>trace to 20</td>
<td>trace to 20</td>
<td>trace to 20</td>
</tr>
<tr>
<td>MBCPK%</td>
<td>trace to 20</td>
<td>trace to 20</td>
<td>trace to 20</td>
<td>trace to 20</td>
<td>trace to 20</td>
</tr>
<tr>
<td>Total LDH</td>
<td>148±28</td>
<td>149±24</td>
<td>134±18</td>
<td>235±96</td>
<td>208±61</td>
</tr>
<tr>
<td>LDH₁%</td>
<td>LDH₁</td>
<td>LDH₁</td>
<td>LDH₁</td>
<td>LDH₁</td>
<td>LDH₁</td>
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<tr>
<td>LDH₂%</td>
<td>greater</td>
<td>Insignificant change</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LDH₃%</td>
<td>than</td>
<td></td>
<td>Slight increase</td>
<td></td>
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<tr>
<td>LDH₄%</td>
<td>LDH₂ and</td>
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<tr>
<td>LDH₅%</td>
<td>LDH₁</td>
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</tbody>
</table>

The results are expressed as mean ±SE. Isoenzymes of CPK (MMCPK, BBCPK, and MBCPK) have been expressed as percent of total CPK.

Abbreviations: CPK, creatine phosphokinase; LDH, lactic dehydrogenase.
and quantification of induced myocardial damage. The myocardial damage was induced by injecting diluted sulfuric acid into the free wall of the left ventricle. In previous studies (Prasad and Gupta, 1980; Prasad et al., 1978b), it was observed that coronary ligation produced an initial increase in the PISA index followed by a return to the control value. The return to control value could have been due to the presence of an extensive collateral circulation in dogs (Cohen et al., 1974; Schaper, 1971). It was therefore decided to produce irreversible and definite myocardial damage by injecting diluted (1:1) sulfuric acid into the free wall of the left ventricle. One could argue that sulfuric acid would circulate in the blood and produce blood pH changes. The acid destroys the tissue immediately, and hence there is very little chance that the acid would circulate in the blood. Various amounts were injected to produce various degrees of myocardial damage.

The myocardial damage was determined with naked-eye observation. Thus the estimate of the extent of the myocardial damage is not truly representative of the myocardial damage. However, this gives a rough correlation between the amounts of acid injected and the extent of myocardial damage.

The present investigation shows that PISA index increased within 10–15 min and remained elevated for the duration of the experiment. Also the increase in the PISA index was related to the extent of myocardial damage or to the amount of acid injected. For the reason given above the PISA index correlated better with the amount of acid injected than with the extent of myocardial damage.

Significant and definite changes were observed in the conventional electrocardiogram with large myocardial damage induced by a large amount of acid. However, no ECG changes were observed with small myocardial damage induced by a small amount of acid. Most of the initial changes in the ECG disappeared 90 min after acid injection.

Total CPK before acid-induced myocardial infarction was higher than reported normal values in dogs (Carlson et al., 1978; Shell et al., 1971). This high level of CPK might be due to the fact that a blood sample was taken after chest surgery to expose the heart and after exposure of the femoral artery and vein for cardiac catheterization. These surgical procedures did produce tissue injury. It is known that a marked rise in CPK activity occurs relative to tissue trauma and postoperative injury (Dixon et al., 1971). Use of electrocautery also makes CPK levels rise (Mostert, 1970). Further increase in CPK after acid-induced myocardial infarction might be due to a progressive increase in the noncardiac tissue damage. It has been shown that total CPK rises progressively and reaches peak at approximately 24–48 h after operation (Dixon et al., 1971). Although total CPK increased, the MB-CPK which is a specific indicator of myocardial infarction did not appear in the blood in any animal serum sample before acid-induced myocardial damage. This is to be expected since MB-CPK makes up a much smaller fraction of total myocardial CPK activity in dogs than it does in man (Roberts et al., 1975). The MB-CPK was not present in a detectable amount after acid-induced myocardial damage. It is possible that the amount of damage could not release enough MB-CPK to be detected in the blood. Also it is possible that appearance of MB-CPK is time-dependent, and 90 min was not enough time for the release of MB-CPK to be detected in the blood. It has been reported that the appearance of a detectable amount of MB-CPK in the blood is too late for early diagnosis of myocardial infarction (Ahumada et al., 1976; Bleifeld et al., 1976; Sobel and Shell, 1972). The other possibility is that sulfuric acid destroyed the enzyme, so it could not appear in the blood. However, the cell surrounding the complete necrotic area must be partially alive to release enzymes.

Total LDH increased but this increase could have been due to tissue damage (Dixon et al., 1971). There was no significant change in LDH after acid-induced myocardial damage.

These studies indicate that the PISA method has the ability to detect and quantify small as well as large myocardial damages. The conventional electrocardiogram was able to detect myocardial damage only when it was large. Biochemical markers failed to detect any of the acid-induced myocardial damage present. Where there was extensive myocardial damage the configuration of the conventional electrocardiogram changed with time, and at the end of 90 min the ECG appeared normal except the T wave was inverted. However, the PISA index increased within 10–15 min and remained elevated for the duration of the experiment. This was expected because whatever damage is produced will stay there.

It appears that the PISA technique is superior to biochemical markers and conventional ECG in detecting and quantifying the myocardial damage. It is envisioned that this method will be a valuable noninvasive diagnostic tool for the detection and quantification of incipient as well as advanced cardiac disorders.

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