



STUDY CODE: HUMET 39-1-08

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## Final Report

**Cardioprotective effects of SHA and HA preparations in  
the isolated working rat heart subjected to  
ischemia/reperfusion.**

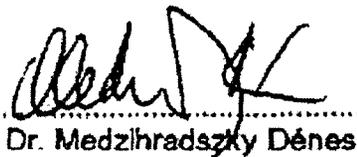
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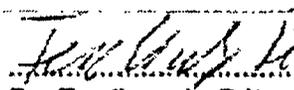
  
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28th April, 1997  
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## 1. Background

Cardiac failure and arrhythmias have particular role in mortality of patients suffering from ischemic heart disease. Therefore, development of therapeutic interventions to improve cardiac function during ischemia, and alleviate arrhythmias upon reperfusion are of great importance. Here, the cardioprotective effect of humic acid (HA) and HA supplemented with micro and macro elements (SHA) was studied. The selenium and HA components of SHA are thought to induce several anti-oxidant enzymes and potentiate antioxidant mechanisms. It is widely accepted in the literature, that the most significant biochemical mechanism underlying the development of reperfusion-induced ventricular fibrillation (VF) is the generation of oxygen free radicals at the onset of reperfusion. Therefore, SHA may protect the heart against VF upon reperfusion.

## 2. Study objective:

To investigate the antifibrillatory effect of chronic peroral treatment with SHA in isolated rat hearts subjected to 25 minutes of ischemia followed by reperfusion.

## 3. Methods:

### 3.1. Isolated heart preparation

Male Wistar rats (300-360 g, fed standard laboratory chow and tap water ad libitum, housed in 12 hours light-dark cycle, 5 animals/cage) (n=32) were anaesthetized with diethylether, and injected intravenously with 500 U/kg heparin. Hearts were excised and cannulated through the aorta, and perfused in the Langendorff mode at a constant perfusion pressure (100 cm water/9.8 kPa) for 10 minutes. During this period, the left atrium was cannulated as described earlier (Ferdinandy et al., 1992). The heart was then converted to a working preparation (Neely et al., 1967; Ferdinandy et al., 1993) perfused at 37°C with oxygenated Krebs-Henseleit bicarbonate buffer. Preload (17 cm water/1.7 kPa) and afterload (100 cm water/9.8 kPa) pressure were kept constant throughout the experiments.

### 3.2. Measurements

Heart rate [HR] derived from the left ventricular pressure curve, coronary flow [CF] measured by collecting effluent from the right atrium in a measuring cylinder for a timed period, aortic flow [AF] measured by a calibrated rotameter (KDG Mobrey, Sussex, England), left ventricular developed pressure [LVDP] counted as peak systolic pressure minus left ventricular end-diastolic pressure [LVEDP], and LVEDP were recorded. Ventricular pressure was measured by a pressure transducer (B. Braun, Melsungen, Germany) connected to a small polyethylene catheter inserted into the left ventricle as described (Ferdinandy et al., 1995). Epicardial electrogram was obtained by using two silver electrodes attached directly to the heart. Ventricular fibrillation [VF], and ventricular tachycardia [VT] were determined from the epicardial electrogram and from the left ventricular pressure curve according to the recommendations of Lambeth Conventions (Walker et al., 1988). Data were on line digitized, recorded, and stored on an IBM PC.

*3.3. Induction of ischemia and reperfusion*

After a 10-min aerobic working perfusion hearts were subjected to a 25-min global no-flow ischemia followed by a reperfusion period of 10 minutes.

*3.4. SHA and HA treatment*

Rats were treated perorally with the vehicle, 10mg/kg and 30 mg/kg SHA and 30mg/kg HA once a day for two weeks, respectively.

*3.5. Statistical analysis*

Data expressed as mean  $\pm$  standard error of the mean [SEM] were analyzed with one way analysis of variance (ANOVA). If a difference was established, each group was compared to the control group using a modified t test corrected for simultaneous multiple comparisons according to the Bonferroni method.

**4. Test conditions and test substances:**

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4.1. The effect of chronic treatment with SHA and HA was studied on reperfusion-induced VF and cardiac function assessed before and after 25 min of global ischemia:

- control, vehicle treated, 25 min ischemia.+10 min reperfusion n=8
- SHA treated (10 mg/kg), 25 min ischemia.+10 min reperfusion n=8
- SHA treated (30 mg/kg), 25 min ischemia.+10 min reperfusion n=8
- HA treated (30 mg/kg), 25 min ischemia.+10 min reperfusion n=8

## 5. Results and discussion

As compared to the control group, neither HA nor SHA treatments changed significantly cardiac function and induced arrhythmias before ischemia (Table 1). This finding shows that HA or SHA exerts no significant effect on nonischemic cardiac function and electrophysiology.

In the control group, upon reperfusion, AF, LVDP,  $+dP/dt_{max}$ , and  $-dP/dt_{min}$  were markedly reduced and LVEDP was significantly increased when compared to preischemic values; and reperfusion triggered 87.5% VF. These are the indicators of the severity of 25 min ischemia. The incidence of VT is the same as the incidence of VF in the present protocol. In the first minutes of reperfusion after 25 min ischemia, if VT occurs, VT terminates soon (after 5-10 beats) and leads to VF.

Treatment with HA significantly improved CF upon reperfusion, and the other cardiac functional parameters (especially AF,  $-dP/dt_{min}$ , and LVEDP) were tended to improve. The incidence of VF was nonsignificantly reduced to 62.5%, which shows a 29% decrease in the occurrence of VF as compared to the control group.

The two week oral treatment with 10mg/kg SHA significantly improved CF, AF,  $+dP/dt_{max}$ , and LVEDP upon reperfusion, whereas LVDP and  $-dP/dt_{min}$  were improved nonsignificantly. SHA markedly decreased the incidence of reperfusion-induced VF to 12.5% ( $p < 0.05$ ), this shows a 86% reduction of VF incidence.

Pretreatment with 30 mg/kg SHA was less effective than 10 mg/kg SHA. CF was significantly improved. Although other functional parameters were improved as well, the improvement did not reach the statistically significant level. Reperfusion-induced VF was decreased to 37.5% which is statistically nonsignificant, however, it shows a 57% decrease in the incidence of VF. Using increased number of experimental animals, this reduction in VF would definitely show a statistically significant change.

### Conclusions:

1. HA and SHA have no effect on the nonischemic myocardium.
2. HA has some beneficial effect on myocardial perfusion after ischemia, and it slightly improves myocardial function. Probably a longer pretreatment, or use of pathological models (e.g. like micro mineral depletion/excess intake, hyperlipidemia etc) would be more appropriate to show the possible beneficial effects of HA.
3. SHA (10 mg/kg over 2 weeks) shows moderate cardioprotective and strong antiarrhythmic effect in the rat heart. The effect of this dose of SHA may be more pronounced in pathological models (see above) or if the pretreatment period would be extended.
4. SHA (30 mg/kg over 2 weeks) although results in a tendency of cardioprotection and antiarrhythmic effect, this dose seems to be too high for a chronic treatment. The dose-response relationship of this compound seems to exhibit a bell shaped curve, and 30 mg/kg SHA may be located in the declining phase of the curve.

The mechanism by which HA and SHA exerts their beneficial effect can not be answered by this study. Nevertheless, the fact that the compounds alleviate reperfusion injury, especially reperfusion-induced VF, may lead to the speculation that an anti-oxidant mechanism is involved.

Table 1. Effects of HA and SHA on myocardial function before and after 25 min global no-flow ischemia in isolated working rat hearts.

treatment	n	HR (bpm)	CF (ml/min)	AF (ml/min)	LVDP (kPa)	+dP/dt <sub>max</sub> (kPa/s)	-dP/dt <sub>max</sub> (kPa/s)	LVEDP (kPa)	VF (%)
before ischemia									
control	8	265±6	22.9±0.9	43.4±1.5	19.2±0.7	1026±45	463±33	0.51±0.04	-
HA 30mg/kg	8	264±7	24.4±0.8	43.9±1.7	19.3±0.5	1058±43	470±26	0.54±0.05	-
SHA 10mg/kg	8	270±4	23.4±0.9	44.5±1.4	19.1±0.4	983±41	451±20	0.58±0.05	-
SHA 30mg/kg	8	263±7	23.0±0.9	44.9±1.6	19.0±0.6	1044±45	445±21	0.49±0.04	-
10 min after ischemia									
control	8	260±3.9	20.4±0.9	13.3±2.5	14.0±0.5	609±53	304±20	1.53±0.09	87.5
HA 30mg/kg	8	257±6	24.3±0.9*	15.4±2.3	14.8±0.7	675±35	339±23	1.35±0.07	62.5
SHA 10mg/kg	8	263±3	24.5±0.8*	24.5±2.8*	15.9±0.8	788±36*	351±20	1.08±0.08*	12.5*
SHA 30mg/kg	8	257±5	23.7±0.9*	19.1±3.6	15.3±0.7	708±44	340±14	1.25±0.08	37.5

HR, heart rate; CF, coronary flow; AF, aortic flow; LVDP, left ventricular developed pressure; +dP/dt<sub>max</sub>, maximum and minimum of first derivative of left ventricular pressure; LVEDP, left ventricular end-diastolic pressure. \* (p<0.05) shows significant difference as compared to the control group.

## 6. Archives

All raw, individual data are stored on disc in SigmaStat worksheet files.

## 7. References

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