The Effects of the Yeast Cytochrome C on the Temporal Occlusion of Coronary Arteries.

By
Koroku Hashimoto & Toshiji Igarashi
Department of Pharmacology, Faculty of Medicine, University of Tokyo

Masahiro Saigusa, Iwo Uei, & Tsuguo Hasegawa
Department of IInd Surgery, Faculty of Medicine, University of Tokyo

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While the technical improvement of the extracorporeal circulation with the harmless and efficient oxygenator unit has brought recent success to the intracardiac operation, it is still an unsolved problem to get a broader safety margin for the temporal interruption of the coronary blood flow and for the temporal induction of the cardiac stillstand.

The technique of cardioplegia1-3) and hypothermia4-7) were already introduced for this purpose. Authors8) also examined previously the influences of cardioplegia. However myocardial damages due to anoxia are not avoidable by these procedures7-9). Then it is of deep concern to find any drug which has a protective property against an expected anoxia during the period of the temporal occlusion of the coronary circulation for the surgical operation.

The effect of cytochrome C to prevent anoxia has been suggested by several reports10-14). Recently quite pure material of the yeast cytochrome C (Sankyo Seiyaku Co.), by the technical development of Prof. K. Okunuki, is introduced for the practical use. Authors tried to evaluate its clinical availability on the isolated Langendorff dog's heart preparation with cross circulation.

EXPERIMENTAL PROCEDURE

Male mongrel dogs were used. Two recipient dogs, weighed 10 kg and one dog weighed over 20 kg as a common donor were prepared for each experiment. An adequate amount of the blood to supply the dead space of perfusion system obtained from the fourth dog.

The experimental arrangement for the isolated Langendorff dog's heart preparation with cross circulation (Fig. 1) was already described in details in the previous paper15). A Dale-Schuster type perfusion pump situated between
the recipient and the donor dog, perfused the blood into the aortic cannula of recipient's heart over the actual demand of the coronary flow. The excess of blood passed by through the pneumatic resistance directly to the donor's jugular vein. By this, mean perfusion pressure was kept in 100 mmHg throughout the experiment. The artificial respiration with oxygen was arranged for the donor dog in 10 ml per kg of the tidal volume and 15 cycles per minute. The same donor dog was used to the paired recipients, this is, the first experiment as a control was done on the recipient without premedication and the second one was done on the recipient with premedication of cytochrome C.

The heart rate, coronary flow and oxygen consumption were measured for 10 to 20 minutes immediately after the experiment was completely set up. Then the blood supply to the recipient heart was excluded instantly by cramping the rubber tube connected to the aortic cannula. The duration of the temporal occlusion of coronary circulation was 10 minutes in 1 case, 15 minutes in 4 cases and 20 minutes in 2 cases. The heart rate and the temperature of the heart was measured at one minute interval during a period of occlusion. After its release, the heart rate and the coronary flow was recorded continuously for 10 minutes, thereafter the records were taken in every 2 minutes for 20 minutes, further in every 5 minutes until the end of one hour observation since the heart had been perfused again. The heart rate and the coronary flow which was measured by the automatic bubble flowmeter were registered simultaneously on ECG. Oxygen consumption was determined at 30 minutes after recirculation.

50 mg of heparin (Takeda Co.) to the donor and 20 mg to the recipient were given initially, afterward 10 mg in a half hour interval. Cytochrome C was given intravenously in 50 mg per kg of the body weight to the recipient dog one hour before the experiment.

Temperature of perfusing blood was kept to 37°C±0.1. However, during
the period of the reactive hyperemia it was impossible to keep just the same temperature because of the sudden change to the tremendous amount of circulating volume.

**RESULTS**

1) The heart rate and coronary flow before occlusion of the coronary flow.
There was no significant difference between non-treated and treated animal with cytochrome C as shown in Table I.

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<th>Non-treated group</th>
<th>Cytochrome C treated</th>
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<tr>
<td></td>
<td>7 cases</td>
<td>group 7 cases</td>
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<tr>
<td>Heart rate</td>
<td>108±S.E. 8</td>
<td>119±S.E. 9</td>
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<tr>
<td>Coronary blood flow</td>
<td>68±S.E. 12</td>
<td>85±S.E. 12</td>
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<td>ml./100gr./min.</td>
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2) Heart rate during a period of occlusion of the coronary circulation.
The heart rate was going to decrease gradually, and finally disappeared after few sporadic ventricular beats. However the auricular beat continued for a while even after the ventricular stillstand. The duration of the persistent rhythmic ventricular beat was not significantly different between two groups, that is, 6 minutes ± S.E. 0.74 in non-treated animal and 5 minutes ± S.E. 0.74 in the treated animal with cytochrome C.

3) The course of the reactive hyperemia and the following period for the recovery.
The heart rate appeared soon after recirculation. The ventricular fibrillation happened once for each group, which was recovered to the normal sinus rhythm by the counter shock by applying momentarily AC current in 100 volt of 50 cycle. The rate of normal sinus rhythm increased over the initial level during a period of reactive hyperemia and settle down to the initial rate in the recovery. The progress was not significantly different between two groups. However, the standard errors of the measurements were definitely smaller in the treated animal than in the non-treated animal (Fig. 2) The reactive hyperemia was at its maximum immediately after the reopening of the occluded coronary arteries. Its magnitude was not so different among 10, 15 and 20 minutes occlusion. So big being as it was, even the biggest supply by perfusion pump could not follow the demand for reactive hyperemia and it was inevitable to obtain a rather bigger standard error of magnitude of reactive hyperemia in either group.

The coronary flow in the recovery period was characteristically different between two groups. It was gradually decreasing to the initial level in the non-treated animal, while in the treated animal with cytochrome C the level was
decreasing further below the initial level for a while and increased again to the initial level. Furthermore the standard error of coronary flow was definitely smaller in the treated animals than in the non-treated animals. (Fig. 3)

Fig. 2. Heart rate in reactive hyperemia and following recovery period. The standard errors in the treated animal was definitely smaller than in the non-treated animal.

Fig 3. Coronary flow and oxygen consumption in the reactive hyperemia and following recovery period. Coronary flow was gradually decreasing to the initial level in the non-treated animal, while in the treated animal with cytochrome C the level was decreasing further for a while and increased again to the initial level. The oxygen consumption in the recovery period was lesser in the non-treated animals than in the treated animals with cytochrome C.

4) The oxygen consumption in the recovery period. The oxygen consumption in the recovery period was 30 per cent lesser than
the initial level in the non-treated animals, while it was higher than the initial level in the treated animals with cytochrome C as shown in Table II. The variation of the level was smaller in the treated animals like in the heart rate and coronary flow.

**TABLE II. The Oxygen Consumption in the Recovery Period.**

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<tr>
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<th>Coronary $O_2$ Uptake ml./100gr./min.</th>
<th>%</th>
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<tr>
<td>Control Group</td>
<td>Before occlusion 3.77</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>After occlusion 2.75</td>
<td>73%(35-127)</td>
</tr>
<tr>
<td>Cytochrome C Group</td>
<td>Before occlusion 3.85</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>After occlusion 4.12</td>
<td>107%(83-123)</td>
</tr>
</tbody>
</table>

5) The histological examination showed no peculiar finding in either the treated and the non-treated group. (Figs. 4, 5, 6, & 7)

Fig. 4. Left ventricular wall in non-treated animal. No peculiar histological change.

Fig. 5. Left ventricular wall in cytochrome C treated animal. No peculiar histological change.
DISCUSSION

Blumgart\textsuperscript{16} found diffuse myocardial fibrosis and persistent electrocardiographic abnormalities after occlusion of the canine left anterior descending coronary artery for 25 minutes. Kardesch\textsuperscript{17} found loss of spontaneous action potentials of the myocardium of rabbits and dogs after 20 minutes of ischemia at 37°C. Greenberg's\textsuperscript{5} experiment resulted in less than 30 per cent decrease of left ventricular work capacity by coronary ischemia more than 15 minutes at 37°C. However, in our studies, it is safe to state that the heart muscle is not so much damaged by the occlusion of coronary circulation for 20 minutes or less. There is not found any particular histological change and the hearts recover well to the state before occlusion of blood supply in an hour or so either in non-treated or in treated animals with cytochrome C. The significantly different points by the use of cytochrome C between two groups are neither in the process of disappear-
ing the ventricular rate, in the number of incidence of ventricular fibrillation induced immediately after reopening the coronary circulation nor in the process of reactive hyperemia.

In the recovery process the level of the oxygen consumption is higher than the initial rate in the treated group while the coronary flow is rather below the initial level. This is definitely different from the non-treated animal which shows the gradual decrease to the initial level even while the oxygen consumption is lower than that in the preocclusion period. The coronary flow in this arrangement is primarily dependent on the extent of oxygen consumption as shown in the previous paper, and then it increases or decreases by the extent of cardiac oxygen demand, when the efficiency of oxygen uptake of the muscle itself is not changed. Thus the result obtained means simply that the cardiac muscle of the treated animal with cytochrome C consumes more easily oxygen and it is not necessary to dilate in excess. In other words the efficiency to consume the oxygen is raised by the cytochrome C treatment clearly in the recovery process. Furthermore the smaller standard errors obtained in the measurements of the heart rate and the coronary flow in the recovery period deserves to be noted because of showing the ability of cytochrome C for the unification of the condition of the heart muscle among animals. This is an apparently beneficial evidence for the clinical usability of this drug, when anoxia is expected in cardiac disease and also in surgical operation, especially when any damage of the heart muscle is suspected.

SUMMARY

The process of cardiac anoxia and its recovery was observed on the isolated Langendorff dog's heart preparation when the coronary circulation was occluded for 10 to 20 minutes. Furthermore the effect of cytochrome C was evaluated in the present procedure.

Cytochrome C improved the recovery process from the anoxia produced by coronary occlusion. This drug let the cardiac muscle take up the oxygen more easily. Furthermore it brings a smaller variation of the heart rate, the coronary flow and the oxygen consumption in the recovery process among individual animals. It indicates quite uniform and stable recovery by cytochrome C premedication.

Authors recommend the use of this drug for the expected anoxia in cardiac surgery on their experimental evaluation.

References

8) Hashimoto, K., Saigusa, M., Uei, I. & Nakamura, K., (to be published)