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Development and Evaluation of a New Apparatus for Continuous Perfusion of Isolated Perfused Pig Heart

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Abstract

To develop a better model of isolated perfused heart, a new apparatus of "coronary artery cannula-fixed-in-aortic tube" was developed for continuous normothermic perfusion and compared to the Casalis apparatus with cold ischemia. Eight mongrel pigs with the body weight of 18 to 24 kg were divided half into two groups.

All the continuous perfusion experimental hearts resumed a spontaneous heart beat and stabilized earlier than the control hearts without the need of defibrillator or pacemaker, indicating no reperfusion injury on the heart. All the experimental hearts did not show fibrillation nor stopped beating during the entire experiment, whereas the control hearts fibrillated. Two control hearts stopped beating, and only one of the two survived with the help of pacemaker. The coronary systolic, diastolic, and mean pressures were more stable with low variation in the experimental hearts than the cold ischemic control hearts. The experimental hearts consumed more oxygen than the control hearts, indicating more cardiac output.

According to these results, the continuous normothermic perfusion method by the new cannula, even though with a short-period of hypothermic perfusion, provided better myocardial protection than the cold ischemia.

Key words : new coronary cannula-fixed-in-aortic tube, continuous normothermic perfusion, short-term hypothermic infusion, cold ischemia, isolated perfused heart, myocardial protection

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Introduction

Animal model of isolated perfused heart is highly recognized to have a heart in vitro to study cardiac function in vitro without the intervention of hormonal and neural effect. A great range of experimental models have been modified with many different preparations of heart isolation is modified and still needs to be improved for a better one[1].

The most ideal method of perfusion that could protect the myocardium effectively from the myocardial reperfusion injury is still controversial and has to be improved as Weisel (1993) stated that "the techniques and constituents of regional cardioplegic protection" against reperfusion injury "have not yet been established"[2]. The purpose of developing a better animal model of isolated perfused heart without myocardial ischemia is receiving more attention; and thus, many strategies have been tried to minimize the ischemia.

Marcus, Wong, and Luisada developed a modified Mann preparation called "Marcus I technique" and a subsequent modification method called "Marcus II technique"[3]. He emphasized that "coronary artery air embolism spells quick and final defeat" and that avoiding ischemic period is the most important thing to improve survival. He attempted to avoid ischemic period through perfusion by a third animal during transfer in order to supply blood to coronary arteries. This method was called "interim parabiotic perfusion" by Marcus group. It was homologous extracorporeal pump. Neptune introduced the concept of "hyperthermia" as means of myocardial preservation of an isolated heart[4]. Webb and Howard provided the idea of "refrigerated heart" in their article titled Restoration of Function of the Refrigerated Heart [5]. In 1960, Lower and Shumway introduced excised heart to be preserved in an iced 4℃ saline solution6.

The most frequently and currently used systems to have an isolated heart perfused are two models which are called the Langendorff preparation (1895)[7] and Neely preparation in (1967)[8]. Leiris et al. discussed the advantages and limitations of the Langendorff's method and Neely's working

heart preparation. They observed longer stability in Langendorff's preparation than Neely's[9]. However, Langendorff system, although it is beating, has the major disadvantage of not performing much or doing any external work. The model of Langendorff requires less oxygen and shows less work output than the ejecting or working heart[9]. The Neely's model performs work like the heart in vivo. Neely's preparation of the isolated working heart is still widely used in the cardiovascular research[10].

Here in this study, the continuous normothermic perfusion combined with a short-term hypothermic infusion was tried to get rid of the period of myocardial ischemia in order to improve myocardial protection. The new aortic cannulating tube has been developed and made in this study with a new coronary cannula tip attached and fixed inside the aortic tube. We compared the modified new perfusion method with this coronary cannula-fixed- in-aortic tube to the ischemic heart perfusion method with the coronary artery cannula of Casali et al.[11].

Materials and Methods

Animals

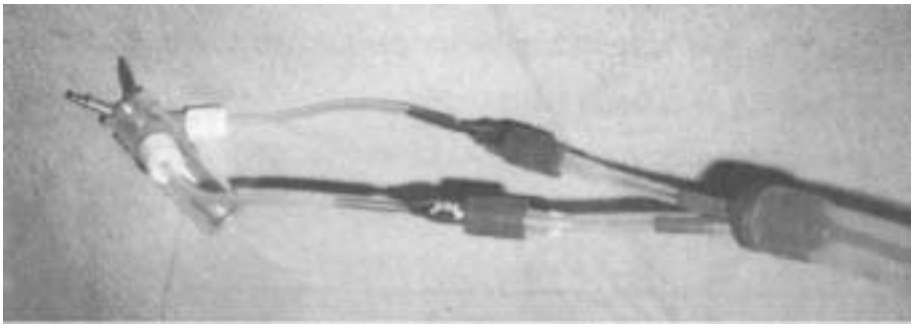
Eight hearts of mongrel swine were studied at the body weight of 18 to 24 kg. They were divided equally into two groups, 4 in a control group and 4 in an experimental group. The experiment procedures and care for the animals complied with the law of Animal Care in France. The pig is widely accepted as an animal model for human cardiovascular physiology studies. Many reasons are given. First, the innate coronary collateral circulation in the pig heart is anatomically sparse just like the extremely low collateral perfusion in humans. A pig has less collateral circulation, about 25% less than a dog has[12]. Secondly, the coronary artery anatomy in the pig heart is similar to that in a human heart[13]. Third, the ratio of the heart size and weight per body weight is the same in pigs as in humans. The fourth reason is that he pig's physiological response to exercise is like to humans[14].

Perfusate Preparation

Cardioplegia called "Solute Cardioplegique SLF 103" (Laboratoire Aguettant, Lyon, France) is used. The cardioplegic solution was infused at 300 mmHg set by Plastimed® (Pressure Infusor, Laboratoire Pharmactique, France).

There had been non-physiological buffer solutions as Tris and Hepes which was described by Mattiazzi et al[15]. Tyrodes solution at 38℃ has been used by Edlund and Wennmalm[16]. For this study, Krebs-Henseleit bicarbonate buffer solution (Krebs) was used as a perfusate. Krebs contained $NaHCO_3$ 25 mmol/L, NaCl 118.9 mmol/L, KH_2PO_4 1.2 mmol/L, KCl 3.75 mmol/L, $MgSO_4$ 2.5 mmol/L, $CaCl_2$ 2.5 mmol/L, and glucose 11 mmol/L. Krebs solutions were mixed and saturated with Carbogene composed of 95% of O_2 and 5% of CO_2. Krebs was perfused at 37℃ by Polystat-thermocontroller(Bioblock Scientific, Avantec Inc.,

A: An's Cannula



B: Casali's cannula

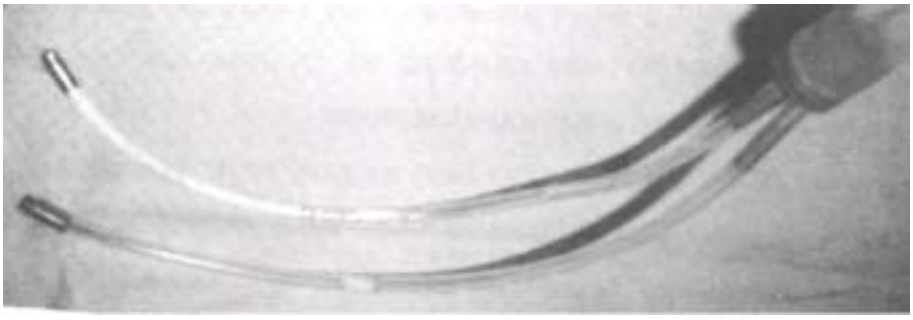


Fig. 1. The new coronary cannula maded for this study (A: An's Cannula) and the coronary cannula used in the work of Casali et al (B: Casali's cannula)

France). The solution was filtered by ABF 40®(40 arterial filter, Sorin Biomedica) and oxigenated by Sorin Biomedica® oxigenator. The perfusate was provided the fresh substrate during three hours of perfusion without recirculation.

New Coronary Cannula-fixed-in-Aortic Tube

The new apparatus was designed as to have the coronary cannula move freely and fix easily in the aortic tube during heart attachment to the perfusion system. As shown in Figure 1, New Coronary Cannula-fixed-in-Aortic Tube have two separated entrances into aortic tube at the degree of 30o, since the ostia of the right coronary and left coronary arteries were located approximately at 30° to 4 0° from the center of the ventral dimension of the aorta.

Perfusion System

A modified perfusion system by Janier and Obadia[11] was used to control a working heart separately from perfusion. The BVS system 5000 Blood Pump (Abiomed®, France) acted as left atrium and left ventricle with two valves simulating the mitral and the aortic valve.

Heart Isolating Surgery

Ketalar 50®(ketamine chlorhydrate, Parke-davis) 10mg/kg BW (body weight) is mixed with Rompun® (xylazine chlorhydrate, Bayer) 2mg/kg and Droleptan® (droperidol, Janssen-Cilag) 0.5mg/kg in one syringe, and injected intramuscularly to induce the sedation just enough to put an intravenous catheter into the auricular vein. Through this intravenous catheter, 3mg/kg of Diprivan® (propofol, Zeneca Pharma) is injected to have the pig in the state of surgical anesthesia. Heparin 660 units/kg is intravenously administered to avoid any formation of microemboli. Tracheostomy was performed immediately with the insertion of endotracheal tube into the trachea. The respiration was set to ventilate mechanically in a constant pressure providing 50% of oxygen mixed with 50% of air. The heart was isolated as described in Table 1.

Table 1. Heart Isolating Surgery Procedure

1. median sternotomy
2. muscle dissected till the 2nd mammary gland
3. sternum opened using bone cutting knife and mallet
4. retractor placed
5. thymus removed
6. Superior Vena Cava(VCS) prepared to be easily cut by dull dissection
7. pericardium cut in the area of aorta
8. cardioplegia cannula placed into aorta
9. Inferior Vena Cava(VCI) and pulmonary vein(PV) localized
10. VCI clamped
11. aorta clamped and 4℃ cardioplegia infused at 300 mmHg simultaneously
12. VCI, PV, and VCS cut in order
13. pericardium cut completely
14. heart removed from body completely and put in the 4℃ sterile normal saline solution

The heart was removed from the body rapidly, less than 30 seconds after the heart beat stopped by cardioplegia infusion. This quick process might need the surgeon's skill and practice. The isolatec heart was weighted and placed in the 4℃ normal saline solution. Both groups had the apex of right ventricle cut to make a small hole for the easy evacuation of perfusate with non-ligated pulmonary artery. Two conductors of pacemaker were placed in the right ventricle before the perfusion of normothermic Krebs solution in both groups for the emergency. This enabled the pacemaker to be connected immediately, if needed, without any damage to the beating heart or any delay. The defibrillator was charged at 10-15 Joules. The pacemaker was Medtronic® 5375 stimulateur cardiaque (Medtronic, Michigan, U.S.A.) set at 110beats/minute in 20mA.

In the control isolated hearts, the attachment of aorta and pulmonary artery is separated. Two coronary arteries were isolated and placed a stay suture material 3-0 silk (Ethicon, U.S.A). The coronary cannula used in the work of Casali[11] was introduced into the coronary arteries and tied. The aorta was attached to the perfusion system by a ligature. The control group had 35-40 minutes of cold ischemia.

In the experimental isolated hearts, the cold cardioplegia was infused continuously but at the pressure of 32 mmHg and stopped two times: when harvesting the heart, and again when mounting the aorta to the perfusion system. Each of these stopping times lasted almost one minute. This entire process took about 10minutes. During these 10 minutes, the fibrous attachment between the aorta and the pulmonary artery was dissected and then connected to the coronary cannula-fixed-in-aortic tube. Once the aorta is well tied to the new aortic tube, the normothermic Krebs perfusate at 37℃ with high dose of KCl (15mmol/L) was perfused in the flow rate of 1ml/min/g HW (heart weight) during 25-30 minutes. The heart is still not beating but perfused. The left main and the right coronary arteries were separated, and a ligature material placed around as near as their ostia. The coronary cannula are inserted and fixed into the coronary arteries. In both groups, the latex balloon was introduced into the left ventricle from the left atrium. The inlet of the balloon was fixed in the mitral valve with the 4 stay sutures.

The major different procedures of the isolated perfused pig heart preparation between the control and the experimental group are summarized in Table 2. The total preparation time was equilibrated to 35-40 minutes in both groups. After the equilibration time, every heart was perfused directly through the coronary arteries by the

Table 2. Major differences in heart preparation

	Control Group	Experimental Group
coronary cannula	Casali's	An's new cannula
Hypotermic cardioplegia	no use (cold ischemia)	continuous for 8 min
cold ischemia	35-40 minutes	2 times for 1 minute
Normothermic Krebs in 15 mmol KCI	no use	25-30 min. of perfusion
role of aortic tube	for hanging heart	for perfusing & hanging
perfusate circulation	coronary artery	aorta & coronary artery

Table 3. Extracorporeal heart response to perfusion by Krebs

Isolated Heart Response to Perfusion								
	Control group				Experimental Group			
	C1	C2	C3	C4	E1	E2	E3	E4
First reponse time(minute)*	4	3	4	4	2	4	3	2
Frequency of fibrillation(number)**	5	2	1	7	0	0	0	0
Stabilization time(minute)***	50	10	5	50	0	0	0	0

* Time of first cardiac response to the normal perfusate.

** Total number of fibrillation at the beginning of normal Krebs perfusion before stabilization of heart beating without fibrillation

*** Time of stabilization of the heart from the first fibrillation to the next measurement time without any more of fibrillation

normothermic physiological Krebs with the concentration of 5 mmol/L KCl at 37℃. After 10 minutes of normal perfusion, the balloon latex was connected to the working heart system. All the extracorporeal hearts were perfused and observed for three hours.

Parameters

The heart's reaction to the control and the experimental perfusion methods were observed by time of first reaction of the heart to the reperfusion of the normothermic Krebs perfusate, on-set of spontaneous heart beat, frequency of left ventricular fibrillation, and time necessary to be stabilized from first fibrillation to the moment of non-fibrillation. From the coronary flow pressure line, we measured the coronary systolic, diastolic, and mean pressures using the 7853 Moniteur (Hewlett Packard, USA) and recorded every 10 minutes. The concentration of gas in the arterial and venous return of the isolated heart was estimated in pH, pCO_2, and pO_2 by 278 Blood Gas System (CIBA-Corning, USA). The heart arterial influx was collected through a coronary pressure line, and venous return was collected through the evacuation hole of the right ventricle. All these parameters were recorded every 10 minutes for three hours. By the end of the study, perfused hearts were weighted and compared to the body weight measured after 10 minutes of a sedative injection and to the heart weight measured right after isolation.

Results

All of the four hearts of the experimental group regained the heart beat spontaneously, smoothly, and quickly after the reperfusion by physiological Krebs in 5mmol/L of KCl, whereas all four control hearts needed a defibrillator to stimulate the heart to beat. None of the experimental group showed any fibrillation. All the control group showed more than 1 fibrillation. The control group needed more time to stabilize the heart beat than the experimental group.

As shown in Table 3, the heart responded to the reperfusion faster in the experimental group than in the control group. The hearts of the experimental group showed a first beating at an average of 2.8 minutes while the hearts of the control group showed the first response of fibrillation at an average of 3.8 minutes. There is a one minute delay in the control group.

The fourth control group, C4 in Table 4, showed a very low heart rate with many fibrillations during the first 50 minutes after the reperfusion and needed a pacemaker to keep the heart beating. The second heart of the control group, C2, had fibrillated after 2 hours of perfusion and stopped beating. The pacemaker and defibrillation were useless in this case. The entire experimental group did not need pacing nor had their hearts stopped beating during three hours of the perfusion by Krebs, indicating that continuous perfusion provided more stable heart beating than the cold ischemia.

Table 5 showed the coronary pressure of the extra-

Table 4. Heart rate of extracorporeal heart

Heart Rate (beats/minute)								
	Control Group				Experimental Group			
Minute	C1	C2	C3	C4	E1	E2	E3	E4
10	108	88	116	72	88	84	84	92
20	80	88	112	68	100	80	84	100
30	84	92	116	68	104	84	104	100
40	84	92	104	Paced	96	84	84	92
50	80	91	104	at 110	80	92	88	92
60	72	88	100	at 110	88	88	92	92
70	72	88	100	at 110	88	88	88	92
80	72	92	96	at 110	100	92	88	84
90	72	88	96	at 110	104	88	84	88
100	84	88	96	at 110	104	92	96	84
110	88	84	100	at 110	108	88	88	64
120	68	88	100	at 110	96	100	84	72
130	80	94	100	at 110	100	104	100	80
140	72	−	96	at 110	100	104	104	80
150	72	−	96	at 110	104	100	100	96
160	72	−	92	at 110	104	104	104	92
170	84	−	96	at 110	104	104	100	72
180	84	−	96	at 110	100	100	100	64
Mean	79	89	101	70*	98	93	93	85

Minute : time of perfusion with normal Kerbs by minute
C : control group
E : experimental group
−: heart fibrillated and stopped
* : paced heart rates not included in the mean

corporeal heart during the left ventricular systole. It was expressed as the systolic coronary pressure. The diastolic coronary pressures of the extracorporeal hearts were also shown in Table 6 during the left ventricular diastole and in Table 7 as the mean coronary pressure. The percentages of the variations in the coronary pressures were calculated by the equation below:

$$\text{Variation } (\%) = \frac{P_{180} - P_{10}}{P_{10}} \times 100$$

The value of "P_{180}" is the last coronary pressure after 180 minutes of perfusion. The value of "P_{10}" is the first coronary pressure after 10 minutes of perfusion.

The mean of these variations is 72% in the control group and 20% in the experimental group, indicating that the experimental group were much more constant in the systolic coronary pressure during the entire experiment. The experimental group had much more stable diastolic coronary pressure than the control group. Much low variation of the experimental group could be an indicator of a good stability of the heart contraction and oxygen consumption. The variations in the experimental group were 26%, 14%, 22%, and 25% with the mean of 22%.

As showed in Table 7, the variations of the mean coronary pressures were 108%, 64%, 37%, and 60% in the control group with the average of 67%. The C4 group is varied to 30% with the 39 mmHg as the last pressure before the pacemaker was turned on. The variations in the experimental group were 25%, 14%, 22%, and 22% with the average of 21%.

All the experimental group showed a higher systolic, diastolic, and mean coronary pressure than all the control group during the early first 30 minutes of the reperfusion. At the end of 3 hours of perfusion, all four hearts of the experimental group showed a higher coronary pressure. All the percentages of the variations in the coronary pressures were below 26% in the experimental group during the left

Table 5. Systolic coronary pressure

Systolic Coronary Pressure (mmHg)								
	Control Group				Experimental Group			
Minute	C1	C2	C3	C4	E1	E2	E3	E4
10	11	29	34	33	50	58	47	46
20	24	37	36	43	51	53	44	44
30	20	38	39	42	50	52	43	42
40	19	39	44	43	55	53	43	43
50	22	34	48	44*	69	52	43	41
60	22	34	49	44*	73	51	43	40
70	22	32	44	43*	63	50	41	39
80	20	32	43	42*	48	50	40	38
90	20	31	40	42*	49	49	39	39
100	18	31	40	41*	47	49	43	39
110	18	33	41	39*	49	53	40	40
120	17	36	39	40*	51	55	43	44
130	18	38	38	40*	56	57	48	45
140	18	46	40	41*	58	60	48	47
150	21	−	40	43*	58	62	51	50
160	22	−	43	48*	59	63	52	52
170	24	−	42	50*	61	63	55	55
180	27	−	44	51*	63	65	56	56
Variation (%)	145	59	29	55*	26	12	19	22

Minute : time of perfusion with normal Kerbs by minute
C : control group
E : experimental group
− : heart fibrillated and stopped
* : pacemaker on at the frequency of 110

ventricular systole, diastole and mean contractile state.

The result of gas analysis was shown in Table 8, 9, and 10. The averages of the atrial influx pH were 7.5, 7.4, 7.4, and 7.4 with their mean of 7.43 in the control group, while they were 7.6, 7.6, 7.5, and 7.5 with their mean of 7.55 in the experimental group. The averages of the venous efflux pH were 7.4, 7.3, 7.3, and 7.4 with the mean of 7.35 in the control group, while they were 7.4, 7.3, 7.3, and 7.3 with the mean of 7.33 in the experimental group. The atrial influx pH was higher in the experimental group than in the control group. But, the venous efflux pH showed no significant difference between the control and the experimental group.

Using the data of the mean from Table 9, we could calculate the percentage of the augmentation of pCO_2 by the perfused heart. The pCO_2 was augmented to 38%, 39%, 31%, and 11% with their average of 30% in the control group. It was increased to 54%, 86%, 70%, and 71% with the average of 70% in the experimental group. The isolated and perfused heart influx pCO_2 had the mean influx of 33.94 mmHg in the control group and 26.84 mmHg in the experimental group. The efflux showed the mean pCO_2 of

Table 6. Diastolic coronary pressure

Diastolic Coronary Pressure (mmHg)								
	Control Group				Experimental Group			
Minute	C1	C2	C3	C4	E1	E2	E3	E4
10	8	20	29	26	46	56	45	44
20	13	28	31	37	48	51	42	42
30	15	27	35	34	46	50	42	40
40	13	29	36	35	50	51	42	36
50	15	25	37	40*	64	50	42	39
60	13	25	38	40*	67	49	42	39
70	13	22	36	40*	58	48	42	37
80	11	21	33	38*	44	48	39	37
90	9	21	33	38*	45	47	36	37
100	9	20	31	36*	45	47	38	37
110	11	20	30	35*	46	51	38	38
120	11	27	31	36*	48	54	41	41
130	12	29	31	36*	53	56	46	44
140	14	36	33	36*	54	59	47	45
150	13	–	32	38*	54	61	49	48
160	17	–	35	45*	55	62	51	51
170	21	–	35	46*	56	62	53	54
180	258	–	37	46*	58	64	55	55
Variation (%)	213	80	28	71*	26	14	22	25

Minute : time of perfusion with normal Kerbs by minute
C : control group
E : experimental group
– : heart fibrillated and stopped
* : pacemaker on at the frequency of 110

43.88 mmHg in the control group and 45.67 mmHg in the experimental group. The increased rate of the pCO_2 was higher in the experimental group than the control group. It indicated that the experimental group had produced much more CO_2 than the control group.

As shown in Table 10, the control group hearts consumed 58%, 71%, 73%, and 63% of O_2 with the average of 66%, whereas the experimental group hearts utilized 73%, 80%, 69%, and 72% of the oxygen with the average of 74%. The experimental hearts consumed more oxygen than the control group, producing the working heart.

The heart weight gains were calculated by subtracting the weight of the heart (HWi) before perfusion from the weight of the heart (HWp) after perfusion.

Using the data of Table 11, the relationship of the heart weight and the body weight was calculated as a percentage shown in Table 12.

The experimental group showed the same heart weight gain of 0.3% among four hearts while the control group showed a variation from 0.4% to 0.2%.

Table 7. Mean coronary pressure

Mean Coronary Pressure (mmHg)								
	Control Group				Experimental Group			
Minute	C1	C2	C3	C4	E1	E2	E3	E4
10	12	25	30	30	48	56	46	45
20	21	33	34	40	50	52	43	43
30	17	33	37	38	47	51	42	41
40	17	34	40	39	52	52	42	42
50	17	30	42	42	66	51	42	40
60	19	30	43	42	65	50	42	39
70	17	28	40	42	58	49	40	38
80	17	28	37	41	46	49	39	38
90	16	27	36	40	47	48	38	38
100	14	26	35	39	46	48	39	38
110	14	27	35	37	47	51	39	39
120	14	32	35	38	50	54	42	44
130	15	34	34	38	54	56	46	45
140	15	41	36	39	56	59	47	46
150	16	−	36	41	56	61	49	49
160	17	−	39	47	57	62	52	51
170	21	−	39	49	58	62	54	54
180	25	−	41	48	60	64	56	55
Variation (%)	108	64	37	60	25	14	22	22

Minute : time of perfusion with normal Kerbs by minute
C : control group
E : experimental group
−: heart fibrillated and stopped
* : pacemaker on at the frequency of 110

Table 8. Isolated heart influx and efflux pH

pH of Heart Influx and Efflux																
	Control Group								Experimental Group							
	C1		C2		C3		C4*		E1		E2		E3		E4	
Minute	Influx	Efflux	Influx	Efflux	Influx	Efflux	Influx	Efflux	Influx	Efflux	Influx	Efflux	Influx	Efflux	Influx	Efflux
10	7.475	7.296	7.443	7.289	7.440	7.294	7.402	7.313	7.510	7.476	7.437	7.262	7.445	7.226	7.530	7.501
20	7.446	7.366	7.428	7.274	7.447	7.346	7.400	7.402	7.531	7.389	7.471	7.377	7.461	7.203	7.533	7.501
30	7.462	7.355	7.445	7.269	7.453	7.326	7.395	7.293	7.566	7.466	7.487	7.369	7.446	7.331	7.531	7.494
40	7.477	7.343	7.464	7.366	7.457	7.321	7.414	7.332	7.550	7.346	7.501	7.314	7.450	7.313	7.527	7.348
50	7.489	7.381	7.458	7.347	7.459	7.255	7.405	7.350	7.555	7.304	7.493	7.288	7.502	7.278	7.554	7.230
60	7.490	7.375	7.406	7.272	7.433	7.284	7.407	7.339	7.558	7.298	7.487	7.283	7.493	7.301	7.539	7.222
70	7.494	7.337	7.406	7.347	7.438	7.327	7.404	7.356	7.553	7.317	7.494	7.279	7.494	7.337	7.544	7.328
80	7.476	7.347	7.422	7.263	7.440	7.305	7.411	7.413	7.587	7.386	7.501	7.285	7.505	7.283	7.547	7.305
90	7.489	7.370	7.432	7.283	7.446	7.290	7.418	7.397	7.625	7.423	7.502	7.295	7.501	7.314	7.544	7.327
100	7.544	7.358	7.427	7.287	7.432	7.294	7.411	7.336	7.640	7.427	7.504	7.288	7.482	7.294	7.548	7.281
110	7.509	7.368	7.423	7.287	7.438	7.309	7.416	7.459	7.726	7.473	7.503	7.323	7.484	7.340	7.543	7.269
120	7.499	7.374	7.436	7.283	7.423	7.364	7.420	7.415	7.493	7.401	7.619	7.327	7.500	7.300	7.538	7.308
130	7.498	7.357	7.449	7.246	7.432	7.285	7.429	7.409	7.549	7.436	7.699	7.341	7.530	7.255	7.558	7.297
140	7.498	7.344	7.536	7.335	7.435	7.400	7.434	7.319	7.569	7.455	7.711	7.360	7.540	7.259	7.549	7.294
150	7.518	7.381	–	–	7.452	7.424	7.430	7.397	7.641	7.439	7.745	7.277	7.528	7.226	7.545	7.324
160	7.518	7.365	–	–	7.437	7.312	7.447	7.434	7.653	7.423	7.736	7.236	7.539	7.242	7.559	7.202
170	7.524	7.369	–	–	7.443	7.363	7.443	7.457	7.649	7.419	7.731	7.241	7.550	7.179	7.564	7.189
180	7.533	7.346	–	–	7.450	7.301	7.440	7.350	7.667	7.400	7.598	7.219	7.597	7.180	7.467	7.142
Mean	7.497	7.357	7.441	7.296	7.442	7.322	7.418	7.376	7.590	7.404	7.568	7.298	7.503	7.271	7.540	7.309

Minute : time of perfusion with normal Kerbs by minute
C : control group
E : experimental group
–: heart fibrillated and stopped
* : pacemaker on at the frequency of 110 from 50 minutes of perfusion

Table 9. Isolated and perfused heart influx and efflux pCO_2

pCO_2 of Heart Influx and Efflux (mmHg)																
	Control Group								Experimental Group							
	C1		C2		C3		C4*		E1		E2		E3		E4	
Minute	Influx	Efflux	Influx	Efflux	Influx	Efflux	Influx	Efflux	Influx	Efflux	Influx	Efflux	Influx	Efflux	Influx	Efflux
10	31.9	48.2	34.6	48.6	33.6	47.2	37.2	46.1	29.4	32.1	34.5	51.8	33.7	56.4	27.7	41.1
20	33.8	41.1	36.2	51.6	33.1	42.0	37.0	36.8	26.8	39.4	31.5	39.5	32.8	59.3	27.8	29.8
30	33.0	41.8	35.5	52.4	33.6	43.4	37.9	47.8	25.6	33.7	31.2	41.2	33.5	43.8	28.0	30.2
40	31.4	43.0	33.6	42.9	32.0	44.6	36.4	43.7	28.7	45.7	30.1	46.5	33.5	46.6	28.1	42.9
50	31.0	40.3	34.1	44.7	33.5	52.2	37.8	40.8	29.8	51.7	30.5	48.3	31.2	51.2	26.4	54.7
60	30.6	39.9	37.6	51.2	35.2	48.7	37.1	43.9	29.0	50.8	30.7	48.9	31.5	48.7	27.4	56.5
70	30.4	43.6	37.3	43.0	35.3	45.1	36.8	42.5	28.5	45.1	30.4	49.5	31.2	43.9	27.3	43.5
80	31.2	43.3	36.7	52.7	35.5	46.5	37.1	36.4	24.7	39.1	30.1	49.5	30.5	49.2	26.4	45.9
90	30.1	39.6	36.5	50.9	34.6	49.2	36.8	37.8	22.0	36.5	29.9	48.6	30.3	46.4	26.9	43.9
100	26.2	41.3	36.2	51.0.	35.8	48.9	37.0	43.9	21.5	35.5	29.3	49.0	30.8	46.8	26.8	49.4
110	29.2	39.4	37.0	50.5	35.0	47.0	36.7	38.2	16.9	32.1	17.6	45.5	30.1	42.0	27.2	49.2
120	29.4	39.9	35.7	48.7	34.9	41.5	36.0	36.3	31.2	37.8	22.9	44.6	29.9	47.1	27.4	45.6
130	28.9	41.5	33.6	53.3	35.6	48.9	35.3	37.3	26.3	34.2	17.9	41.8	28.3	52.9	26.6	46.1
140	28.9	41.5	28.2	43.0	35.6	37.3	35.6	46.3	24.9	33.3	17.6	40.5	27.5	52.0	26.9	45.5
150	28.0	37.9	–	–	33.9	34.2	35.0	37.8	21.2	33.9	16.2	47.7	27.8	55.3	26.9	41.3
160	28.2	39.9	–	–	34.4	45.6	33.5	34.4	20.2	35.0	16.3	51.0	27.4	53.3	26.0	56.9
170	27.8	39.5	–	–	34.2	41.5	35.0	33.2	20.4	35.4	16.3	51.7	26.4	60.1	25.6	54.9
180	27.5	41.7	–	–	33.7	46.7	34.7	41.8	19.8	36.6	22.4	49.8	23.4	61.2	30.8	60.8
Mean	29.9	41.3	35.2	48.9	34.4	45.0	36.3	40.3	24.8	38.2	25.3	47.0	30.0	50.9	27.2	46.6

Minute : time of perfusion with normal Kerbs by minute
C : control group
E : experimental group
– : heart fibrillated and stopped
* : pacemaker on at the frequency of 110 from 50 minutes of perfusion

Table 10. Isolated and perfused heart influx and efflux pO_2

pO_2 of Heart Influx and Efflux (mmHg)																
	Control Group								Experimental Group							
	C1		C2		C3		C4*		E1		E2		E3		E4	
Minute	Influx	Efflux	Influx	Efflux	Influx	Efflux	Influx	Efflux	Influx	Efflux	Influx	Efflux	Influx	Efflux	Influx	Efflux
10	524.5	183.5	538.3	144.3	623.5	176.2	603.1	229.7	579.5	205.6	610.0	111.4	596.5	127.2	578.8	227.5
20	575.7	209.5	590.1	193.8	628.3	156.4	620.8	205.8	549.6	178.2	597.2	121.2	564.7	111.2	596.2	323.7
30	600.3	269.8	616.7	150.1	641.4	151.7	621.4	271.0	552.9	125.0	605.8	133.7	565.7	158.4	593.0	284.8
40	577.1	290.4	584.6	146.1	624.4	138.5	600.8	256.8	529.8	101.5	585.3	111.0	563.5	164.2	588.2	159.6
50	590.2	248.5	590.9	135.6	597.3	138.1	636.7	231.7	515.0	90.3	558.4	100.4	556.3	185.4	592.0	118.3
60	558.6	223.6	555.9	129.4	619.4	147.6	618.4	207.3	500.7	85.8	543.0	108.7	557.5	201.3	591.4	129.7
70	549.1	263.5	539.4	134.6	632.7	147.8	606.9	196.6	463.6	154.7	536.5	121.6	560.9	205.2	574.1	145.4
80	568.7	268.5	550.6	132.9	624.9	170.8	626.8	219.3	527.8	154.7	543.5	129.4	540.1	217.4	553.1	133.9
90	566.2	262.7	568.6	163.5	577.4	170.2	620.4	189.1	540.6	148.7	537.4	138.7	524.5	200.9	549.4	128.7
100	469.7	243.5	563.3	183.9	627.0	180.3	625.7	297.6	520.1	135.9	544.6	137.2	529.9	175.7	531.2	118.5
110	566.2	182.0	566.1	184.8	616.2	183.3	614.9	195.6	482.7	123.0	289.3	107.3	530.6	184.0	545.1	124.5
120	562.7	255.1	583.7	187.7	597.0	180.7	617.2	201.9	618.0	207.0	571.1	154.2	518.8	170.6	554.0	126.8
130	576.3	209.6	546.8	177.1	631.7	179.1	604.4	214.4	622.4	198.7	611.8	126.5	530.8	170.3	563.3	116.2
140	576.3	225.1	576.2	247.2	624.9	176.9	612.5	267.5	610.5	192.8	595.7	111.2	510.3	156.8	568.5	125.2
150	570.3	211.2	–	–	588.8	170.3	600.9	212.4	585.5	165.8	569.6	78.2	499.5	160.1	572.0	139.7
160	570.9	258.8	–	–	610.5	163.0	569.8	225.8	545.6	141.5	535.0	66.8	512.5	151.6	569.0	135.0
170	572.3	252.9	–	–	609.0	170.5	607.4	193.7	526.4	131.5	523.6	67.4	510.6	117.2	570.3	126.1
180	576.0	212.0	–	–	602.4	172.3	597.5	218.8	525.1	130.1	563.2	81.4	512.2	112.2	573.7	169.1
Mean	564.0	237.2	569.4	165.1	615.4	165.2	611.4	224.2	544.2	148.4	551.2	111.5	538.1	165.0	570.2	157.4

Minute : time of perfusion with normal Kerbs by minute
C : control group
E : experimental group
–: heart fibrillated and stopped
* : pacemaker on at the frequency of 110 from 50 minutes of perfusion

Table 11. Body and heart weight

Heart Weight and Body Weight (unit : gram)								
	Control Group				Experimental Group			
	C1	C2	C3	C4	E1	E2	E3	E4
BW	18000	20000	20000	20000	23000	24000	22000	20000
HWi	101.6	115.6	112.0	126.0	130.0	140.0	114.0	106.6
HWp	170.5	163.5	179.4	182.6	206.8	220.0	180.0	168.0
HWg	68.9	47.9	67.4	56.6	76.8	80.0	66.0	61.4

HWi : isolated heart weight before perfusion by Krebs
HWp : perfused heart weight at the end of the experiment
HWg : heart weight gains from the isolated heart to the perfused one
BW : body weight measured after 10 minutes of sedative injection
* : pacemaker on at the frequency of 110 from 50 minutes of perfusion

Table 12. Relationship of heart weight and body weight

Percentage of Heart Weight Per Body Weight (unit : %)								
	Control Group				Experimental Group			
Percentage	C1	C2	C3	C4	E1	E2	E3	E4
HWi / BW	0.564	0.578	0.56	0.63	0.565	0.583	0.518	0.533
HWp / BW	0.947	0.818	0.897	0.913	0.899	0.917	0.818	0.84
HWg / BW	0.383	0.240	0.337	0.283	0.334	0.334	0.30	0.307
HWg / HWi	67.8	41.4	60.2	44.9	59.1	57.1	57.9	57.6

HWi : isolated heart weight before perfusion by Krebs
HWp : perfused heart weight at the end of the experiment
HWg : heart weight gains from the isolated heart to the perfused one
BW : body weight measured after 10 minutes of sedative perfusion

Discussion

The cardioplegia has been used in a hypothermic or a normothermic state and in continuous or intermittent infusion. The question is raised on what will happen when giving hypothermic cardioplegia continuously to the heart even for a short period of time. Will the continuous normothermic perfusion combined with hypothermic cardioplegia infusion reduce the side effects of cold ischemia? To answer this question, we conducted the study of continuous perfusion by the short-term hypothermic cardioplegia and normothermic cardioplegic Krebs perfusate and compared it with the cold ischemia. The modified perfusion preparation used in this study could be one of solutions to protect the isolated and perfused heart from the reperfusion injury.

Lee and his team found a continuous warm blood cardioplegia infusion in the human cardiac surgery provided a better rate of the pericardial closure than hypothermic infusion[17]. When the warm krebs reperfusion was carried out, the sinus rhythm returned spontaneously. The experimental group here showed no ventricular fibrillation with very stable coronary pressures throughout the experiments. Cheon et al. found ischemic preconditioning increased the ability of the heart to overcome reperfusion injury[18], whereas our ischemic control hearts failed to overcome the reperfusion injury, by showing many fibrillation early and an inability to beat spontaneously.

Sack informs that the pig heart weights 0.5% of the body weight before forming the subcutaneous and fatty tissues. After formed, the weight of the adult pig heart is 0.3% of the body weight[19]. In our study, the mean percentage of a heart weight per body weight (HWi/BW) before the reperfusion was 0.58% in the control group, while 0.55% in the experimental group. According to Sack, it could be said that all of our pigs studied here were undergoing the formation of the subcutaneous and fatty tissues. Aziz et al. found that heart swellings will cause an impairment of the cardiac compliance and function after the use of hypothermic cardioplegia[20]. That explains why the hearts of the control and the experimental group

gained weight after the end of three hours of the Krebs perfusate without blood.

There is a coronary flow reserve which is diminished by the epicardial and intramyocardial stenosis[21]. The mean coronary pressure in the experimental hearts were much more in the normal range than the control hearts were. Here, the coronary flow rate was fixed at 2ml/min/g heart weight. The coronary flow is very important in keeping a normal viability, function, and metabolism of the heart.

The very short period of ischemia, which is not sufficient enough to cause infarction, may bring a transient dysfunction at the time of the reperfusion period, and delay the recovery phase[22]. The prolonged recovery phase will cause the postischemic reperfusion dysfunction without myocardial necrosis. The cardiac contractile function will restore very slowly. The slow or gradual recovery period of 100% contractile function is called postischemic period or stunning. When the heart is perfused by cardioplegia, it stops immediately and draws a contractile function graph line dropping steeply. When it is reperfused before necrosis, its contractile function is supposed to restore very quickly, making the graph line steeply go up to 100% theoretically, as illustrated in Figure 2(A). However, in practice, the recovery time of normal contractile function is longer and slower than the theory, as shown in Figure 2(B). The experimental group resumed the cardiac contractile function faster than the control group without any fibrillation. Then, the experimental group could be referred to not have a postischemic reperfusion dysfunction or a very short one.

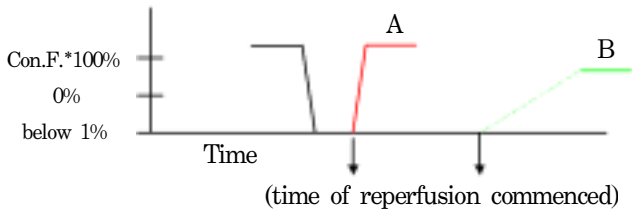


Fig. 2. Recovery time of contractile function; (A) theory o the recovery time of contractile function (B) postischemi reperfusion dysfunction (* contractile function)

For the pig with a low collateral perfusion, the ischemia is caused more profoundly by a coronary occlusion than for any other animals. Guth and Ross used the term of the "perfusion-contraction matching" to illustrate the fact that the ischemia reduced cardiac contractile function and its energy demand[23]. Only reducing the coronary flow[24] or lowering the perfusion pressure of Langendorff perfused hearts[25] can make a graded ischemia be formed. By the ligation of the coronary arteries, the ischemia can be induced regionally[26]. Therefore, a new coronary cannula-fixed-in-Aorta tube is made to have a multiporous coronary cannula tip to avoid any blockage of coronary branches, providing a non-ischemic heart.

As the heart consumes and needs oxygen and energy to function, the oxygen amount used can be calculated to show the index of the cardiac metabolism[8]. The cardiac output and oxygen consumption increase at the rate of 100ml/min/m^2 of oxygen with 590ml/min/m^2 of cardiac output[27,28,29]. Cardiac output can be predicted by the equation of cardiac index that is "Cardiac Index (L/min/m^2) = 0.0059 × A + 2.99", where "A" is the measurement of oxygen consumption expressed in ml/min/m^2. From this equation, the experimental group was predicted to have higher cardiac output and better working hearts than the control group because the experimental group hearts consumed much more oxygen than the control group.

In humans, the left main coronary artery is about 2cm in length; but, our pigs arteries seem to have less length than human arteries. In a young swine with the body weight of 15 to 25 kg, the left main coronary artery seems to be around 1cm, or even less than 1cm in length.

The perfusion system used in our study could not provide the left ventricle (LV) systolic, diastolic, and mean pressures and LV ejection volume due to the early stage of development of this system. For the future study, the coronary effluent can be collected from pulmonary artery instead of the right atrium to analyze the coronary vein return or recirculation, as in the work of Bergmann[30].

According to these results, the continuous normothermic perfusion method by the new cannula, even with a short-period of hypothermic perfusion, provided better myocardial protection than the cold ischemia.

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References

1. Obadia, J.F., Casali, C., Chassignolle, J.F. and Janier, M.: Mitral subvalvular apparatus: Different functions of primary and secondary chordae. *Circulation*, 96(4): 3124-3128, 1997.
2. Weisel, R.D.: Models of myocardial stunning. *J Card Surg*, 8(Suppl): 287-290, 1993.
3. Marcus, E., Wong, S.N.T., and Luisada, A.A.: Homologous heart grafts. *AMA Arch Surg*, 2: 179-191, 1951.
4. Neptune, W.B., Cookson, B.A., Bailey, C.P., Appler, R., and Rajkowski, F.: Complete homologous heart transplantation. *AMA Arch Surg*, 66: 174-178, 1953.
5. Webb, W.R. and Howard, H.S.: Restoration of function of the refrigerated heart. *Surg Forum*, 8: 313, 1957.
6. Lower, P.R. and Shumway, N.E.: Studies orthotopic homotransplantation of the canine heart. *Surg Forum*, 11: 18, 1960.
7. Langendorff, O.: Untersuchungen am uberlebenden

saugethierherzen. *Pflugers Archiv*, 61: 291-332, 1895.
8. Neely, J.R. and al., e.: Effect of pressure development on oxygen consumption by isolated rat heart. *American Journal of Physiology*, 212(4): 804-814, 1967.
9. de Leiris, J., Harding, D.P. and Pestre, S.: The isolated perfused rat heart: A model for studying myocardial hypoxia or ischemia. *Basic Research in Cardiology*, 79: 313-321, 1984.
10. Schneider, C.A., Nguyen, V.T.B. and Taegtmeyer, H.R.: Feeding and fasting determine postischemic glucose utilization in isolated working rat hearts. *American Journal of Physiology*, 260: H542-H548, 1991.
11. Casali, C., Obadia, J.-F., Canet, E., Bendjelid, K., André-Fouet, X., Revel, D. and Janier, M.F.: Design of an isolated pig heart preparation for positron emission tomography and magnetic resonance imaging. *Invest Radiol*, 32(11): 713-720, 1997.
12. Bloor, C.M., White, F.C. and Roth, D.M.: The pig as a model of myocardial ischemia and gradual coronary artery occlusion, edited by Swindle, M.M., Moody, D.C. and Phillips, L.D. (1st ed.), *Iowa State University Press*, pp.163-175, 1992.
13. Baroldi, G., Mantero, O. and Scomazzoni, G.: The collaterals of the coronary arteries in normal and pathologic hearts. *Circulation Research*, 4: 223-229, 1956.
14. Hastings, A.B., White, F.C., Sanders, T.M. and Bloor, C.M.: Comparative physiological responses to exercise stress. *J Appl Physiol (the American Physiological Society)*, 52: 1077-1083, 1982.
15. Mattiazzi, A.R., Cingolani, H.E. and Gonzalez, N.C.: Primary negative inotropic effect of tris(hydroxymethyl) aminomethane. *Am J Physiol*, 222(1): 10-15, 1972.
16. Edlund, A. and Wennmalm, A.: Oxygen consumption in rabbit Langendorff hearts perfused with a saline medium. *Acta Physio. Scand*, 113: 117-122, 1981.
17. Lee, C.K., Park, S.I., Cho, J.M. and Won, J.H.: Clinical experiences of continuous warm blood cardioplegia; valvular heart surgery. *Korean J Thorac Cardiovasc Surg*, 31(4): 353-361, 1998.
18. Cheon, Y.J., Lee, I.S., Kim, Y.S., Choi, Y.H., Kim, K.T., Kim, H.M., Kim, H.J. and Lee, G.: Effect of global ischemic preconditioning after cardioplegic arrest; Langendorff isolated heart study. *Korean J Thorac Cardiovasc Surg*, 31(2): 95-101, 1998.
19. Sack, W.O.: Essentials of Pig Anatomy. Veterinary Textbooks, Ithaca, New York, pp. 1-22, 1982.
20. Aziz, S., Tada, Y., Jaffery, S., Mori, Y., Reichenback, D.D., Gronka, R., Kushmerick, M. and Verrier, E.D.: University of Wisconsin solution provides superior myocardial preservation compared with Stanford cardioplegic solution. *J Heart Lung Transplant*, 13: 1099-1108, 1994.
21. Hodgson, J.M., Cohen, M.D., Murphy, J.H., Szentpetery, S. and Thames, M.D.: The dilated left ventricle: Atrial Natriuretic peptide. *J Am Coll Cardiol*, 9(2): 116A, 1987.
22. Vroom, M.B. and van Wezel, H.B.: Myocardial stunning, hibernation, and ischemic preconditioning. *J Cardiothor and Vasc Anesth*, 10 (6): 789-799, 1996.
23. Guth, B.D., Schulz, R. and Heusch, G.: Time course and mechanisms of contractile dysfunction during acute myocardial ischemia. *Circulation*, 87(suppl IV): IV-35-IV-42, 1993.
24. Manning, A.S., Hearse, D.J., Dennis, S.C., Bullock, G.R. and Coltart, D.J.: Myocardial Ischaemia: an isolated, globally perfused rat heart model for metabolic and pharmacological studies. *Europ J Cardiol*, 11: 1-21, 1980.
25. Weisfeldt, M.L. and Shock, N.W.: Effect of perfusion pressure on coronary flow and oxygen usage of nonworking heart. *Am J Physiol*, 218(1): 95-101, 1970.
26. Kannengiesser, G.J., Lubbe, W.F. and Opie, L.H.: Experimental myocardial infarction with left ventricular failure in the isolated perfused rat heart. Effects of isoproterenol and pacing. *Journal of Molecular and Cellular Cardiology*, 7(1): 135-151, 1975.
27. Grossman, W.: Cardiac Catheterization and Angiography. (2nd ed.). Lea & Febiger, Philadelphia, pp. 1-86, 1980.
28. Grossman, W. and Mclaurin, L.P.: Dynamic and isometric exercise during cardiac catheterization. In Book. edited by Grossman, W. Lea & Febiger, Place published, pp. 215-222, 1980.
29. Dexter, L., Whittenberger, J.L., Haynes, F.W., Goodale, W.T., Gorlin, R. and Sawyer, C.G.: Effect of exercise on circulatory dynamics of normal individuals. *J Appli Physio*, 3(8): 439-453, 1951.
30. Bergmann, S.R., Clark, R.E. and Sobel, B.E.: An improved isolated heart perparation for external assessment of myocardial metabolism. *American Journal of Physiology*, 236(4): H644-H651, 1979.