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Effect of Ibuprofen and Indomethacin on the O_2 Supply/Consumption Balance in Ischemic Rabbit Myocardium (42175)

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Abstract. The purpose of this study was to determine if differences in the cardioprotective abilities of ibuprofen and indomethacin were due to their differing abilities to alter the O_2 supply/consumption ratio in the ischemic myocardium. Experiments were done on 21 anesthetized open-chest rabbits. Regional flow (using radioactive microspheres), O_2 extraction, O_2 consumption, and O_2 supply/consumption ratio were determined 1 hr after occlusion of the left anterior descending coronary artery in controls and animals given iv 10 mg/kg ibuprofen or iv 3 mg/kg indomethacin. Myocardial blood flow was depressed in the occluded region compared to the nonoccluded region after occlusion for all treatments. O_2 extraction in the occluded region was elevated compared to the nonoccluded region for all treatments after occlusion. No differences in O_2 consumption were noted between any treatment within the occluded or nonoccluded regions. The O_2 supply/consumption ratio was lower in the occluded region compared to the nonoccluded region for all treatments. No differences in this ratio were noted between any treatment. Thus, the effects of indomethacin or ibuprofen on ischemia are not related to acute changes in myocardial O_2 supply/consumption balance.

Myocardial ischemia can elicit an inflammatory response which may act to increase the severity of the ischemia and ultimately increase infarct size (1, 2). Such sequelae include the activation of leukocytes (2) and complement (1), aggregation of platelets (3), and release of lysosomal enzymes and other inflammatory mediators such as prostaglandins (4, 5). Any of these factors can be associated with an increased severity of an ischemic episode. One mechanism for this increased severity of ischemia due to an elicited inflammatory response may be that the O_2 supply/demand relationship may be depressed due to microvascular obstruction or to release of vasoactive substances (2, 6, 7). Thus the treatment of ischemic myocardium with anti-inflammatory agents may have a beneficial effect in salvaging viable myocardial tissue. The effects of some nonsteroidal anti-inflammatory drugs on the severity of ischemia have not been conclusive, however. Indomethacin (cyclooxygenase inhibitor) has been shown either not to improve or actually increase the severity of myocardial ischemia (6, 7). Conversely, ibuprofen (another cyclooxgenase inhibitor) appears to decrease infarct size or severity of ischemia (8–10).

One mechanism for the beneficial effect of ibuprofen may be an improvement in the regional O_2 supply/demand ratio in the ischemic area. This may be due to its ability to inhibit thromboxane production and platelet aggregation, thus possibly improving coronary flow or flow distribution (11, 12). Ibuprofen and indomethacin may have differing abilities to improve the ischemic region O_2 supply/demand ratio and this may result in their different effects on the ischemic zone in myocardial tissue. Thus, the purpose of this study was to determine the effect of ibuprofen and indomethacin on the regional O_2 supply/demand ratio in the ischemic myocardium in rabbits.

Materials and Methods. In this study, 21 New Zealand white rabbits (1.4–2.4 kg) were used. The animals were anesthetized with iv sodium pentobarbital (30 mg/kg) and polyethylene catheters were inserted into the right femoral artery and vein. The trachea was cannulated and artificial respiration was instituted such that eucapnia was maintained. A left thoractomy was performed at the fifth intercostal space and the pericardium was resected. A catheter was inserted into the left atrium for injection of radioactive microspheres. After allowing ample time for stabilization in all

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groups, blood pressure and heart rate were recorded via the arterial catheter on a Beckman R-411 recorder. Regional blood flows were then determined using radioactive microspheres. Blood samples obtained from the femoral artery were analyzed for blood gases and pH electrometrically (Radiometer Model BMS3Mk2) while hemoglobin was determined spectrophotometrically using a cyanmethemaglobin method. For the coronary blood flow determinations, approximately 7.5×10^5 microspheres (3M Co.), 15 ± 3 μm in diameter, labeled with ^{85}Sr or ^{141}Ce were shaken for 5 min and injected as a 0.2 ml bolus into the left atrial catheter. A reference sample method was used for the flow determinations (13). The blood sample was withdrawn from the femoral artery with a peristaltic pump set at 1 ml/min. Withdrawal was begun 30 sec before injection and was continued for 3 min.

After these measurements, all rabbits were subjected to left anterior descending coronary artery (LAD) occlusion. After 10 min of LAD occlusion, the animals were divided into three groups: (a) controls ($N = 7$), (b) animals injected iv with 10 mg/kg ibuprofen (Upjohn; $N = 7$), (c) animals injected with iv 3 mg/kg indomethacin (Merck, Sharp, and Dohme; $N = 7$). These were injected as a bolus and the animals were maintained for another 50 min, well within the half-life of these drugs (14). At the end of the 50-min period, hemodynamic variables and blood flows were again determined. Thus regional blood flows were measured before LAD occlusion and 60 min post-LAD occlusion.

After these measurements, the hearts were rapidly removed by cutting the heart at the atrioventricular ring with shears, then quick-frozen in liquid nitrogen and stored at −70°C. For flow determinations, four tissue plugs were taken from the left ventricle; subepicardium in occluded and nonoccluded regions and subendocardium in the occluded and nonoccluded regions.

In order to measure O_2 saturation in frozen arterial and venous blood vessels a 3-wavelength microspectrophotometric method was used as described previously (15, 16). Hearts were cut on a band saw at −20°C and adjacent plugs were obtained from the left ventricular free wall in the occluded and nonoccluded regions of the heart for microspectrophotometry and flow determinations. After the plugs were cut to a convenient size, they were mounted with an embedding medium (O.C.T. Compound, Lab-Tek Products, Naperville, Ill.). Twenty micra sections were cut on a microtome at −20°C in a nitrogen atmosphere. They were transferred to glass slides and covered with degassed silicone oil and a coverglass. These slides were placed on a Zeiss microspectrophotometer fitted with a N_2-flushed cold stage to obtain readings of optical density at 560, 524, and 507 nm. The slit width was set at a 5-nm band pass and size of the measuring spot was 8 μm. Readings were obtained to determine O_2 saturation in the first five arteries and veins, 20 to 100 μm in diameter, found in four left ventricular regions; subepicardium in the nonoccluded and occluded areas and subendocardium in the nonoccluded and occluded regions. The O_2 content of the blood was obtained by mutiplying the percentage O_2 saturation by the hemoglobin concentration times 1.36. The difference between the average arterial and venous O_2 contents was then obtained. For flow, the activity of the microspheres in these regions, as well as in the reference blood samples, was then determined on a Hewlett-Packard Autogamma spectrometer.

By use of the Fick principle, the paired product of O_2 extraction and blood flow was obtained to determine O_2 consumption on a regional basis within the heart. The regional ratio of O_2 supply to O_2 consumption was determined by $SaO_2/(SaO_2 - SvO_2)$, where SaO_2 and SvO_2 are the percentage oxyhemoglobin in the arterial and venous blood, respectively.

A factorial analysis of variance was used to determine whether differences existed in hemodynamic or blood gas parameters before and after occlusion and between treatments. This analysis was also used to determine differences between treatments and regions for all O_2 supply/consumption parameters. Duncan's prodecure was used for multiple comparisons (17). A value of $P < 0.05$ was accepted as significant.

Results. Hemodynamic variables for all treatments are shown in Table I. Aortic diastolic pressure was reduced after LAD occlusion in the control group compared to their own preocclusion values. Occlusion resulted in an increased systolic and diastolic pressure

TABLE I. HEMODYNAMIC VALUES FOR CONTROL, IBUPROFEN, AND INDOMETHACIN GROUPS[a]

	Control		Ibuprofen		Indomethacin	
	Before occlusion	After occlusion	Before occlusion	After occlusion	Before occlusion	After occlusion
Systolic blood pressure (mm Hg)	94 ± 14	86 ± 13	84 ± 11	84 ± 10	86 ± 12	96 ± 9[b]
Diastolic blood pressure (mm Hg)	71 ± 13	64 ± 11[b]	64 ± 9	67 ± 6	69 ± 9	76 ± 6[b,c]
Heart rate (beats/min)	276 ± 19	265 ± 19	271 ± 16	269 ± 24	270 ± 23	274 ± 23

[a] All values are mean ± SD ($N = 7$).
[b] Significantly different from respective before occlusion value ($P < 0.05$).
[c] Significantly different from respective control group value ($P < 0.05$).

in indomethacin-treated animals compared to their own preocclusion values. In this group, postocclusion diastolic pressure was significantly higher compared to control animals. Hemoglobin concentration was lower in ibuprofen-treated animals compared to control animals. No other changes in hemodynamic or blood gas variables were seen for any treatment.

Myocardial blood flow values are shown in Table II. Before occlusion, no differences in flow were observed for any treatment or region. After occlusion, myocardial blood flow decreased significantly in the occluded region compared to the nonoccluded region for all treatments. In the nonoccluded region no differences were noted between flows, pre and postocclusion within treatment groups. After occlusion, no differences in flow were noted between treatments in both occluded and nonoccluded regions, thus drug treatment had no effect on myocardial blood flow. Before occlusion, no subepicardial–subendocardial differences existed in flow for any treatment. After occlusion no subepicardial–subendocardial differences existed in the nonoccluded region for any treatment, although in the occluded region subendocardial flow was greater than subepicardial flow in controls and ibuprofen-treated animals.

Mean SaO_2 in the nonoccluded region in the control group was 89.9 ± 3.0% (mean ± SD) which was significantly higher than the occluded region SaO_2 (71.2 ± 4.1%). Also, for

TABLE II. REGIONAL MYOCARDIAL BLOOD FLOWS IN OCCLUDED AND NONOCCLUDED REGIONS FOR CONTROL, IBUPROFEN, AND INDOMETHACIN GROUPS[a]

Myocardial blood flow (ml/min/100 g)	Control		Ibuprofen		Indomethacin	
	Nonoccluded region	Occluded region	Nonoccluded region	Occluded region	Nonoccluded region	Occluded region
Before occlusion						
Mean	253 ± 46	244 ± 50	244 ± 61	273 ± 80	218 ± 80	224 ± 57
Subepicardium	242 ± 46	234 ± 40	234 ± 69	251 ± 87	224 ± 85	237 ± 56
Subendocardium	264 ± 47	254 ± 59	253 ± 56	295 ± 72	213 ± 84	211 ± 58
After occlusion						
Mean	214 ± 62	135 ± 70[b]	232 ± 71	159 ± 68[b]	225 ± 90	164 ± 70[b]
Subepicardium	206 ± 58	112 ± 63[b]	225 ± 82	125 ± 49[b]	232 ± 88	166 ± 70[b]
Subendocardium	222 ± 65	158 ± 78[c]	240 ± 60	193 ± 87[b,c]	218 ± 91	161 ± 71[b]

[a] All values are mean ± SD ($N = 7$).
[b] Significantly different from respective nonoccluded value ($P < 0.05$).
[c] Significantly different from respective subepicardial value ($P < 0.05$).

ibuprofen and indomethacin-treated groups, mean SaO_2 was higher in the nonoccluded region compared with the occluded region. Within both the occluded and nonoccluded regions, no differences in SaO_2 were noted between any treatment. Subendocardial SaO_2 was slightly but significantly lower in both occluded and nonoccluded regions compared to the subepicardium in the control group (Fig. 1).

Mean SvO_2 in control animals was 40.9 ± 2.6% in the nonoccluded region which was significantly higher than the value in the occluded region (28.4 ± 3.7%). Mean SvO_2 was significantly depressed in the occluded regions of the ibuprofen and indomethacin-treated groups compared to their respective nonoccluded regions. Within both the occluded and nonoccluded regions, treatment had no effect on SvO_2. In all treatment groups, SvO_2 was significantly lower in the subendocardium compared to the subepicardium in the nonoccluded region (Fig. 1). In the occluded region, SvO_2 was significantly lower in the subendocardium compared to the subepicardium for control and indomethacin groups, while no such difference existed in the ibuprofen group.

Mean O_2 extraction was higher in the occluded region of controls compared to the value observed in the nonoccluded region (Table III). This was also true for the ibuprofen and indomethacin groups. Within both the occluded and nonoccluded regions, O_2 extraction was slightly though not significantly lower for the ibuprofen and indomethacin groups compared to the control group. Subendocardial O_2 extraction was significantly higher compared to the subepicardium in the occluded region of controls, while no other such difference existed for any other group.

Mean O_2 consumption was not different in the occluded region compared to the nonoccluded region for any treatment (Table III). Within both the occluded and nonoccluded regions, no differences in O_2 consumption were noted for any treatment. Subendocardial values of O_2 consumption were higher compared to subepicardial values in the occluded regions of control and ibuprofen groups. No other regional differences in O_2 consumption were noted.

Mean O_2 supply/consumption ratio was significantly lower in the occluded regions compared to the nonoccluded regions for all treatments (Table III). Within the occluded and nonoccluded regions, no differences in this ratio were noted between treatments. The subendocardial O_2 supply/consumption ratio was significantly lower compared to the subepicardial region in the occluded regions of the control and indomethacin groups. These

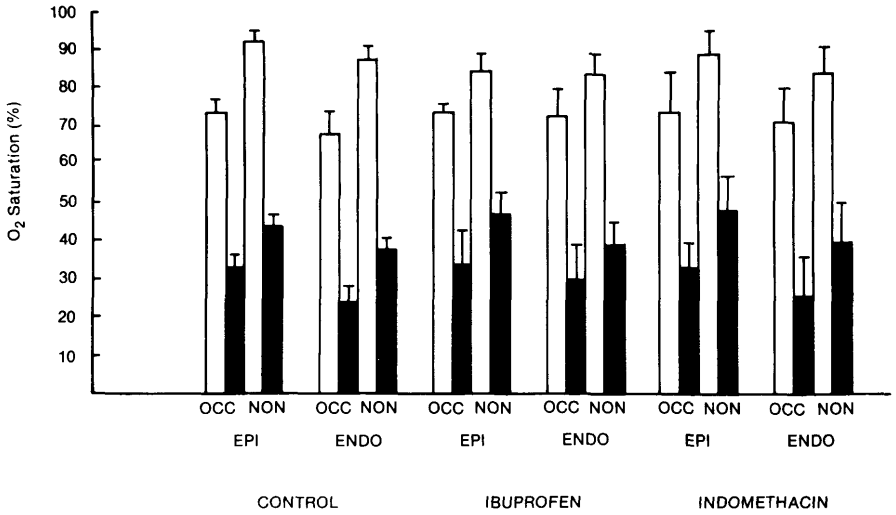

FIG. 1. Arterial and venous (shaded) O_2 saturations in subepicardial (Epi) and subendocardial (Endo) regions in occluded (Occ) and nonoccluded (Non) areas. These values are shown for control, ibuprofen, and indomethacin animals. All values are means ± SD (N = 7).

TABLE III. REGIONAL O_2 EXTRACTION, O_2 CONSUMPTION, AND O_2 SUPPLY/CONSUMPTION RATIO IN THE OCCLUDED AND NONOCCLUDED REGIONS OF CONTROL, IBUPROFEN, AND INDOMETHACIN GROUPS[a]

	Control		Ibuprofen		Indomethacin	
	Nonoccluded region	Occluded region	Nonoccluded region	Occluded region	Nonoccluded region	Occluded region
O_2 extraction (ml O_2/100 ml)						
Mean	6.9 ± 1.2	8.6 ± 1.4[b]	5.5 ± 1.0	6.9 ± 1.5[b]	5.0 ± 0.6	6.8 ± 0.7[b]
Subepicardium	6.7 ± 1.2	8.1 ± 1.3[b]	5.0 ± 1.0	6.6 ± 1.5[b]	4.8 ± 0.5	6.7 ± 0.8[b]
Subendocardium	7.1 ± 1.1	9.1 ± 1.4[b,c]	6.0 ± 0.9	7.1 ± 1.5[b]	5.3 ± 0.7	6.8 ± 0.6[b]
O_2 consumption (ml O_2/100 g/min)						
Mean	14.4 ± 2.6	11.2 ± 5.4	11.5 ± 5.0	11.3 ± 5.5	11.2 ± 4.5	10.9 ± 4.9
Subepicardium	13.3 ± 1.9	8.7 ± 4.4[b]	11.4 ± 5.0	8.2 ± 3.2[b]	11.1 ± 4.4	10.9 ± 4.7
Subendocardium	15.4 ± 3.4	13.8 ± 6.5[b,c]	14.6 ± 5.0	14.6 ± 7.8[c]	11.4 ± 4.6	10.9 ± 5.9
O_2 Supply/consumption ratio ($SaO_2/(SaO_2 - SvO_2)$)						
Mean	1.8 ± 0.1	1.5 ± 0.1[b]	2.1 ± 0.3[b]	1.6 ± 0.3[b]	2.1 ± 0.3	1.5 ± 0.1[b]
Subepicardium	1.9 ± 0.1	1.6 ± 0.1[b]	2.3 ± 0.4	1.7 ± 0.3[b]	2.3 ± 0.4	1.6 ± 0.2[b]
Subendocardium	1.8 ± 0.1[c]	1.4 ± 0.1[b,c]	1.9 ± 0.4[c]	1.6 ± 0.3[b]	1.9 ± 0.4[c]	1.4 ± 0.2[b,c]

[a] All values are mean ± SD ($N = 7$).
[b] Significantly different from nonoccluded value ($P < 0.05$).
[c] Significantly different from respective subepicardial value ($P < 0.05$).

regional differences disappeared with ibuprofen. Subendocardial O_2 supply/consumption values were significantly lower compared to subepicardial values in the nonoccluded regions for all treatments.

Discussion. Myocardial ischemia is characterized by a decreased flow to the ischemic region and a depressed O_2 supply/consumption balance especially in the subendocardium (18). Typically, ischemic region O_2 extraction increases while O_2 consumption decreases (18). In addition to the deleterious effects of an O_2 supply deficit, an inflammatory response may be initiated which in itself may act to increase the severity of the ischemic episode by increased microvascular obstruction and tissue degradation (3, 5).

Previous studies have indicated that ibuprofen is effective in mitigating damage during an ischemic episode in the myocardium (8, 9, 10). Other nonsteroidal anti-inflammatory drugs such as indomethacin have not proven to be as effective (6, 7). Differences in the protective action of ibuprofen and indomethacin may also be a result of their differing abilities to alter the O_2 supply/demand relationship in ischemic myocardium. Apstein and Vogel (19) showed that clinical doses of ibuprofen could result in some degree of coronary vasodilation in rabbits, thus raising the possibility that an increased flow to the ischemic region may be one mechanism for its action. However, some studies (8–10) have indicated that doses of ibuprofen that can protect the myocardium alter neither coronary blood flow, its distribution, nor O_2 consumption. Indomethacin does not appear to alter ischemic region collateral flow, but can increase O_2 demand (8). The studies above determined O_2 consumption primarily by using the heart rate–arterial blood pressure product and thus a local measure of consumption was not performed. Thus it is still possible that differences in the ability of ibuprofen and indomethacin to protect the myocardium may lie in their differing ability to regionally affect the acute O_2 supply/demand balance.

In the present study, we used a dose of ibuprofen well within the range used to protect the ischemic myocardium (11). It is also within the range known to induce vasodilation in the coronary bed of rabbits (19). The dose of indomethacin used in this study was also sufficient to inhibit synthesis of prostaglandins in the rabbit heart (20). We also found that ibuprofen and indomethacin had no effect on regional flow or distribution of flow within the occluded region. Occlusion did depress flow in the occluded region while no change was recorded within the nonoccluded region. Previous results using this occlusion model have demonstrated a similar decrement in flow in the occluded region and that an overlap of flow of 21–22% from noncollateral sources exists in the ischemic region (21). Our results in rabbits are similar to those previously reported by Jugdutt *et al.* (6, 8) for dogs.

Despite slight decreases in O_2 extraction neither ibuprofen nor indomethacin changed O_2 consumption in any region compared to control animals. This is in agreement with studies by Jugdutt *et al.* (8) who also found no change in overall myocardial O_2 consumption with ibuprofen. In our study this slight decrease in extraction is compensated by a slight increase in flow in the occluded region compared to control. This may also be a result of lower Hb concentration in the two treated groups. Jugdutt *et al.* (6) found a slight increase in O_2 consumption with indomethacin but speculated that the changes observed may not have been sufficient to significantly alter the O_2 supply/demand ratio.

Occlusion resulted in a depressed myocardial O_2 supply/consumption ratio in the occluded region for all treatments. Neither ibuprofen nor indomethacin altered this ratio in any region compared to control. The subendocardial O_2 supply/consumption ratio was depressed compared to the subepicardial value in control and indomethacin group occluded regions. Weiss (18) reported similar findings in ischemic myocardial tissue.

Thus it appears as if a difference in the ability to alter the O_2 supply/consumption balance in acutely ischemic hearts is not the mechanism for the different actions of indomethacin and ibuprofen on these hearts. Instead if ibuprofen is effective it may work to increase the ability of myocardial cells to survive a given acute O_2 supply/demand deficit. That this may be a mechanism for its action is suggested by studies showing its ability to diminish the postischemic inflammatory response, including stabilization of lysosomal membranes (9, 11).

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