Biphasic Accumulation of Leukocytes in Rat Cardiac Infarct Tissue Caused by Leukotriene B4 and Complement

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Accepted March 30, 1989

Abstract—An initial increase (3–12 hr) in the polymorphonuclear leukocytes (PMN) counts after ligation of the rat left main coronary artery was reduced by 49.4% (at 12 hr) by a 5-lipoxygenase inhibitor, AA-861 (80 mg/kg, p.o., 1 hr before ligation). Depletion of the complement components induced by cobra venoma factor (CVF) (i.v.), given one day before, resulted in significant reduction in the PMN accumulation after 12 hr (by 63.6% at 24 hr). The combined treatment (CVF+AA-861) suppressed the PMN accumulation by 69.7% (24 hr). The infarct size at 48 hr was also reduced by approximately 36% by either AA-861, CVF or combined treatments.

We reported (1) that permanent ligation of the left main coronary artery of rats induced ischemia in the left ventricle, but resumption of blood flow from surrounding healthy tissues through collateral vessels allowed the reduction of the ischemic area from 50.1 to 27.1% of the left ventricle (LV). Thus, this rat model can be considered to be a type of reperfusion model, although the left main coronary artery was not reperfused. The infarct area became a plateau (41.6% LV) at 12 hr after ligation, and the same level was kept up to 48 hr. Polymorphonuclear leukocytes (PMN) counts in the cardiac tissue were increased biphasically: an initial increase of 3–12 hr was followed by a later large increase occurring from 12 to 24 hr during the progress of the cardiac necrosis. Leukotriene (LT) B4, not immunoreactive LTC4, increased in the cardiac tissue and reached a plateau at 8 hr after ligation. Oral administration of a selective 5-lipoxygenase inhibitor (AA-861) one hr before ligation decreased the LTB4 level to that of the sham-operated rats and suppressed the PMN counts by 49.4% at 12 hr and by 41.2% at 24 hr, indicating that a part of the PMN migration was attributable to LTB4 generated in the cardiac tissue (1).

The peptide fragment of C5 of the complement (C5a, anaphylatoxin) is known to be a potent chemoattractant, and highly purified human C5a exhibits chemotactic activity toward human neutrophils at a concentration as low as 0.1 nM (2, 3). On the other hand, it was also reported that cobra venom anti-complement protein (cobra venom factor), 140K dalton protein, isolated from the venom of Naja naja Kaouthia, is functionally analogous to C3b (4) and forms an effective C5 convertase, and this formation results in the consumption of all of the terminal components of the complement (5). Accordingly, multiple injections of cobra venom factor to mice caused marked depletion of the early components of the alternative pathway and suppression of the functional activity of the pathway (6, 7). The present experiments were performed to examine the possibility of the involvement of the complement system in addition to LTB4.

In the present experiments, myocardial infarction was induced in male rats (Sprague-Dawley strain, 8 weeks old, body weight of 240–270 g, specific pathogen free) by ligation.
of the left main coronary artery under light ether anesthesia, using the method described previously (1). The occlusion of the left main coronary artery was confirmed by ST elevation in the standard limb lead II of the electrocardiogram (ECG 6611, Nihon Kohden, Tokyo). The operated rats were kept in a cage at constant temperature (25±0.5°C) and humidity (60±1%) up to 24 or 48 hr and were given water and food ad libitum.

Cardiac arrest was induced by an intravenous injection of 1 M potassium chloride; then the heart was removed, and the third transverse ring (ring C) of four rings from the base of the heart was fixed for about 4 hr in 10% formal saline, left in 0.05 M phosphate-buffered saline at 4°C overnight and then embedded in paraffin. The basal side of ring C was cut at 4 μm thickness with a microtome (Yamato Koki Industries, Tokyo). The chloroacetate esterase activity in the granules of PMNs in the cardiac tissue was stained by the method reported previously (1). PMNs with red-stained granules and blue nuclei were counted under a microscope (BH-A, Olympus Optics, Tokyo) (objective x100, oil immersion, eyepieces x10).

The infarct areas of the hearts at 48 hr after ligation were evaluated by the previously described method (1): All three transverse rings (ring B, C and D) were incubated at 37°C for 20 min with 1% triphenyltetrazolium chloride (TTC) solution in a 0.09 M phosphate buffer (pH 8.5–8.6), in incubation tubes shielded from the light. The necrotic areas of the cardiac tissue were devoid of dark purple color, which would be due to dehydrogenase activity in the cardiac muscle (1).

A selective 5-lipoxygenase inhibitor, 2,3,5-tri-methyl-6-(12-hydroxy-5,10-dodecadinyl)-1,4-benzoquinone (AA-861) (Takeda Chemical Industries, Osaka), was suspended in 5% gum arabic and administered orally (80 mg/kg) by a metal oesophagal catheter. The blood concentration of AA-861 and its active metabolite (AA-1777) was maintained over 24 hr (personal communication from Dr. Yoshitaka Maki, Takeda Chemical Industries, Osaka).

The serum complement titers were measured in terms of 50% hemolysis (CH50) by a slight modification of the original method of Mayer (8). Cobra venom factor (CVF, from Naja naja Kaouthia, Sigma, St. Louis, MO) (Lot 37F-4015) (7.5 μg/100 g/0.1 ml) was injected intravenously from the tail vein one day before the coronary ligation. Twenty-four hours after the injection of CVF, the serum CH50 titers in three rats became less than the detection limits (<5 u/ml), showing a striking contrast with the titers in three non-treated normal rats (42, 52 and 47 u/ml). Activated partial thrombin time and prothrombin time in plasma were not changed after CVF injection.

Figure 1A indicates the PMN counts in ring C of the cardiac tissue of untreated and treated rats. The PMN counts in the untreated rats were increased after ligation (1059±166 cells/slice, at 3 hr) (n=4), but the rate of increase was blunted until 12 hr after ligation (2531±924 cells/slice, at 12 hr) (n=3). Then the PMN counts began to increase sharply and reached the value of 5926±683 cells/slice (n=4) at 24 hr after ligation. These results were similar to that obtained in the previous paper (1). Oral administration of AA-861 (80 mg/kg) to rats one hr before ligation caused significant inhibition of the PMN accumulation at 6 and 12 hr. This inhibitor, however, became ineffective for the inhibition of the accumulation, and the increase of the PMN counts after 12 hr in the AA-861 treated rats was parallel to that of the untreated rats (Fig. 1A). By contrast, the PMN counts at 3, 6 and 12 hr after ligation were not reduced by depletion of the serum complement, but at 24 hr, the PMN count was markedly suppressed.

Figure 1B indicates percent inhibition of the PMN counts after two kinds of pretreatment of the animals. The inhibition by AA-861 reached a peak (49.4%) at 12 hr after ligation. The inhibition curve well-coincided with the initial increase in the PMN counts up to 12 hr. When the increase in the LTB4 levels over the sham-operated hearts, which were calculated from the data obtained in the previous experiments (1), were superimposed in Fig. 1B, the initial increase agreed very well with that of LTB4 in the cardiac tissue, indicating that the first increase was largely attributable to LTB4 generated in the cardiac tissue. In contrast, the percent inhibition by
CVF became gradually evident with time after ligation and reached 63.6% at 24 hr. This implies that the large increase in the PMN counts from 12 to 24 hr is attributable to the action of the complement, probably C$_{5a}$. LTB$_4$ was reported to be very potent in attracting PMN to the tissue, and we confirmed this in the study of microcirculation in hamster cheek pouch (9). As shown in the present experiment, the PMN accumulation was not induced solely by LTB$_4$. The initial PMN accumulation by LTB$_4$ was succeeded by the late activation of the complement system, which may require some period of time before the activation occurs. Failure of PMN reduction in rat myocardial infarction by CVF as reported in another paper (10) may be due to i.v. injection of CVF shortly after ligation. Failure of the detection of LTB$_4$ in the perfusate from rat hearts during reperfusion (11) is not inconsistent with our data, since they did not determine this level in the cardiac tissue.

The PMN counts in the complement-depleted animals treated with AA-861 (1798±171/slice) were not significantly different from those in the animals treated with CVF alone (2156±171/slice), but the value was significantly lower than that in the animals treated with AA-861 (3280±514/slice) (Fig. 2A). This suggests that the mechanism of attraction of PMN by LTB$_4$ may be slightly different from that by C$_{5a}$. Further experiments are required to draw a conclusion. The residual accumulation of the PMN in the cardiac tissue after the combined pretreatment may be due to unknown chemoattractants.

Furthermore, the infarct size of the cardiac tissue at 48 hr after ligation was reduced by 36.8, 33.2 and 38.2% after pretreatment of the animals by AA-861, CVF and CVF+AA-861, respectively. This may indicate that the exacerbation of the infarction caused by PMN migrating into the cardiac tissue comprised approximately 36% of the infarct size of the untreated rats and the rest of the necrosis was directly induced by the ischemia. Furthermore, the initial attraction of PMN by LTB$_4$ before 12 hr may have a more marked
enlarging effect on the infarct size than that induced by complement, since the infarct size reached a plateau from 12 hr after ligation. Thus, the earlier treatment of the patients with cardiac infarction by 5-lipoxygenase inhibitor may be more effective.

Acknowledgments: This study was partly supported by Grants-in-Aids from the Ministry of Education, Science and Culture of Japan (No. 5848013 and No. 61480442). We wish to thank Mr. O. Katsumata for his skillful technical assistance for staining of the PMN leukocytes.

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