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INFLUENCE OF A LYMPHAGOGUE, CLS 2210, ON REGIONAL CARDIAC LYMPHATICS AND THE ELECTROCARDIOGRAM AFTER CORONARY ARTERY OCCLUSION IN THE DOG

L Szlavy, I Repa, A De Courten

Institute of Cardiovascular Surgery, Semmelweis University Medical School, Budapest, Hungary (LS, IR) and the Scientific Department, OM Laboratories Ltd., Geneva, Switzerland (ADeC)

ABSTRACT

To examine the role of cardiac lymphatic drainage in myocardial infarction, we quantified the effect of a lymphogogue, CLS 2210, on the number and appearance of myocardial lymphatics as well as the electrocardiogram following coronary occlusion in the dog. Thirty minutes and six hours after intravenous administration of the benzenesulfonate compound, (CLS 2210) cardiac lymphatics in the distribution of the left anterior descending coronary artery (LAD) were determined and further delineated by postmortem cardiac lymphangiograms. The results were compared with treated and untreated dogs without and with descending coronary artery ligation including the noninfarcted zone; that is, myocardium within the distribution of left circumflex coronary (LCC) artery. After 30 minutes in dogs receiving CLS 2210 without LAD ligation, number of lymphatics (point count/cm², see Methods) were respectively — LAD zone: 2.62 ± 0.11 or 10.9% of left ventricular (LV) surface; LCC zone: 2.87 ± 0.10, whereas after six hours—LAD zone 8.04 ± 0.03 or 32.3% LV surface; LCC zone—8.13 ± 0.06 compared with untreated controls—LAD zone 1.71 ± 0.11 or 6.6% of LV surface; LCC zone 1.65 ± 0.12 ($p < 0.0001$). At similar intervals in dogs with LAD ligation, the findings were at 30 minutes LAD zone 0.78 ± 0.07 or 3.1% of LV surface and at 360 minutes was 0.80 ± 0.08 or 3.3% of LV surface. In conjunction with CLS 2210 administration, however, LAD zone showed at 30 minutes 2.50 ± 0.12 or 10% of LV surface ($p < .01$) and at 360 minutes was 10.34 ± 0.03 or 35.1% of LV surface. Moreover, in dogs with LAD ligation and CLS 2210 administration, electrocardiogram at six hours showed diminished ST-segment elevation.

The data suggest that CLS 2210 facilitates preservation of myocardial lymphatics and hence cardiac lymph transport and with its other "angioprotective" affects (antagonizes platelets and lowers blood viscosity) limits myocardial infarct size following coronary artery occlusion.

Reduction of infarct size is a principal goal of therapy in acute myocardial infarction, because the ultimate outcome depends to a large extent on the amount of myocardium damaged. Many methods, pharmacologic and mechanical, for reducing necrosis have been recommended, but none have led to consistent myocardial salvage (1,2). Thrombolysis by infusion of streptokinase into the occluded coronary artery has yielded promising results (3), but its scope is limited by the need for early application and invasive manipulation aided by coronary arteriography. Studies of the relationship between cardiac lymph flow and myocardial dysfunction (4-8) have suggested that an intact lymphatic system limits destruction of myocardium after coronary occlusion by facilitating removal of fluids and toxic byproducts that aggravate necrosis. A decrease in lymphatics in infarcted myocardium, and increase after administration of hyaluronidase both in infarcted zones and normal hearts have previously been demonstrated experimentally (5). In normal dog hearts, CLS 2210 (OM Laboratories, Geneva, Switzerland) a synthetic benzenesulfonate compound (10),

which is a new formulation of calcium dobesilate, increases the number of lymphatics to a greater extent than hyaluronidase (9).

In this study we quantified cardiac lymphatics by post-mortem lymphangiography after administration of CLS 2210 in dogs with normal myocardia and after experimental coronary artery occlusion to ascertain the drug's effectiveness in maintaining lymphatic integrity following coronary artery occlusion.

MATERIALS AND METHODS

Forty-four mongrel dogs of both genders (22kg) were used. Anesthesia was initiated with sodium pentobarbital (30mg/kg) and ventilation maintained through an endotracheal tube with a Harvard respirator which provided a tidal volume of 200-300 ml of room air at a rate of 18-20/minute. Catheters were placed in the aorta via the right femoral artery for blood pressure monitoring and in a peripheral vein for supplemental anesthesia. The coronary artery was approached through a left lateral thoracotomy through the third intercostal space. The pericardium was incised parallel to the phrenic nerve. The left main coronary artery was dissected with special care to minimize disruption of adjacent lymphatic trunks. In dogs undergoing ligation of the left anterior descending coronary artery (LAD) ligation was accomplished just distal to the first septal branch. The left circumflex coronary artery (LCC) was not disturbed and its myocardial territory being non-infarcted was used as an internal control (see below).

Group 1: Eighteen dogs underwent sham-operation and the coronary artery was not occluded. Six were untreated and served as controls (a) while the other 12 dogs (b) received CLS 2210 in a single dose of 100 mg/kg by intravenous infusion in 100 ml of physiological saline. At 30 minutes (6 dogs) and 360 minutes (6 dogs) after administration of CLS 2210 the dogs were sacrificed and cardiac lymphangiography performed immediately thereafter.

Group 2: In 13 untreated dogs the coronary artery was occluded and lymphangiography performed post-mortem at 30 minutes (7 dogs) and 360 minutes (6 dogs) after occlusion (controls for Groups 2A and 2B respectively). An additional 13 dogs were treated with CLS 2210 in a single dose of 100 mg/kg by intravenous infusion, immediately after coronary artery ligation. In six of these dogs, post-mortem lymphangiography was performed at 30 minutes (Group 2A), and in the other seven at 360 minutes (Group 2B). The infarcted zone of myocardium was identified by direct inspection after coronary artery ligation using the indices of color change and disordered muscular contraction.

Post-mortem cardiac lymphangiography: As previously described (5), lymphatics in the apex of the left ventricle were cannulated using a 30-gauge Becton-Dickinson lymphangiography set with a 2.5 magnification lens. Using 30 g of barium sulfate, 3 to 5 ml of Iodamide 420 (Bracco, Milan, Italy), and five drops of different food colors for contrast, separate injections into individual lymphatics were made with gentle manual pressure until the lymph channels were filled as indicated by contrast backflow. Attempts at further filling would have led to lymphatic disruption. Roentgenograms of the hearts were made in five-fold magnification using a 0.1mm focal spot Siemens tube and exposure factors of 50 kV, 0.01 sec, and 30 mA. This technique visualized lymphatics with diameters as small as 25 to 35 microns. After lymphangiography the hearts were opened and photographed.

Analysis of the lymphangiograms: A grid system consisting of horizontal and vertical lines 1cm apart was superimposed on the lymphangiograms. An area of 5x5 cm^2 on the grid corresponded to 1 cm^2 of ventricular myocardium (because of five-fold magnification used in preparing the lymphangiogram), and this area was taken as a single basic unit with each containing 25 intersections. A grid intersection that coincided with a lymphatic on the lymphangiogram scored one point. The number of lymphatics visualized in an arterial distribution was expressed in terms of point counts per cm^2 and percentage of ventricular surface area occupied by the lymph vessels. This approach provided an index of lymphatic volume

relative to that of ventricular myocardium and not just a simple count of lymphatics. The infarcted (LAD) and non-infarcted (LCC) zones were compared. Mean values with standard error were used as an index of dispersion. Statistical analyses were done, where appropriate, with Student's t-test or by non-parametric methods in the form of the Wilcoxon rank sum test or Fisher's exact test. The null hypothesis was rejected when p was less than 0.05.

RESULTS

Group 1A (control dogs): **Cardiac lymphangiograms:** The appearance of the lymphangiograms in the normal dog heart was similar to that we reported previously (5) with the point count/cm² as 1.71 ± 0.11 (mean ± SEM) in the LAD zone and 1.65 ± 0.12 in the LCC zone (Fig. 1 and 3A). The proportion of left ventricular surface occupied by visible lymphatics in these untreated dogs (LAD zone) was 6.6%.

Group 1B **Effect of CLS 2210 on cardiac lymphangiograms:** CLS 2210 augmented myocardial lymphatic visualization in the normal dog. In 12 dogs the point counts per cm² following CSL 2210 was increased after 30 minutes in the LAD zone to 2.62 ± 0.11 or 10.9% of left ventricular (LV) surface and in the LCC zone to 2.87 ± 0.10. After 360 minutes the point counts in the LAD zone was 8.04 ± 0.03 or 32.3% of the LV surface while in the LCC zone it was 8.13 ± 0.06 (Fig. 1, 3B and 3C). The differences were significant ($p < 0.001$) when compared with untreated dogs (Group 1A above).

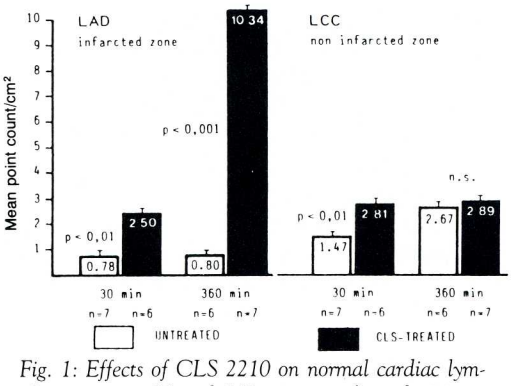

Fig. 1: Effects of CLS 2210 on normal cardiac lymphatic system, 30 and 360 minutes after administration (Group 1).

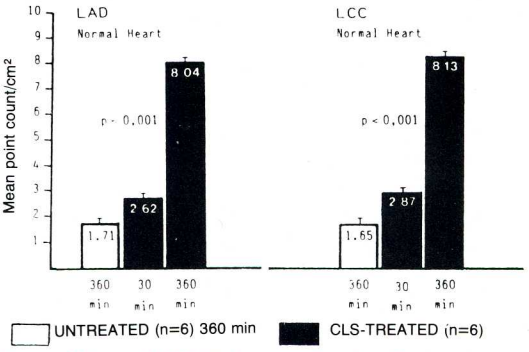

Fig. 2: Effect of CLS 2210 on cardiac lymphatic system, 30 and 360 minutes after administration and coronary artery ligation (LAD) (Group 2).

Group 2A **Changes of lymphangiograms in dogs with experimental myocardial infarction:** In the lymphangiograms taken 30 minutes after coronary artery ligation in untreated dogs (Group 2A) there was a strikingly consistent reduction in visualization of lymphatics (0.78 ± 0.07 points/cm²) in the infarcted (LAD) zone (Fig. 2 and 4A). 3.1% of the LV surface was occupied by lymphatics filled with contrast media. On the other hand, in the non-infarcted zone (LCC) visualization of lymphatics was unchanged (1.47 ± 0.09 points/cm²).

With CLS 2210 treatment there was a notable increase in lymphatics 30 minutes after coronary artery ligation (Fig. 2 and 4B), both in the infarcted (LAD) zone (2.50 ± 0.12 points/cm² or 10% of LV surface) and in the non-infarcted (LCC) zone (2.81 ± 0.10 points/cm²). The differences were significant when compared with untreated dogs (2A above) at 30 minutes ($p < 0.01$).

Group 2B: In the lymphangiograms of untreated dogs studied 360 minutes after coronary artery ligation there was decreased lymphatic filling in the infarcted (LAD zone (0.80 ± 0.08 points/cm²), comparable to that at 30 minutes (see 2A). The non-infarcted zone (LCC) showed a small but significant increase ($p < 0.01$) in visible lymphatics, (2.67 ± 0.07 points/cm²) when compared to the infarcted zone (LAD) (Fig. 2).

With CLS 2210 treatment, there was a

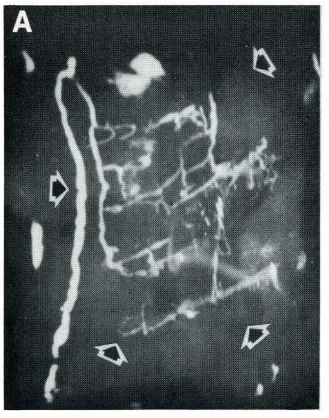

Fig. 3A: Lymphangiogram for normal untreated dog showing in the LAD area (delimitated by arrows) few lymphatic vessels filled with contrast media.

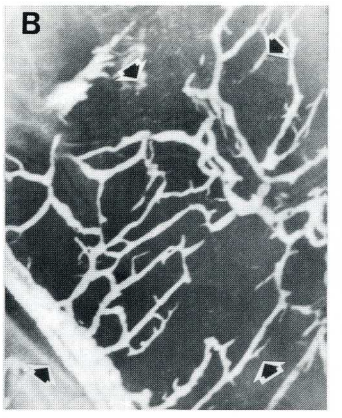

Fig. 3B: Lymphangiogram from normal dog taken 30 minutes after CLS 2210 treatment showing in the LAD area (delimitated by arrows) a greater number of lymphatic vessels.

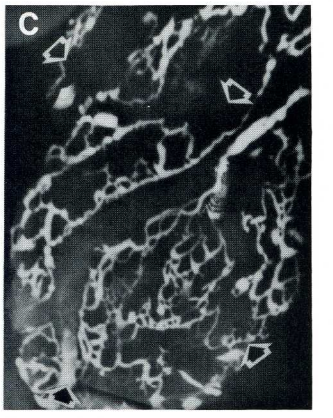

Fig. 3C: Lymphangiogram from normal dog taken 360 minutes after CLS 2210 treatment showing in the LAD area (delimited by arrows) a much greater number of lymphatic vessels.

striking increase of lymphatic visualization 360 minutes after coronary artery ligation in the infarcted (LAD) zone (10.34 ± 0.03 points/cm²) or 35.1% of LV surface) (Fig. 2,5). The non-infarcted (LCC) zone also showed an increase in lymphatic filling (2.89 ± 0.11 points/cm²) which was similar to the non-infarcted (LCC) zone in untreated dogs, (2.67 ± 0.07 points/cm²) at 360 minutes (see above).

In electrocardiographic tracings from CLS 2210-treated dogs observed during six hours (Fig. 6), the most notable change was reversal of ST-segment elevation.

DISCUSSION

A reduction in lymphatics in the infarcted myocardial zone after coronary artery ligation in dogs has previously been shown with post-mortem cardiac lymphangiography (5). The interval between coronary occlusion and lymphangiography is too short for lack of lymphatics to be ascribed to regional coagulation necrosis. Intravenous administration of hyaluronidase increases the number of lymphatics in both infarcted and in normal heart (5,9), but administration of CLS 2210 intravenously substantially increases

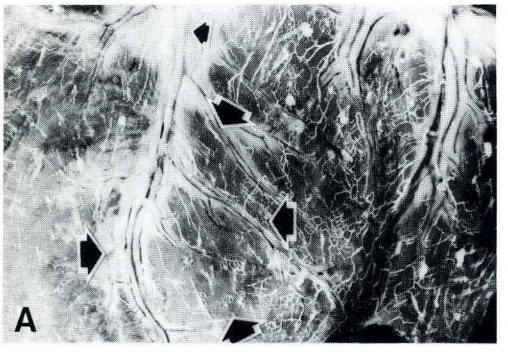

Fig. 4A: Lymphangiogram from untreated dog taken 30 minutes after coronary artery occlusion showing in the infarcted LAD area (delimitated by arrows) fewer lymphatic vessels than in the noninfarcted area. The upper small arrow indicates the site of occlusion.

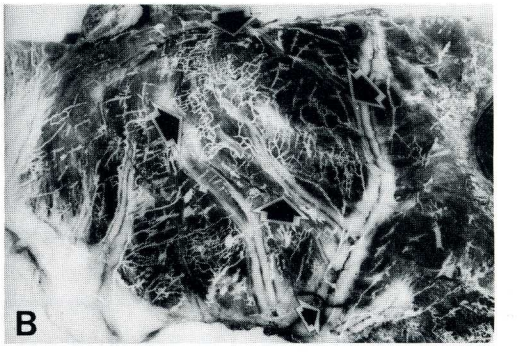

Fig. 4B: Lymphangiogram taken 30 minutes after coronary artery occlusion and CLS 2210 treatment showing in the infarcted LAD area (delimitated by arrows) a greater number of lymphatics.

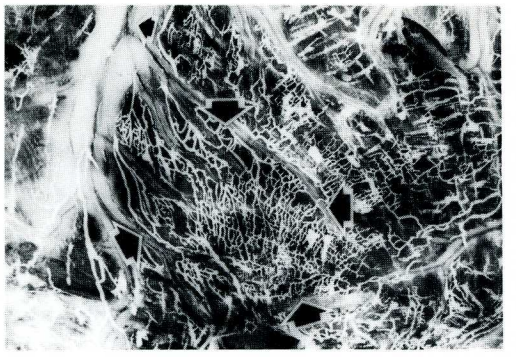

Fig. 5: Lymphangiograms in CLS 2210 treated dog taken 360 minutes after occlusion of the coronary artery showing in the infarcted LAD area (delimitated by arrows) increased lymphatic vessels. The upper small arrow indicates site of occlusion.

visualization of lymphatics beyond that achieved by hyaluronidase (9).

Numerous macromolecules including erythrocytes enter cardiac lymph after coronary artery occlusion as shown by detection of labelled microspheres injected into the left atria of dogs in cardiac lymphatics within two hours of coronary artery occlusion lasting 10 to 20 minutes (4). This finding is consistent with altered myocardial blood capillary permeability secondary to ischemic injury. Lymph withdrawn from the main cardiac lymph trunk two to four hours after coronary occlusion injected into the coronary artery of normal dogs induces serious arrhythmias (ventricular tachycardia and fibrillation) whereas lymph withdrawn prior to two hours has little affect (5), suggesting that injured myocardium releases toxic byproducts absorbed by draining lymphatics. An impaired cardiac lymph circulation induces interstitial edema, myocardial hypoxia and fatty degeneration (6), damaged small capillaries (6), elongated capillaries with dilated pre- and post-capillary sections, poor capillary filling with empty "vessel-free" spots, and arteriovenous shunts (7)) as well as cardiac arrhythmias and other electrocardiographic changes similar to the sick-sinus syndrome (8).

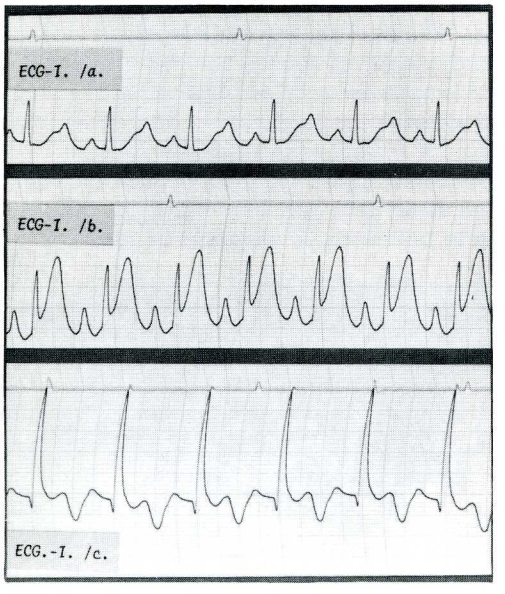

Fig. 6: Electrocardiographic changes in dog with experimental occlusion of the left anterior descending coronary artery: (a) before occlusion; (b) 60 minutes after occlusion and CLS 2210 infusion; (c) 360 min after occlusion and CLS 2210 administration. Note the sharp fall of ST-segment elevation.

In this study of dogs with both an intact myocardium and after experimental myocardial infarction produced by coronary artery ligation, the number of lymphatics in infarcted myocardium without treatment was diminished. Intravenous administration of CLS 2210 was associated with a dramatic increase in the number of lymphatics in both normal and infarcted myocardium and, in the latter, by a less deranged electrocardiogram. The finding that CLS 2210 decreased ST-segment elevation in "infarcted myocardium" is similar to that observed after administration of hyaluronidase both in experimental animals and patients after acute myocardial infarction (19). As a decrease in the ST-segment elevation is an accurate index of myocardial salvage (19), it is reasonable to conclude that these two agents limit or reduce infarct size.

In summary, CLS 2210 a recognized "angioprotective" agent (11,12), a lymphagogue (13,14) and an antagonist of platelet hyperaggregability (15,16) and of blood hyperviscosity (17,18) contains features that limit infarct size after experimental coronary artery ligation. CLS 2210's angioprotective effect (11, 12) restricts fluid extravasation into the cardiac interstitium. By reducing plasma viscosity (17,18) it salvages

ischemic but not necrotic myocardium (20). By increasing the number of functioning lymphatics and by its lymphagogue property (13), it facilitates lymph drainage thereby minimizing fluid accumulation and accelerating removal of degradation products, and other toxic macromolecules that are generated by disordered myocardial metabolism and which aggravate injury and promote arrhythmia.

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Dr. A. deCourten
P.O. Box 95
1211 Geneva 7
SWITZERLAND