Temporality Changes in 12-HETE Formation in Two Models of Canine Myocardial Infarction

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ABSTRACT

Arachidonic acid (AA) metabolism by infarcted canine myocardium was studied and correlated with matched histologic analyses following permanently occluded or reperfused infarction. Histologic analysis of tissues from reperfused infarcts showed a marked acceleration of inflammatory cell invasion and of granulation tissue formation when compared to the occlusive infarct. In the reperfused infarct, polymorphonuclear leukocytes (PMNs) were very prominent at one day after infarction while in the occlusive infarcts the neutrophilic invasion was less intense but more sustained. At one day following reperfused infarction the major arachidonate product, which co-migrated by thin layer chromatography with the mono-hydroxyeicosatetraenoic acids (HETEs), was significantly elevated (254 ± 49 pmoles/gm wet weight, n=3) when compared to normal tissue (48 ± 6 pmoles/gm n=19). This occurred at a time when the number of PMNs was maximal in the infarcted tissue. Addition of the calcium ionophore A23187 caused a further marked stimulation in HETE production in the one day reperfused infarct but not at the other time points studied. The production of HETE was not significantly different in the infarcted tissue than in the normal tissue at three and seven days following reperfused infarction or at one, three, or seven days after occlusive infarction. The identity of this HETE product was investigated using reverse phase high performance liquid chromatography (RP-HPLC) and gas chromatography-mass spectrometry (GC-MS) and found to be predominantly 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) with a small amount of 15-HETE. Thus the production of 12-HETE parallels the number of neutrophils invading the infarcted area of the heart.

INTRODUCTION

Enhanced arachidonic acid (AA) metabolic potential in infarcted areas of canine myocardium following permanent circumflex coronary artery occlusion has been previously demonstrated in our laboratory (1). We hypothesize that invading inflammatory cells bring into an infarcted area of tissue the ability to metabolize AA into active metabolites which could potentially affect the function of the adjacent undamaged myocardium. To test this hypothesis, the
histologic progression of invading inflammatory cells and the ability of the tissue to metabolize [14C]-AA were compared in areas of infarcted myocardium subjected to permanent left anterior descending (LAD) coronary artery ligation or to two hours of coronary artery ligation followed by reperfusion.

Histologically, the reperfused infarct is a better model for the situations which exist following bypass surgery (2), or following global ischemia during cardiac surgery (3,4). The reperfused infarct differs from a totally occluded infarct in several ways. Reperfused infarcts show an accelerated inflammatory cell response (5) when compared with permanently occluded infarcts (6). They also contain extensive areas of hemorrhage (7) and regions of viable myocardium throughout the infarcted area (8).

Many studies have neglected the histologic changes which occur in the first few days following reperfused infarction and have only looked at the histological progression of inflammatory cells several days or weeks following an ischemic insult. Recently, the accumulation of leukocytes (PMNs) has been reported in reperfused infarcts as early as one hour following reperfusion (9). Since agents which inhibit the metabolism of AA by the lipoxygenase pathway (10,11) have recently been shown to reduce the eventual infarct size, we have studied the histologic progression of cellular invasion during the first week following either occlusive or reperfused LAD coronary artery ligation and have correlated this with the production of AA metabolites in myocardial minces from both infarcted and non-infarcted areas of myocardium. We have found that there is an acceleration of PMN invasion in the reperfused compared with the occlusive infarct and that there is an enhanced production of the monohydroxy AA metabolite 12-HETE in the reperfused infarct one day following infarction.

METHODS

Myocardial infarction was produced by the method of Michelson et al. (12). Adult male mongrel dogs weighing between 10 and 20 kg were anesthetized with pentobarbital sodium (30 mg/kg i.v.), intubated with a cuffed endotracheal tube and ventilated with a Harvard respirator on room air supplemented with oxygen. Flocillin (600,000 units - i.m.) was administered preoperatively. Body temperature was maintained using a water-filled thermal mattress. The heart was exposed by a transverse thoracotomy at the fourth intercostal space and the heart suspended in a pericardial cradle. The left anterior descending coronary artery was dissected free at the level of the first major diagonal branch and a silk ligature passed around it. The artery was then occluded in two stages according to the method of Harris (13) with 20 minutes of partial occlusion followed by total occlusion. The coronary ligature was either tied and left permanently occluded (OCCLUSIVE) or formed into a snare that was drawn tight for 2 hours and then released and removed (REPERFUSED). Lidocaine (2 mg/kg i.v.) was given 5 minutes
before reperfusion to reduce arrhythmic deaths and five minutes after reperfusion if needed. The chest was closed in layers and the dogs were allowed to convalesce for 1 day, 3 days or 7 days.

Following convalescence the dogs were re-anesthetized, the hearts removed while still beating and washed in ice cold Krebs-Henseleit buffer. The areas of infarction were identified by gross inspection. Extensive sampling for histologic analysis was done by taking transmural slices of myocardium from control and infarcted regions adjacent to those sampled for arachidonate metabolic potential. Tissue samples were fixed in 10% formalin (sodium phosphate buffered), dehydrated in ethanol, embedded in paraffin and 5 microns sections were cut. Sections were stained with hematoxylin and eosin and examined by light microscopy.

The arachidonate metabolic potential was studied using a modification of the method of Mullane et al. (10). Transmural cores of myocardium (16 mm dia) were obtained from the center of the infarcted area and from a normal area of the posterior ventricular wall. The epicardium was removed from these pieces to avoid surgical inflammation. The transmural plugs of myocardium were finely minced with scissors on ice in isotonic tris-buffered saline (pH 8.0) containing 1% fatty acid-free bovine serum albumin (Calbiochem) to adsorb any free fatty acids liberated during mincing. The tissues were spun down, the supernatants removed and the tissue washed with tris-buffered saline (pH 8) without bovine serum albumin. [14C]-AA incubations were run in 2 ml of tris-buffered saline (pH 8.0) by incubating the 1 gram of tissue for 15 minutes at 37°C with 10 nmols of [14C]-AA (New England Nuclear [1-14C]-AA 55 mCi/mmol - 3.3 μg, 10⁶ cpm). At the end of the 15 minute incubation period, the tissue was spun down and the supernatant was removed and extracted as described below. The tissue was then resuspended in 2 ml of the same tris-buffered saline containing the calcium ionophore A23187 (Calbiochem) and an additional 10 nmols of [14C]-AA. A23187 was dissolved in DMSO (10mg/ml) and aliquots were diluted into the tris buffer (final A23187 concentration = 10μM) immediately before use with vigorous shaking. The maximum concentration of DMSO did not exceed 0.02%. After 15 minutes at 37°C the tissue was again spun down and the supernatant removed. The supernatants were extracted by adjusting the pH to 3.5 with 4 M formic acid followed by the addition of 1 volume of 100% ethanol, vortexing, and extracting twice with 2 ml of chloroform. These extracts were evaporated to dryness under a stream of nitrogen, resuspended in 25 μl CHCl₃:MeOH 2:1, and plated on silica gel thin layer chromatography plates (Brinkmann). The products were separated in benzene:ethyl ether:ethanol:acetic acid; 50:40:2:0.2 v/v. Autoradiographic images were produced using Kodak XAR X-omat film. Product areas were defined using the radiographic image and the corresponding zones were cut out and quantitated using a Packard liquid scintillation spectrometer. Data are expressed as picomoles of product formed based on the percent conversion of the original substrate. Values are expressed as mean
The data were evaluated with a two-tailed Student's t test and a P value of 0.05 was considered significant.

Samples for HPLC were prepared using similar incubation conditions followed by extraction of ethanol precipitated samples into chloroform. These chloroform extracts were evaporated to dryness with nitrogen and resuspended in 50 µl methanol (MeOH) followed by 950 µl water. This 5% MeOH solution was loaded on a pre-activated Baker octadecylsilyl mini-column (C-18 - 1 ml), washed with 15% MeOH and then eluted with 1 ml 100% MeOH. This eluate was evaporated to dryness and resuspended in 35 µl acetonitrile plus 65 µl water (35% ACN). This sample was loaded on the 500 µl injection loop of a Varian Model 5000 Liquid Chromatograph. Separation was performed using an Altex Ultrasphere-ODS 4.6 X 250 mm analytical 5 micron reverse phase column. Products were detected using a Varian UV-50 variable wavelength detector set at 194 nm and 0.1 absorbance units full scale (AUPS) for 0-24 minutes to detect prostaglandins, 280 nm for 24-45 minutes to detect the conjugated trienes of leukotrienes and 235 nm for 45-90 minutes to detect the conjugated dienes of mono-hydroxy AA metabolites. Radioactive products were detected with an in-line Berthold LB 503 flow-through scintillation detector. The solvent system used was that of Peters et al. (14) and consisted of a programmed mix of acetonitrile (ACN) and acidified water (0.01% glacial acetic acid). The program was as follows: 36% ACN for 0-23 minutes, 50% for 28-30 minutes, 56% for 32 to 75 minutes and 100% from 82-95 minutes. Concentration changes were achieved with linear gradients. The solvent flowed at 1.5 ml/minute. This system allowed baseline separation of authentic standards of 12-HETE (retention time = 53 minutes), 15-HETE (50 minutes) and 5-HETE (60 minutes) standards (the generous gifts of A.R. Morrison and A.S. Taylor).

Methyl esters were prepared by dissolving HPLC purified material in a few drops of methanol and incubating for 5 min at room temperature with excess diazomethane in diethyl ether. The trimethylsilyl derivative was then produced by incubating the methyl ester in 10 µl of pyridine and 10 µl of bis(trimethylsilyl)trifluoroacetamide at room temperature for 24 hr. Mass spectrometry was carried out on the methyl ester trimethylsilyl ethers using a Hewlett-Packard 5985B quadrupole spectrometer equipped with a Grob type injector. The column was a wide bore capillary column of crosslinked OV-1, 25 meters long. The initial temperature was 85°C and programmed to 250°C at 30°C/min. The carrier gas was helium and the injection temperature was 250°C.
RESULTS

HISTOLOGIC ANALYSIS Sections from normal regions (Fig. 1) of the infarcted hearts showed a compact arrangement of myocytes, capillaries, and scattered interstitial fibroblasts. In hearts examined one day after coronary artery occlusion, an acute inflammatory infiltrate consisting largely of polymorphonuclear leukocytes (PMNs) was present in the infarcted regions. In the reperfused infarcts, PMNs were diffusely present throughout the central zones of hemorrhagic necrosis. In more peripheral zones of the reperfused infarcts, PMNs appeared in clumps and as dense band-like infiltrates. In the occluded infarcts at one day, PMNs were sparsely scattered in the central zones of coagulative necrosis but arranged in bands about the infarct periphery. Thus one day after coronary occlusion the distribution of the acute inflammatory infiltrates was distinctly different in the two types of infarcts; more diffuse and concentrated in the center of the hemorrhagic reperfused infarct, more zonal and peripheral in the occluded infarct.

In the occluded infarcts examined three days after coronary occlusion, PMNs persisted as the predominant inflammatory cell type. In contrast, in the reperfused hearts, only scattered neutrophils remained in areas of hemorrhagic necrosis but were joined, three days after occlusion, by large numbers of macrophages, fibroblasts and other mononuclear cells. The reperfused infarct zone was surrounded by a broad band of intense fibroblast proliferation accompanied by large numbers of macrophages. The densely packed fibroblasts in this zone were enlarged, elongated, and displayed basophilic (RNA-rich) cytoplasm with large vesicular nuclei and multiple prominent nucleoli. Several interstitial cells in this area showed mitotic figures.

Seven days after coronary occlusion, the reperfused and occluded infarcts had similar chronic inflammatory changes with marked ingrowth of fibroblasts and blood vessels at the periphery of the infarct. Numerous macrophages created a resorption front extending from this broad zone of fibroplasia into the remaining central necrotic zone. The resorptive reparative processes seemed somewhat more advanced in the reperfused infarcts, and there was early evidence of hemosiderin conversion in the remaining central zone of hemorrhagic necrosis.

ARACHIDONIC ACID METABOLIC POTENTIAL The products of $[^{14}C]$-AA metabolism in normal left ventricle and in one day reperfused infarct minces are shown in Fig. 3. Minces of normal myocardium were relatively inactive in metabolizing $[^{14}C]$-AA (Lane 1). The calcium ionophore A23187 was added to allow redistribution of endogenous calcium and thus stimulate Ca++ dependent phospholipases or lipoxygenases. Even with the addition of 10μM A23187 (Lane 2), very few products were formed. The infarcted tissue showed the production of a non-polar product comigrating with 12-HETE standard in this thin layer chromatography system (Lane 3) and a striking
Fig. 1 HISTOLOGY OF NORMAL AND ONE DAY INFARCTED CANINE MYOCARDIUM. The upper panel shows normal myocardium at high power (40x). The lower panels show the distinctive features of one day old reperfused and occlusive infarcts at 16x (middle) and 40x (bottom).
Fig. 2 HISTOLOGY OF INFARCTED CANINE MYOCARDIUM AT 3 AND 7 DAYS following reperfused (left) and occlusive (right) infarction. By 3 days, fibroblasts can be seen in the reperfused infarct while the occlusive infarct is still dominated by PMNs. At 7 days both reperfused and occlusive infarcts show chronic inflammatory cells.

increase in the production of this product with the addition of A23187 (Lane 4). This product was not inhibited (lanes 5,6) by the cyclooxygenase inhibitor indomethacin (20 μg/ml) but was inhibited (lanes 7,8) by the lipoxygenase and cyclooxygenase inhibitor eicosatetraynoic acid (ETYA - 25μM). This product was quantitated by cutting the thin layer chromatography plate and counting the radioactivity in each zone (Table 1). Without stimulation, the reperfused infarct tissue was five times more active in producing the product that comigrated with 12-HETE than was the normal control tissue (254 vs 48 pm/gm). Production of this metabolite by tissue from the occlusive infarct, although elevated, was not significantly different than normal.
Fig. 3 AUTORADIOGRAM OF TLC PLATE showing products of canine cardiac mince incubated with $[^{14}C]$-Arachidonic Acid. Migration of cold standards is shown in the empty lane. Lane 1: One gram of normal myocardium incubated with 10 nmols AA. Lane 2: Normal + AA + 5μM A23187. Lane 3: Infarct + AA. Lane 4: Infarct + AA + A23187. Lanes 5 and 6 include 20 μg/ml indomethacin. Lanes 7 and 8 include 25μM ETYA (eicosatetraynoic acid).

The inclusion of 10μM A23187 in the one day reperfused infarct more than doubled HETE production to 741 ± 131 pm/gm. Again there were no significant increases in the permanently occluded infarct. There were slight elevations at the other time points in reperfused infarcts and at all time points in the occlusive infarcts but these differences were not significant. Thus the one day reperfused infarct was the only tissue which showed a significant elevation in the production of this HETE product when compared with normal myocardium. As is shown in Fig. 1, there was an extensive accumulation of PMNs in the infarcted area at this time.
PROSTAGLANDINS

PRODUCTION OF "MONO-HETE" IN NORMAL MYOCARDIUM AND IN REPERFUSED AND OCCLUSIVE INFARCTS

<table>
<thead>
<tr>
<th></th>
<th>Normal 1 Day</th>
<th>3 Days</th>
<th>7 Days</th>
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<tr>
<td>Normal</td>
<td>48 ± 6</td>
<td></td>
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<tr>
<td>+A23187</td>
<td>§ 87 ± 12</td>
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<tr>
<td>Reperfused</td>
<td>* 254 ± 49</td>
<td>82 ± 32</td>
<td>50 ± 11</td>
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<tr>
<td>+A23187</td>
<td>§ * 741 ± 131</td>
<td>130 ± 56</td>
<td>177 ± 86</td>
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<tr>
<td>Occlusive</td>
<td>104 ± 30</td>
<td>100 ± 35</td>
<td>86 ± 20</td>
</tr>
<tr>
<td>+A23187</td>
<td>149 ± 31</td>
<td>113 ± 20</td>
<td>128 ± 28</td>
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* = p<.05 compared to normal myocardium
§ = p<.05 comparing with and without A23187
pmoles/gm wet weight

TABLE 1: The production of the [\(^{14}\text{C}\)]-AA metabolites co-migrating on TLC with mono-HETE. Data are expressed as picomoles of product per gram wet tissue weight. Normal data are pooled. Values are mean ± SEM.

By three days, the PMNs had diminished and had been replaced with monocytes and activated fibroblasts as seen in Fig. 2. A similar progression occurred in the occlusive infarct but over a longer period of time. The maximum number of the PMNs in the occlusive infarct occurred in our study at three days as has been previously reported by Mallory et al. (6). Although there were PMNs in the permanently occluded infarcts, they were fewer in number at any given time and concentrated in the periphery of the infarcted zone. The production of the mono-HETE product thus followed the relative number of PMNs in the tissue with the one day reperfused tissue showing the highest production.

The identity of the AA metabolite co-migrating with mono-HETE on the TLC plate was investigated using HPLC. Incubations were prepared and extracted as before and the products separated as shown in Figure 4. As can be seen, the major AA metabolite from both normal and infarcted tissues co-migrated with 12-HETE. A small amount of 15-HETE was also seen.
The major radioactive peak from a one day reperfused infarct was collected, derivatized as described in the methods section and studied by mass spectrometry. The spectrum obtained was consistent with previously published spectra (15,16) for authentic 12-HETE with a 2 mass unit increase consistent with \(^{14}\text{C}\) in the original substrate. The mass ion (408) was not seen but characteristic peaks were seen at \(m/z=393\) (M-15: -CH\(_3\)), \(377\) (M-31: -OCH\(_3\)), and the base peak at 297 (M-111). Characteristic peaks were also seen at 231, 207 (297-90), and 175 (297-(90+32)). The relative abundance of endogenous substrate can be estimated from the relative peak heights of the base peak (297) and the corresponding peak for the naturally occurring isotope (295). The unlabeled substrate therefore represented approximately 40% of the \(^{14}\text{C}\)-labelled substrate.

DISCUSSION

The purpose of our study was to compare occlusive and reperfused myocardial infarcts and to correlate the histologic changes in the progression of inflammatory cells with the changes in the production of AA metabolites in the early days after an initial ischemic event. We have shown the histologic progression of inflammatory cell invasion in reperfused infarcts to be distinctly different than what was seen in occlusive infarcts and that the ability to metabolize \(^{14}\text{C}\)-AA into 12-HETE paralleled the number of PMNs in the tissue.

Other investigators have studied the reperfused infarct but have focused either on the acute changes occurring within minutes or hours following occlusion and reperfusion or on the histologic changes occurring several days or weeks following infarction. The "acceleration" of the inflammatory response following reperfusion of an ischemic area of myocardium was initially described by Sommers and Jennings (5). Their study looked only at changes up to 4 hours following 40 minutes of ischemia. A recent study by Roberts et al. (17) examined histologic differences only after 3 days. Recently, Mullane and co-workers (9) have reported that PMN invasion can be seen as early as one hour following reperfused infarction and that tissue minces from these hearts produce 12-HETE from \([^{14}\text{C}]\)-AA. In our study we have focused on the period of time previously unstudied by other investigators and have seen that the peak of the PMN invasion occurs at one day following reperfusion in the days that were studied. We also have observed the same major products of AA metabolism (12-HETE and a small amount of 15-HETE) as reported by Mullane et al. (9) and report here that the production of these metabolites are time and infarction model dependent, being most pronounced in our study in the one day reperfused infarct and decreasing in the subsequent days.

In our study we did not attempt to restrict the flow in the reperfusing vessel using the "critical stenosis" technique employed by Lucchesi et al. (18) and thus our reperfused infarcts were
Fig. 4 Reverse Phase – High Performance Liquid Chromatography separation of [14C]-AA metabolites from (a) normal and (b) infarcted areas of 1 day reperfused canine myocardial infarct. Solid line (—) represents optical density. Dashed line (--) represents [14C] radioactivity. The arrows below the figure show the retention times of authentic standards as indicated. Dotted line (...) shows elution profile of % acetonitrile.

variably hemorrhagic. The limitation of flow (and therefore hemorrhage) has the advantage that it results in fewer experimental deaths due to reperfusion tachyarrhythmias (18). It is possible that limitation of flow could produce an infarct that more closely approximates situations where a fixed stenosis is maintained during
Fig. 5 Mass Spectrum of the methyl ester trimethylsilyl ether derivative of the major radioactive AA metabolite which co-migrated on RP-HPLC with 12-HETE. The abscissa represents the mass scale m/e and the ordinate represents the relative abundance of the ion fragments expressed as a percentage of the base peak (297).

reperfusion. This might occur for example during the recanalization of an atheroscleriotically restricted vessel following treatment with a thrombolytic agent (19,20). Our model of reperfused infarct more closely parallels the situations described following cardiac surgery (3,4) or in human post-mortem studies of infarction treated with aortocoronary revascularization (2).

The source of the 12-HETE seen in the infarcted tissues and its physiologic significance were not directly addressed in this study. Unlike human (21) or rabbit PMNs (22), which metabolize 14C-AA predominantly into 5-HETE, dog peripheral blood or elicited peritoneal PMNs are unusual in that they metabolize 14C-AA into 12-HETE (9). Very little 5-HETE was seen either with or without
A23187 stimulation in either occlusive or reperfused infarcts.

In our study, the presence of PMNs in the tissue and the ability of the tissue to metabolize [14C]-AA into 12-HETE were temporally correlated, although other sources of 12-HETE cannot be excluded. Platelets accumulate in both permanently occluded and reperfused infarcts (23,24) and can also produce 12-HETE (15). However, depletion of platelets with goat anti-dog platelet serum (25), did not decrease infarct size, suggesting that platelets do not play a central role in infarct size determination. Mullane has also noted that the lack of thromboxane suggests that platelets are not major contributors to AA metabolism (9). A minor role in infarct size determination is also suggested for platelets by the observation that a dose of ibuprofen which decreases infarct size in a reperfused infarct does not alter the accumulation of in-platelets (26). Recently, it has been shown that erythrocytes can metabolize AA to 12-HETE (27) so it is conceivable that the elevated 12-HETE which we have studied originates from the hemorrhage in the reperfused infarct. By studying the metabolism of [14C]-AA in tissues in which specific antibodies are used to selectively deplete either PMN or platelets or by comparing comparable reperfused infarcts produced with or without a critical stenosis (and thus without or with intramyocardial hemorrhage), it may be possible to resolve the source and the role of the 12-HETE seen in these infarcts.

Another factor which may influence the production of 12-HETE by the infarcted myocardium is the level of calcium in the tissue. With ischemia and reperfusion a more than 10-fold accumulation of calcium occurs in the tissue with levels as high as 24 mmoles/kg wet weight (28). Similarly, levels of calcium as high as 4.2 mmoles/100g dry weight in reperfused myocardium have been reported by Shen and Jennings (28). In their experiments, no increases in calcium content were seen in permanently occluded infarcts when compared with non-infarcted control myocardium. The difference in the unstimulated production of 12-HETE seen between normal (48 ± 6) and 1 day reperfused infarct (254 ± 49) may be due to these differences in availability of Ca++ in the tissues. Since A23187 may be acting by mobilizing tissue Ca++ stores and providing Ca++ cation to calcium dependent enzymes such as phospholipase or lipoxygenase, the accumulation of Ca++ may explain the further enhancement of 12-HETE production seen with A23187 and may be acting in the tissue as a tonic stimulus to the release of AA and its metabolism into 12-HETE.

It has been recently reported that ibuprofen (29,30,31) and BW755c (10,11) have a cardioprotective effect in a variety of animal models. BW755c inhibits both cyclooxygenase and lipoxygenase (32) and can reduce myocardial infarct size even if it is given after reperfusion (10). This precludes a protective or membrane stabilizing effect during ischemia and reperfusion. Treatment with BW755c also inhibits the production of 12-HETE, decreases the number of PMNs in the infarcted tissue, and reduces
the number of ventricular ectopic beats seen following reperfusion. Since 12-HETE is chemotactic (33), BW755c may be acting to prevent further PMN accumulation in the infarcted tissue by inhibiting the production of this recruiting signal. This may also be advantageous since PMN activation is accompanied by release of lysosomal proteases (34) and generation and release of toxic oxygen metabolites such as $O_2^-$ and $H_2O_2$ (35). The importance of these metabolites in the final determination of infarct size has been demonstrated by using an infusion of the inactivating enzymes catalase and superoxide dismutase (33). Ibuprofen can also decrease the accumulation of PMNs (without affecting platelet accumulation) in reperfused infarcts with a similar reduction in infarct size (31). The mechanism of this effect is not known. It is also not yet known whether the production of 12-HETE is merely a marker for the presence of PMNs in the tissue or whether it plays an integral role in the pathophysiology of the evolving myocardial infarct. One possibility is that 12-HPETE, the hydroperoxide formed as a precursor of 12-HETE (15), is acting to stimulate leukotriene production by another cell population (36). A final possibility is that 12-HETE is interconverted to 12,20-diHETE (37,38) which as yet has unknown biological activity.

The long term therapeutic value of limiting infarct size with agents such as ibuprofen and BW755c has yet to be investigated. If sufficient wound repair and fibroblast activation could occur in the absence of prominent neutrophilic invasion it might be possible to limit infarct size using this type of agent. Specific drugs capable of blocking the production of chemotactic AA metabolites may have an advantage over the use of steroids which not only decrease AA metabolite release but are also directly cytotoxic to a number of leukocyte sub-populations. Specific agents would allow direct manipulation of the inflammatory response in the tissue and presumably increase survival following reperfused myocardial infarction. Whether the inhibition of leukocyte function is beneficial for long term survival and damaged tissue repair following myocardial infarction remains to be seen. Experience with corticosteroid treatment has shown that by inhibiting the wound repair process (39), a "mummified" infarct (40) is produced with a weakened myocardial wall leading to the possibility of myocardial wall rupture. If the pharmacological manipulation of myocardial infarction with lipoxygenase inhibitors also jeopardizes the structural integrity of the myocardium, then their clinical use will be limited to the reduction of infarct size in the early minutes of a developing infarct. Thus understanding the effect of arachidonic acid metabolites on the inflammatory response to myocardial infarction may provide useful avenues of pharmacological manipulation for therapeutic benefit.

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