Prevention of Myocardial Lesions in JCR:LA-corpulent Rats by Nifedipine

James C. Russell, Dorothy G. Koeslag, Peter J. Dolphin, and Roger M. Amy

Male rats of the JCR:LA-corpulent strain spontaneously develop atherosclerosis and myocardial lesions if corpulent. The corpulent rats exhibit a marked very low density hyperlipidemia and insulin resistance. The incidence of both vascular and myocardial lesions correlates strongly with the hyperinsulinemia, but not with the hyperlipidemia. Corpulent male rats were chronically treated with nifedipine or acetylsalicylic acid to explore the roles of smooth muscle spasm and platelet activity in induction of the myocardial lesions. Acetylsalicylic acid treatment was associated with no significant changes in fasting glucose, insulin, or lipid concentrations. Nifedipine caused no significant changes in glucose concentration but was associated with mildly increased insulin levels. Treatment with nifedipine resulted in significant decreases in serum triglyceride concentrations. The decreases were confined to longer-chain triacylglycerol molecular species with no change in the concentration of molecular species with 48 or 50 acyl carbon atoms. There was no effect on myocardial lesion frequency with acetylsalicylic acid treatment. In contrast, nifedipine prevented the development of old organized scarred lesions. This effect is similar to that seen with treatments that markedly reduce the insulin resistance. These findings suggest that platelet-initiated thrombus formation is not an important factor in lesion formation in the JCR:LA-cp rat, but that smooth muscle spasm is probably important. (Arteriosclerosis 10:658-664, July/August 1990)

One of the critical end points of atherosclerotic disease is the induction of ischemic myocardial lesions. These may vary from small discrete lesions with loss of myocytes and scarring to massive areas of infarction leading to rapid death. The precise sequence of mechanisms of events leading from the atherosclerotic lesion to myocardial damage is often not clear. The major possible mechanisms that have been discussed are simple occlusion of the artery by the atherosclerotic lesion, intramural hemorrhage leading to an enlarged lesion and occlusion, thrombus formation on the surface of the atherosclerotic lesion, and vasospasm induced or promoted by the vascular lesion.1,2

The JCR:LA-corpulent rat is a unique strain that has been shown to spontaneously develop both atherosclerotic and ischemic myocardial lesions.3,4 The strain incorporates the corpulent (cp) gene first isolated by Koletskey5,6 and later bred by Hansen7 into two congenic strains, the LA/N-cp and SHR/N-cp. These strains have been described8,9 and are regarded as models for the study of obesity. At the fifth backcross to the parent LA/N strain, a colony was established in our laboratories and at the seventh backcross, an analogous SHR/N-cp colony was established elsewhere. These two substrains have now been assigned the symbols JCR:LA-cp and SHR/Mcc-cp to distinguish them from the parent strains, although we originally referred to ours as LA/N-cp. These strains are both susceptible to different cardiac diseases, with the SHR/Mcc-cp rat being prone to cardiomyopathy progressing to fatal congestive heart failure.10

The cp gene is autosomal recessive. The rats may thus be bred by conventional techniques to be homozygous cp (cp/cp) and obese, heterozygous cp (cp/+ ) or homozygous normal (+/+), both lean.3,4 The cp/cp rats of the JCR:LA-cp strain have a marked very low density lipoprotein (VLDL) hyperlipidemia and severe insulin resistance.11,12 The hyperlipidemia is more extreme in the cp/cp female rat than in the cp/cp male. In contrast, the insulin resistance and accompanying hyperplasia of the pancreatic B cells is more severe in the cp/cp male rat.12,13 The cp/cp male rats spontaneously develop both atherosclerotic and myocardial lesions while +/+ and female rats do not.3,4 The lesions develop in parallel in an age-dependent manner, and the frequency correlates strongly with the hyperinsulinemia, but not the hyperlipidemia.

Long-term consumption of ethanol by cp/cp male rats causes a marked reduction of fasting insulin levels and little change in serum lipid concentrations.14 Prolonged running and moderate food restriction cause similar decreases in the hyperinsulinemia together with a major reduction in the VLDL hyperlipidemia.15 Both treatments resulted in the elimination of large scarred myocardial lesions. These results are consistent with the proposal that the pathophysiological process leading to vascular and myocardial damage is initiated by high insulin levels or some related factor. However, the mechanism leading...
from vascular lesions to myocardial damage remains obscure.

Nifedipine is one of the group of calcium channel antagonists. It thus inhibits smooth muscle contraction and has been the drug of choice in treatment of presumed coronary artery spasms.16 Nifedipine has also been reported to suppress the development of atherosclerosis under some circumstances, particularly in the cholesterol-fed rabbit, but not in the Watanabe heritable hyperlipidemic rabbit.17 Recent studies have shown that the aorta from cp/cp male rats, but not +/+ or female rats, has a defect in endothelium-dependent relaxation and abnormal vascular tone in response to norepinephrine (McNamee, Kappagoda, and Russell, unpublished results). This would suggest that vasospasm, secondary to atherosclerotic injury, may play a role in the induction of myocardial lesions in the cp/cp rat. This concept is further supported by the finding that the isolated perfused heart of the JCR:LA-cp rat is intolerant of normal circulating calcium concentrations (Lopaschuk and Russell, unpublished observations). Previous observations of occlusive thrombi in coronary and abdominal arteries of cp/cp rats would also suggest that platelet aggregation and thrombus formation on arterial lesions could also be important. Acetylsalicylic acid (ASA) inhibits the cyclooxygenase system, and thus indirectly platelet function, and has been reported to reduce the incidence of myocardial infarction, but not infarct size.18,19 We report here on long-term treatment with nifedipine and ASA as prophylactic agents.

**Methods**

Male cp/cp and +/+ rats were bred in our established colony of JCR:LA-cp rats as previously described.3,4,14 They were housed individually in 48×26×16 cm polycarbonate cages with stainless steel tops and wood chip bedding. Food was Wayne Lab Blox (Continental Grain, Chicago, IL) and was initially available ad libitum together with tap water. The temperature was maintained at 22°C and the relative humidity, at 40% to 50%. Lighting was on a 12:12 hour cycle, on at 03:00 and off at 15:00. At 6 weeks of age, the rats were started on treatment with nifedipine. The drug was kept in the dark at all times, and solutions were made and handled in a dark room. Nifedipine was dissolved in ethanol at 0.5 to 1.5 g/100 ml, and this solution was adsorbed onto preweighed 6 g food pellets so that each rat would receive a daily dosage of 15 mg/kg body weight. Treated food pellets were made fresh each week and kept refrigerated in light-proof containers. Control rats received food pellets that had been treated with the ethanol vehicle only. ASA was added to drinking water to a final concentration of 1.54 g/l. This gave a consumption in the range of 10 mg/kg body weight per day. The rats were maintained in the protocol to 9 months of age without disturbance other than routine cage changes.

The rats were sacrificed under halothane anesthesia after an overnight fast. Blood was obtained from an open chest cardiac puncture and was separated into serum for lipid analyses and heparinized plasma for glucose and insulin measurements. A complete postmortem examination was made, and the heart was removed and cut transversely into three segments: base, mid-heart, and apex. After fixation in 10% neutral buffered formalin, the samples were processed by standard histologic techniques and embedded in paraffin. Two adjacent sections were cut from each block and were stained with hematoxylin and eosin and Masson’s trichrome. The slides were examined blind by an experienced pathologist, and lesions were categorized and recorded as previously described: type A, muscle scar or dropout with chronic inflammatory cell accumulation; type B, necrosis of myocytes with reactive inflammatory cells; type C, nodule of inflammatory cells; and type D, muscle scar with or without chronic inflammatory cells. Lesions were summed from the three sections from each heart with the two stains used to confirm identification, giving a relative frequency of occurrence.

The serum was frozen and maintained at −78°C until lipids were analyzed by the total lipid profile technique of Kuksis et al.20 This technique involves preliminary digestion of the plasma sample with phospholipase C (Sigma Chemical, St. Louis, MO) followed by derivatization with N,O-bis(trimethylsilyl)-acetamide (Pierce Chemical, Rockford, IL). Gas chromatographic analysis yields the concentration of free cholesterol, individual cholesterol esters, diacylglycerols, and ceramides of the phospholipids and triglycerides, with individual results by fatty acid carbon numbers. Thus the triglyceride molecular species may be specified according to their number of acyl carbon atoms as follows:

- C:48, triglycerides with C-16:16:16 or C:14:16:18 fatty acyl chains;
- C:50, triglycerides with C-16:16:18 or C-14:16:20 fatty acyl chains;
- C:52, triglycerides with C-16:18:18 or C-16:16:20 fatty acyl chains;
- C:54, triglycerides with C-16:18:20 or C-16:16:22 or C-18:18:18 fatty acyl chains;
- C:56+>, triglycerides with C-18:18:20 or C-16:18:22 fatty acyl chains and triglycerides with greater acyl carbon numbers that were not resolved.

The results reported for cholesterol esters, phospholipids, and triglycerides represent the sum of the concentrations of the particular molecular species. We also report separately on the individual triglyceride molecular species in appropriate instances.

Plasma glucose was measured by a glucose oxidase procedure (Beckmann Instruments, Brea, CA) and insulin by a radioimmunoassay method with rat insulin standards.21 Statistical analysis was by unpaired t test or Wilcoxon rank sum test as appropriate, with p<0.05 in a two-tailed test considered to be significant.

All care, treatment, and handling of the animals was in accordance with the guidelines of the Canadian Council on Animal Care. These procedures were subject, as specified in the guidelines, to prior approval by the Health Sciences Animal Welfare Committee of the University of Alberta.
Table 1. Body Weight and Plasma Concentration of Glucose and Insulin In 9-month-old Male JCR·LA-cp Rats

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Group</th>
<th>Body weight (g)</th>
<th>Plasma glucose (mg/dl)</th>
<th>Plasma insulin (mU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>Control</td>
<td>386±17</td>
<td>161±19</td>
<td>34.1±22.2</td>
</tr>
<tr>
<td>cp/cp</td>
<td>Control</td>
<td>786±39</td>
<td>227±55</td>
<td>327±58</td>
</tr>
<tr>
<td>cp/cp</td>
<td>Nifedipine</td>
<td>676±23‡</td>
<td>218±34</td>
<td>411±86*</td>
</tr>
<tr>
<td>cp/cp</td>
<td>Acetylsalicylic acid</td>
<td>767±70</td>
<td>157±22‡</td>
<td>334±110</td>
</tr>
</tbody>
</table>

The values are the means±SD. There were 10 rats in each group.

Table 2. Whole Serum Lipid Concentrations in 9-month-old Male JCR·LA-cp Rats

<table>
<thead>
<tr>
<th>Genotype/group</th>
<th>Cholesterol (mg/dl)</th>
<th>Cholesteryl esters (mg/dl)</th>
<th>Phospholipids (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+ control</td>
<td>11.2±16</td>
<td>60.6±10.7</td>
<td>55.3±10.7</td>
<td>19.9±8.3</td>
<td>48.7±7.5</td>
</tr>
<tr>
<td>cp/cp control</td>
<td>31.3±12.7</td>
<td>180±64</td>
<td>225±89</td>
<td>305±163</td>
<td>137±50</td>
</tr>
<tr>
<td>cp/cp/nifedipine</td>
<td>27.7±3.2</td>
<td>144±15.4</td>
<td>180±23.4</td>
<td>157±44*</td>
<td>112±12</td>
</tr>
<tr>
<td>cp/cp/ASA</td>
<td>34.0±5.4</td>
<td>174±20</td>
<td>230±27.0</td>
<td>278±49</td>
<td>136±17</td>
</tr>
</tbody>
</table>

Values of lipid concentrations are in mg/dl (means±SD). There were 10 animals in each group.

Table 3. Concentration of Triglyceride Molecular Species in cp/cp Male Rats Treated with Nifedipine and Acetylsalicylic Acid

<table>
<thead>
<tr>
<th>Group</th>
<th>C:48</th>
<th>C:50</th>
<th>C:52</th>
<th>C:54</th>
<th>C:56+&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=7)</td>
<td>15.1±6.8</td>
<td>49.3±24.7</td>
<td>135±71</td>
<td>70.2±34.9</td>
<td>81.29±36.76</td>
</tr>
<tr>
<td>Nifedipine (n=10)</td>
<td>16.2±4.3</td>
<td>39.1±11.5</td>
<td>58.1±18.2</td>
<td>34.1±11.7*</td>
<td>21.89±6.32†</td>
</tr>
<tr>
<td>ASA (n=10)</td>
<td>14.4±8.2</td>
<td>46.7±12.9</td>
<td>100±16</td>
<td>56.8±8.5</td>
<td>60.3±10.3</td>
</tr>
</tbody>
</table>

The values are in mg/dl, means±SD.

Results

The rats tolerated both the nifedipine and ASA treatments without any apparent symptoms. Table 1 shows both the twofold greater weight of cp/cp control rats compared to +/+ control rats and the significantly lower (14%) weight of the nifedipine-treated rats. The origin of this is not clear and was not related to decreased food intake. Table 1 also shows the markedly higher insulin concentrations in the cp/cp rats. These were significantly higher in the nifedipine-treated group. The cp/cp control rats showed moderately raised fasting glucose concentrations that were significant (p<0.01 vs. +/+ control) and not seen in previous studies. The nifedipine-treated rats had virtually identical glucose concentrations to the cp/cp control rats. The ASA-treated group, in contrast, had essentially identical fasting glucose concentrations to the +/+ control rats, and the difference compared to the cp/cp control rats was significant.

Table 2 shows the fasting whole serum lipid concentrations in the four groups. The concentrations of all lipid classes in the +/+ or cp/cp control rats are similar to those previously reported. Treatment of cp/cp rats with nifedipine caused decreases in cholesteryl esters and thus total cholesterol. The decreases in cholesteryl esters and total cholesterol were significant on a one-tailed test (p<0.05), but the variance was such that the differences did not satisfy the more appropriate two-tailed test. The triglyceride concentrations were more markedly reduced, and this was significant (p<0.02, two-tailed). In contrast, treatment with ASA caused negligible changes in serum lipid concentrations.

The results in Table 3 show that the decreases in triglycerides in the nifedipine-treated rats were in the longer-chain fatty acids. There was no effect on C:48 concentrations and little effect on C:50 triglycerides. There were marked reductions in the concentrations of C:52, C:54, and C:56+> molecular species. These changes in absolute concentration were reflected in highly significant changes (p<0.001) in the relative concentrations of all molecular species except C:54 as shown in Table 4. This resulted in a quite different distribution of triglyceride molecular species that was weighted toward shorter-chain fatty acids in the nifedipine-treated group. The ASA-treated rats had decreased triglyceride concentrations and, as shown in Table 3, this was also confined to longer-chain fatty acids, but none of these differences were statistically significant.

The incidence of myocardial lesions in the rats is shown in Table 5. No lesions were seen in the hearts of the +/+ control rats. The cp/cp control rats exhibited similar fre-
Table 4. Relative Concentration of Triglyceride Molecular Species in cp/cp Male Rats Treated with Nifedipine

<table>
<thead>
<tr>
<th>Group</th>
<th>C:48</th>
<th>C:50</th>
<th>C:52</th>
<th>C:54</th>
<th>C:56+&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.39±0.36</td>
<td>14.0±0.99</td>
<td>38.0±1.2</td>
<td>20.1±0.46</td>
<td>23.5±1.6</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>9.7±1.0</td>
<td>23.1±1.7</td>
<td>34.2±0.91</td>
<td>20.0±1.7</td>
<td>13.1±1.8</td>
</tr>
</tbody>
</table>

Values are the percentages of total triglyceride concentration, means±SD, calculated individually for each rat. All values except for C:54 are significantly different in the two groups (p<0.001).

Table 5. Myocardial Lesion Frequency in 9-month-old Male JCR:LA-cp Rats

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Group</th>
<th>Type of lesion</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>cp/cp</td>
<td>Control</td>
<td>0.90±0.31</td>
<td>0</td>
<td>0.40±0.22</td>
<td>0.70±0.26</td>
<td></td>
</tr>
<tr>
<td>cp/cp</td>
<td>Nifedipine</td>
<td>0.70±0.21</td>
<td>0.10±0.10</td>
<td>0.20±0.13</td>
<td>0*</td>
<td></td>
</tr>
<tr>
<td>cp/cp</td>
<td>ASA</td>
<td>1.50±0.48</td>
<td>0</td>
<td>0.60±0.27</td>
<td>0.70±0.21</td>
<td></td>
</tr>
</tbody>
</table>

The values are means±SE of the number of lesions observed in the three sections from each rat. Lesion types: A. Muscle scar or cell dropout with chronic inflammatory cell accumulation; B. Necrosis of myocytes with reactive inflammatory cells; C. Nodules of chronic inflammatory cells; D. Old scars.

Significance of difference vs. cp/cp control: *p<0.05.

ASA=acetylsalicylic acid.

Figure 1. Section from the heart of a cp/cp control rat at 9 months of age showing a typical type A lesion with cell loss and chronic inflammatory cell infiltration. Hematoxylin and eosin stain. × 61

Frequency of lesions to those found in previous studies. Figure 1 shows a typical type A lesion in the heart of a cp/cp control rat. It is characterized by extensive local cell loss and active chronic inflammatory cell activity. In contrast, the type D lesions, as illustrated in Figure 2, are mature organized lesions with extensive collagen deposits. The ASA-treated group showed a frequency of types B, C, and D lesions similar to the control group and a nonsignificantly higher incidence (p>0.05) of recent lesions. The nifedipine-treated rats showed similar frequency of types A, B, and C to the cp/cp control group. However, there was a complete absence of old scarred lesions, and this was statistically significant. In addition the type A lesions, while present at a similar frequency in the hearts of nifedipine-treated rats to that of control rats, were qualitatively different. The type A lesions were markedly smaller and more circumscribed as illustrated by the typical lesion from a nifedipine-treated rat shown in Figure 3.

Discussion

ASA caused little general effect on the rats, as would be expected at the dose employed. There was no signif-
sicant change in fasting insulin or body weight nor of lipid status. The significantly lower glucose concentrations are difficult to explain, especially in view of the unaltered insulin levels and the higher glucose concentrations in the cp/cp control rats as compared to rats in previous studies. They may simply reflect unusual random variation and not be of biological significance.

The dose of ASA consumed by the rats was distributed throughout the day as the animals drank. Thus, a reasonably consistent blood level should have been maintained. The dose is sufficient to markedly inhibit thromboxane A2 synthesis and platelet function in rats or humans. The failure to inhibit myocardial lesion frequency suggests that platelet aggregation and consequent thrombus for-
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formation are not critical mechanisms in the cp/cp rat. This is consistent with other findings14 and the effects of nifedipine reported here.

Nifedipine clearly had significant metabolic effects on the rats as revealed by the lower body weights, increased fasting insulin levels, and halved triglyceride concentrations. The mechanisms leading to these changes are not obvious, and increased insulin levels were not accompanied by either decreased glucose levels or an increased fasting glucose secondary to a greater insulin resistance. The cause of the decreased body weight may be related to the increased insulin levels, but this possibility will require more detailed metabolic studies. The increased insulin insensitivity might be expected to exacerbate the VLDL hypersecretion and lead to higher triglyceride concentrations. However, in the nifedipine-treated rats, it was associated with a large decrease in the triglycerides that are essentially present only in the VLDL fraction.11 The selective reduction in C:52, C:54, and C:56 + triglyceride molecular species may reflect inhibition (or stimulation) of a specific enzyme. A preference by this enzyme for longer-chain fatty acids could yield the observed changes in triglyceride molecular species. There were no corresponding changes of any significance in either absolute or relative concentrations of phospholipid fatty acid molecular species or of the fatty acid composition of cholesteryl esters (data not shown). This suggests that the effect is confined to the triacylglycerol synthetic pathway, although phospholipid composition is conserved and the origin of the changes in the triglycerides may lie in an alteration of fatty acid flux to the liver or of chain elongation.

Reduction by clofibrate of cholesterol esters by 50% and triglycerides by 22% in the cp/cp male rat was not associated with any reduction in myocardial lesion frequency (Russell, Koeslag, Amy, and Dolphin, unpublished results). Thus, the effect of nifedipine on lesion frequency is unlikely to be solely due to the reduction in lipid concentrations. Reduction of plasma insulin concentration in the cp/cp male rats has been associated with reduced myocardial lesion frequency.14 Increased insulin levels should then cause increased endothelial damage24-26 and consequent vascular damage and increased myocardial lesion frequency.14 Increased insulin levels are related to low density lipoprotein (LDL) cholesterol metabolism. The cp/cp rat has only moderate elevation of LDL cholesterol secondary to a VLDL hypersecretion,28 and thus atherogenesis in this model is not likely to be related to LDL metabolism. Nonetheless, it will clearly be important to clarify the effect of nifedipine on atherogenesis in further studies. Since the effects of nifedipine are not consistent with the previously demonstrated relationships between insulin levels, hyperlipidemia, and atherogenesis, they may be affected through direct effects on calcium metabolism in several cell types.

The evidence from this and previous studies12,14,15 is consistent with a pathophysiological process in the male cp/cp rat that is initiated by high insulin levels. Damage to the endothelium leads to intimal thickening with subendothelial smooth muscle and other cells (Heisler, Amy, and Russell, unpublished observations). This develops with time and perhaps with the presence of hyperlipidemia into large raised atherosclerotic lesions as previously reported.2 The underlying smooth muscle becomes hyperreactive to norepinephrine and the endothelium-dependent relaxation becomes weakened (McNamee, Kappagoda, and Russell, unpublished observations). Vasospasm can then lead to localized myocardial ischemia and to myocardial lesions. Interference with any one element of this process, that is, through lowered insulin levels or inhibition of smooth muscle contraction with nifedipine, will block the process. The details of the effects of calcium channel antagonists on insulin metabolism, arterial wall responsiveness, and vascular lesions remain to be elucidated. Understanding these factors in this animal model may prove valuable in understanding and preventing human myocardial disease.

Acknowledgments

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References

8. Elwood KE, Michaelis OE, Emberland JJ, Bhathena SI. Hormonal and lipogenic and gluconeogenic enzyme


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