THE PROTECTIVE EFFECT OF PROPRANOLOL ON ISCHEMIC MYOCARDIUM: AN ELECTRON MICROSCOPIC STUDY

BY

Noriaki Yamamoto*1, Ryoji Hahano*2 and Toshiaki Sunaga*3

ABSTRACT

The protective effect of propranolol on ischemic myocardium was studied experimentally and clinically by electron microscope. In an animal experiment, ischemic changes were produced in the posterior papillary muscle of the rabbit following 3, 15, and 30 minutes of occlusion of the circumflex coronary artery. Prolpranolol (0.25 mg/kg) was injected into the left atrial cavity before occlusion of the artery. The posterior papillary muscle was excised and examined by electron microscope. In clinical experience, propranolol (20 μg/kg) was given intravenously to 6 patients who underwent open heart surgery. Transmural left ventricular myocardial biopsy was performed after the anoxic cardiac arrest and the material, particularly the subendocardium, was examined by electron microscope. It was shown that propranolol was effective, both in the experiment and in the clinical experience, in preserving ischemic myocardium. The possible mechanisms through which propranolol might act were considered to be (1) indirect effect of altered oxygen supply vs. demand, effected by reducing heart rate and reducing cardiac output due to the drug's function as a beta blocker, (2) direct cellular effect, i.e., reducing myocardial substrate metabolism along with stabilization of cellular structure, and (3) increase collateral circulation to the subendocardium.

INTRODUCTION

According to the theory by Ahlquist1, two different kinds of adrenergic receptors exist in the circulatory system, designated as alpha and beta. Propranolol selectively blocks beta adrenergic receptors2) and decreases many of the measurable hemodynamic parameters of myocardial mechanical efforts, such as heart rate, cardiac output, and external left ventricular work3). Concomitant with these changes, coronary flow and myocardial oxygen consumption have been shown to be reduced4,5). Much attention has been directed towards various types of therapeutic intervention which prevent the development of myocardial cell necrosis following coronary artery occlusion6–8). However, no direct observations have been made with respect to the effects of propranolol on the ultrastructure of ischemic myocardium.

The present study was undertaken to observe these latter effects. In experimental animal studies, myocardial ultrastructure was assessed in two groups of rabbits. The circumflex branch of the left coronary artery (CXB) was occluded for 3 minutes, 15 minutes, and 30 minutes in each group,
but only one group was pretreated with propranolol. In clinical material, a transmural left ventricular myocardial biopsy was taken to examine the subendocardium after anoxic cardiac arrest, during open heart surgery with or without propranolol.

**Materials and Methods**

Experimental Study:

Forty-five male albino rabbits weighing 1.9 to 2.8 kg were used in the experiment. Each rabbit was anesthetized with 25% urethane (ethyl carbamate) administered intraperitoneally, the total doses varying between 5 and 8 ml (1.25 and 2 mg). The rabbit was tracheostomized and respiration was maintained by room air through an endotracheal tube attached to a respirator pump. Continuous electrocardiograms were recorded from standard limb leads.

The chest was opened through the third intercostal space using 3 to 5 ml of 0.5% Novocaine (procaine hydrochloride) as a local anesthetic. The left lung was retracted gently and the pericardium widely incised to expose the heart. The CXB was occluded near its origin with a 5-0 polyester suture. Occlusion was confirmed by ECG and the development of cyanosis in the region of the left ventricular wall supplied by this artery.

Of the original 45 rabbits, eight died from ventricular fibrillation during occlusion and 37 were divided into three groups, designated A, B, and C. Group A: Control rabbits. Seven rabbits were killed immediately after the heart was exposed. The rest of the 30 rabbits were divided into six subgroups, Groups B-1, B-2, B-3, and Groups C-1, C-2, C-3. Each subgroup consisted of five rabbits. Group B: Occlusion of the CXB with no pretreatment with propranolol. Group C: Pretreatment with propranolol (0.25 mg/kg) and occlusion of the CXB. In Group B and Group C, the CXB was occluded for 3 minutes (Groups B-1 and C-1), 15 minutes (Groups B-2 and C-2), and 30 minutes (Groups B-3 and C-3). Propranolol (0.25 mg/kg) was injected directly into the left atrial cavity as a single bolus before the occluding suture had been in place for two minutes.

The left ventricle was immediately opened in situ and the posterior papillary muscle (PPM) of the left ventricle was excised. Two or three sagittal slices were cut from the PPM, each 1 mm thick and 2 mm long, and were immediately placed in ice-cold 2.5% glutaraldehyde-2% paraformaldehyde solution (pH 7.5, 5% sucrose) and fixed for 60 minutes. Tissue samples were rinsed in a buffered cacodylate solution in 8% sucrose. Following fixation in 1% osmic acid, they were dehydrated with 50% ethanol and stained with 50% ethanol-uranyl acetate for from 60 to 90 minutes. After dehydration with graded ethanol, they were embedded in Epon 812. They were cut ultrathin on a Porter Blum MT-2 ultramicrotome using diamond knives. The ultrathin sections were further stained with lead citrate and were examined in a Hitachi HS-9 electron microscope.

Clinical Materials:

Propranolol was given to 6 patients who underwent open heart surgery. Six patients who did not receive propranolol served as control subjects. Table 1 shows summary of clinical material. Cardiopulmonary bypass with a bubble oxygenator was used. The unit was primed with lactated Ringer’s solution. Normothermia was employed except in the cases of aortic insufficiency in which moderate general hypothermia to 25°C (rectal temperature) and topical hypothermia, achieved by pouring 1000 ml of a cooled lactated Ringer’s solution upon the heart, were used. Propranolol (20 μg/kg) was given directly into the right atrial cavity.
Table 1. Summary of Clinical Material

<table>
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<th>Patient No.</th>
<th>Initial</th>
<th>Age</th>
<th>Sex</th>
<th>Dx.</th>
<th>Op.</th>
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<th>AA (min.)</th>
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5 minutes before instituting cardiopulmonary bypass. Transmural left ventricular myocardial biopsy was taken before cross-clamping the aorta, after the termination of the anoxic cardiac arrest, and at 60 minutes after restoring coronary circulation if possible. The biopsy specimens were divided into the epicardial layer, endocardial layer, and midmyocardium, all prepared for electron microscopic examination by the same method used in the experimental study. Aortic pressure, left ventricular pressure, and central venous pressure were measured during operation. Electrocardiograms were recorded during and after operation. DPT1/TT1 ratio \(^{10,11}\) was calculated by planimetry on the aortic and left ventricular pressure curves.

RESULTS

Experimental Study:
A: Nonischemic Control Myocardium
Ultrastructure of Group A is shown in Figs. 1, 2, and 3. The mitochondria were abundant, were surrounded by intact sarclemma, and showed tightly packed cristae. Sarcomeres were in register and contracted well. The mitochondrial membrane was well preserved. Glycogen was abundant and was distributed throughout the cell. Intercalated disks had a narrow interspace. Interstitial spaces were narrow. Nuclei had irregular shallow indentations and evenly dispersed chromatin.

B: Ischemic Myocardium
Ultrastructure of Group B-1 is shown in Figs. 4 and 5. Striking alterations were present. Nuclear chromatin was clumped at the membrane with clearing of the central region of the nucleus. Glycogen was almost totally absent. Cells appeared moderately swollen with empty spaces between myofibrils. Alterations of the mitochondria were remarkable, with enlarged matrix spaces and disrupted membranes. Intercalated disks appeared to be separated and unclear in part.
Interstitial edema was severe and the capillary endothelium showed prominent swelling.

Ultrastructure of Group B-2 is shown in Fig. 6 and 7. Almost all the mitochondrial membranes were disrupted. Matrix was swollen and indistinct. Intermysobrillar spaces were enlarged and sarcoplasmic reticulum was dilated. Glycogen was virtually absent. Intercalated disks appeared to be dissolved. Interstitial spaces were enlarged and several lipid droplets were recognized. Capillary endothelium was swollen and endothelial nuclear chromatin clumping and margination were present.

Ultrastructure of Group B-3 is shown in Fig. 8 and 9. Striking ultrastructural abnormalities were demonstrated. Glycogen was completely absent. There was intermyofibrillary edema. The mitochondrial membranes were swollen and almost all mitochondrial membranes were disrupted. Many vacuoles were present. Interstitial spaces were enlarged and capillary endothelium was markedly swollen with clumping and margination of endothelial nuclear chromatin. Giant mitochondria were present.

C: Ischemic Myocardium Pretreated with Propranolol

Ultrastructure of Group C-1 is shown in Fig. 10 and 11. Nuclear chromatin was evenly distributed. The mitochondrial membrane was well preserved and mitochondria had tightly packed cristae. Glycogen appeared decreased compared with that of the control myocardium but was much more abundant than in Group B-1. Interstitial spaces and capillary appeared normal.

Ultrastructure of Group C-2 is shown in Fig. 12 and 13. This was the group in which myocardial ultrastructure appeared best protected. These two sections were virtually identical to those of control myocardium, showing only a slight reduction in glycogen.

Ultrastructure of Group C-3 is shown in Fig. 14 and 15. Although the ultrastructure was severely damaged, it was better preserved than that observed in Group B-3. A significant difference as compared with Group B-3 was that a few glycogen granules were present in Group C-3.

A total of 4,292 mitochondria, 774 from the control group, 1,588 from Group B, and 1,930 from Group C, was selected at random. They were classified according to the state of membrane and matrix (Table 2). The mitochondrial membrane was evaluated as to whether its outer membrane was ruptured or not. Matrix changes were classified according to the degree of swelling. In one group the matrix was either normal or moderately swollen. In the other, the matrix

<table>
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<th>Group</th>
<th>Mitochondria Total</th>
<th>Mitochondrial Membrane</th>
<th>Mitochondrial Matrix Normal to Moderately Swelling (%)</th>
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<td>A</td>
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<td>560 (72.4%)</td>
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<td>694 (89.7%)</td>
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<td>B-1</td>
<td>635</td>
<td>101 (15.9%)</td>
<td>534 (84.1%)</td>
<td>318 (50.1%)</td>
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<td>B-2</td>
<td>485</td>
<td>57 (11.8%)</td>
<td>428 (88.2%)</td>
<td>244 (50.3%)</td>
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<tr>
<td>B-3</td>
<td>468</td>
<td>78 (16.7%)</td>
<td>390 (83.3%)</td>
<td>226 (48.3%)</td>
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<tr>
<td>C-1</td>
<td>375</td>
<td>89 (23.7%)</td>
<td>286 (76.3%)</td>
<td>177 (47.2%)</td>
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<tr>
<td>C-2</td>
<td>910</td>
<td>342 (37.6%)</td>
<td>568 (62.4%)*</td>
<td>605 (66.5%)</td>
</tr>
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<td>C-3</td>
<td>645</td>
<td>133 (20.6%)</td>
<td>512 (79.4%)</td>
<td>374 (58.0%)</td>
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</tbody>
</table>

* p<0.001, ** p<0.05
showed severe swelling and some of them, additionally, exhibited fluffy deposits. Both mitochondrial membranes and matrices were preserved in Group C (pretreatment with propranolol), but the effect of propranolol on membrane and matrix was statistically significant in only Group C-2 (p<0.001 in membrane and p<0.05 in matrix, respectively). The percentage of ruptures in mitochondrial membrane among the groups was fairly constant rather than gradually increasing with time of occlusion. Eighty-four per cent of mitochondrial membrane from Group B-1 (3 minutes occlusion) were ruptured, 88% in Group B-2 (15 minutes occlusion), and 83% in Group B-3 (30 minutes occlusion). Thus rupture of the membrane following 3 minutes occlusion was strikingly severe and remained virtually constant. The same tendency was noted regarding matrix swelling.

Clinical Materials:

Fig. 16 shows ultrastructure of biopsy from patient No. 5, A. M. The specimen was taken from the left ventricular subendocardium after 22 minutes of anoxic cardiac arrest. Striking ischemic alterations were present. The nucleus showed chromatin clumping. Interstitial edema was noted and a contraction band was demonstrated. The mitochondrial matrix was swollen and indistinct. Fig. 17 shows ultrastructure of biopsy from patient No. 7, M. H. Compared with Fig. 16, mitochondria were well preserved and sarcomeres were in register.

Discussion

Occlusion of the CXB is followed by ischemia in the PPM of the left ventricle. Electron microscopic study of this ischemic PPM provides useful information with respect to early changes in the myocardial ultrastructure. This lesion is well suited for electron microscopic studies because of its uniformity and its location in an easily identifiable region of the myocardiun. Moreover, the left ventricular subendocardium, particularly that of the papillary muscle, is preferentially damaged by ischemic arrest. Changes, such as loss of glycogen granules, intracellular edema, relaxation of myofibrils, swelling of mitochondria and of the sarcoplasmic reticulum, and clumping of the nuclear chromatin are considered to be evident after 15 minutes of ischemia, comprising the earliest detectable ischemic changes. As the period of ischemia is prolonged these changes progress rapidly, particularly in the mitochondria. The dense granules in the matrix disappear and the matrix becomes indistinct. The percentage of dying cells increases rapidly after 30 minutes and irreversible injury develops. Occlusion for 40 minutes results in the death of about half the cells of the PPM. In this study, ultrastructural changes following 3 minutes of occlusion are severe and we observe that the percentage of ruptures in mitochondrial membrane and the degree of matrix swelling are fairly constant rather than gradually increasing. Kowada et al. reported the production of cerebral ischemia by occlusion of the carotid artery in a rabbit, the ischemia associated with a temporary rise in systemic arterial pressure followed by marked sustained hypotension. Within 3 minutes the systolic pressure fell between 20 and 40 mmHg. It remained low and did not recover after ischemic periods lasting 7.5 minutes or longer, even when the ischemia was terminated. Ames and Gurian recorded the compound optic nerve response of a rabbit to light flashed at the isolated retina. After oxygen was removed, the fall in response was rapid and disappeared entirely in 3 minutes. Those phenomena observed in the rabbits are apparently due to neurologically mediated changes. We suggest
that the same phenomena of hypotension and subsequent ischemic changes appear to occur in the PPM of a rabbit whose CXB is occluded for 3 minutes.

The dog has been used most frequently in experiments with occlusion of the CXB, rabbits having been employed previously only by Caulfield\textsuperscript{23}. The CXB in rabbits is not easily identifiable and immediate mortality following occlusion is high. Eight of 45 rabbits (18\%) died in the course of the experiment. In an experiment preliminary to this study, India ink was injected into the left atrium after occlusion of the CXB and was subsequently found throughout the myocardium except in the PPM, suggesting that occlusion of the CXB in the rabbit would be followed by infarction of the PPM. Moreover, the 2 kg male rabbit is suitable for this study because of the ease of standardization.

Myocardial cells are almost completely dependent on aerobic mitochondrial metabolism for their energy requirement. When the supply of the oxygen is severely reduced by ischemia, mitochondrial function is greatly reduced and there is a consequent marked change in mitochondrial fine structures, especially in the mitochondrial membranes, where the sites of the greatest concentrations of the oxidative electron transfer systems exist\textsuperscript{24,25}. Therefore, it is appropriate to observe mitochondrial changes to detect early ischemic changes of the myocardium. The severity of cell damage in early stages of infarction varies from one area to another and rarely do all cells in a given region show exactly the same changes\textsuperscript{15}. The reasons for these variations are unclear. It may be due to variation in cellular response to a fairly uniform collateral flow or to a variable collateral flow among fairly uniform cells\textsuperscript{8}. Accordingly, quantitative (morphometric) techniques of electron microscopy, as used in this study, are required\textsuperscript{26,27}.

It has been demonstrated electron microscopically in this study that propranolol has a protective effect on ischemic myocardium. The effect of propranolol was manifested most clearly after 15 minutes of occlusion. The dose of propranolol (0.25 mg/kg) employed in this study, for animals subjected to 30 minutes of occlusion, was lower than that used by other authors\textsuperscript{6,7,28}.

We should like to explain, if possible, the various mechanisms through which propranolol might protect ischemic myocardium. First of all, propranolol works indirectly by lowering oxygen requirements, through reducing heart rate and cardiac output\textsuperscript{8}. Sommers and Jennings\textsuperscript{7} observed the effect of propranolol on myocardial necrosis, which developed in dogs whose CXB's were occluded for 20 minutes. Only one small focus of necrosis was found in the 16 animals pretreated with propranolol, while easily identifiable areas of necrosis were found in nine of the 16 surviving control animals. Reimer et al.\textsuperscript{8} reported the effect of propranolol on the severity of myocardial necrosis following 40 minutes CXB occlusion and subsequent reperfusion. Dogs were killed two to five days later and the areas of necrosis were quantified. Dogs pretreated with propranolol showed significantly less necrosis than untreated controls. Maroko et al.\textsuperscript{8} demonstrated, using the epicardial ECG, that propranolol decreased the severity and extent of ischemic injury produced by occlusion of a branch of the left anterior descending coronary artery.

Sakurada et al.\textsuperscript{20} found that propranolol depressed the oxidative phosphorylation and oxidation of NAD\textsuperscript{+}-linked substrates by myocardial mitochondria and also decreases myocardial metabolism. Myocardium uses free fatty acids more than it does glucose as an energy source and propranolol reduces the
myocardial uptake and utilization of free fatty acids\textsuperscript{30,31}.

Propranolol reduces total coronary blood flow by decreasing myocardial oxygen consumption\textsuperscript{32-34}. However, Becker \textit{et al.}\textsuperscript{25} measured the changes in regional myocardial blood flow occurring during acute myocardial ischemia using radioactive microspheres and found that propranolol caused a significant increase in the endocardial to epicardial flow ratio in an ischemic area. These local changes in myocardial blood flow ratio are also reported by other authors\textsuperscript{35,36} and are believed to be beneficial for ischemic sub-endocardium.

Propranolol also protects ischemic myocardium through primary stabilization of cellular structure, the so-called membrane-stabilizing effect\textsuperscript{6}. Further, propranolol decreases both the height and the rate of rise of the action potential and has local anesthetic effects\textsuperscript{37}.

One of the significant effects of propranolol on ischemic myocardium is the preservation of glycogen granules. Ischemia is known to augment glycogenolysis in the myocardium\textsuperscript{38,39}. The transformation of phosphorylase \textquotedblleft b\textquotedblright to \textquotedblleft a\textquotedblright is an important role in this process and pronethanol, less active than propranolol as an adrenergic beta blocker\textsuperscript{40}, has been found to block this transformation\textsuperscript{41}.

Anoxic cardiac arrest by cross-clamping the ascending aorta has been widely used in open heart surgery. Although it produces a quiet and dry operative field, the entire myocardium is, nonetheless, exposed to anoxia. Protection of the myocardium has been provided through generalized or local hypothermia, coronary artery perfusion, instillation of various solutions\textsuperscript{9,42-44} into the aortic root and induced cardioplegia. Clinical application of propranolol for myocardial protection has been reported only by Reul \textit{et al.}\textsuperscript{45}. A single intravenous bolus of propranolol (6 \textmu g/kg) was given to 60 patients who underwent aortic valve replacement. Despite the high-risk profile, none of the patients died or sustained a postoperative myocardial infarction. Propranolol apparently protected the anoxic myocardium by decreasing the ventricular fibrillation time, thereby decreasing myocardial metabolism. Three of six patients in this study showed absolute cardiac standstill following aortic occlusion and three showed the finer and slower pattern of fibrillation, in which myocardial energy demand was less\textsuperscript{46}.

Therapeutic plasma levels of propranolol to abolish ventricular ectopic beats and to slow isoproterenol-induced tachycardia are 40 to 85 ng/ml and 10 to 20 ng/ml, respectively\textsuperscript{47,48}. Shand \textit{et al.}\textsuperscript{49} measured plasma levels in five Caucasian men after 10 mg of propranolol was infused intravenously at a rate of 1.03 mg/min. Samples were obtained at 2.5-minute intervals during infusion and peak plasma levels were 200 ng/ml. Therefore, the 20 \textmu g/kg (1 mg/50 kg) does used in this study was not too small but was within the therapeutic range. The reason for not giving a large dose was to avoid the high plasma levels which could conceivably be harmful to cardiac function on terminating cardiopulmonary bypass\textsuperscript{50}. No patient needed a beta adrenergic receptor stimulator such as epinephrine and isoproterenol during or after operation, indicating that no deleterious side effect was produced by the dose employed. However, the optimum dose of propranolol to afford the highest degree of myocardial protection remains undetermined.

Acknowledgment

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tude to Prof. Kenichi Asano of the Second Department of Surgery, Prof. Tadashige Murakami of the First Department of Surgery, and Prof. Hidenori Maezawa of the Third Department of Internal Medicine for their valuable advice and guidance.

References


Fig. 1. Nonischemic control posterior papillary muscle (PPM). The mitochondria (m) have tightly packed cristae and membranes are well preserved. Glycogen granules (gl) are abundant. (×11,500)

Fig. 2. Nonischemic control PPM. The nucleus (n) appears normal with evenly dispersed chromatin. (×11,500)

Fig. 3. Nonischemic control PPM. Intercalated disks (id) are intact. (×11,500)

Fig. 4. PPM occluded for 3 min. Nuclear chromatin clumping and margination are noted. Glycogen is absent. Many mitochondria show enlarged matrix spaces and disrupted membranes. Inset—Intercalated disks appear unclear. (×11,500)

Fig. 5. PPM occluded for 3 min. Intermyofibrillar edema (me) is present. Capillary endothelium (ce) is swollen and interstitial edema (ie) is noted. (×11,500)

Fig. 6. PPM occluded for 15 min. Most of the mitochondrial membranes are disrupted and matrices are swollen and indistinct. Interstitial edema (ie) is obvious. (×11,500)

Fig. 7. PPM occluded for 15 min. Capillary endothelium is swollen and endothelial nuclear chromatin clumping and margination are noted. (×11,500)

Fig. 8. PPM occluded for 30 min. There is severe intermyofibrillar and interstitial edema. (×11,500)

Fig. 9. PPM occluded for 30 min. Vacuoles (v) are considered to be mitochondrial remnants.

Capillary endothelium is markedly swollen. (×11,500)

Fig. 10. PPM pretreated with propranolol and occluded for 3 min. Nucleus appears intact with evenly dispersed chromatin and indentations. The mitochondrial membrane and cristae are normal. Glycogen granules are present. (×11,500)

Fig. 11. PPM pretreated with propranolol and occluded for 3 min. A capillary and interstitial space appear to be normal. (×11,500)

Fig. 12. PPM pretreated with propranolol and occluded for 15 min. There is no intermyofibrillar edema or interstitial edema. The mitochondria are well preserved. (×11,500)

Fig. 13. PPM pretreated with propranolol and occluded for 15 min. Capillary endothelium is thin. Sarcomeres are in register. (×11,500)

Fig. 14. PPM pretreated with propranolol and occluded for 30 min. The mitochondrial membrane and matrix are better preserved than those in Figs. 8 and 9. (×11,500)

Fig. 15. PPM pretreated with propranolol and occluded for 30 min. A few glycogen granules are seen. (×11,500)

Fig. 16. Ischemic left ventricular subendocardium. A contraction band (cb) is noted. (×11,500)

Fig. 17. Ischemic left ventricular subendocardium pretreated with propranolol. Mitochondria are well preserved and sarcomeres are in register. (×11,500)

Correction

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On page 11 Table 1, Patient No. 3 and No. 4 should appear as below:

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On page 12 Table 2, headline Ruptured and Not Ruptured are reversed. They should be corrected as below:

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<td>560 (72.4%)</td>
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