The Effects of Carbenoxolone on Human Myocardial Conduction
A Tool to Investigate the Role of Gap Junctional Uncoupling in Human Arrhythmogenesis

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OBJECTIVES
This study assessed the effects of carbenoxolone on human myocardial conduction and refractoriness.

BACKGROUND
Carbenoxolone, an antipeptic ulcer drug, has been shown to reduce gap junctional coupling without affecting cellular ion channels. Gap junctions (GJ) are considered to be determinants of cardiac action potential propagation. The effects of GJ uncoupling in the human heart are unknown.

METHODS
Right atrial (RA) and ventricular (RV) activation mapping (Carto, Biosense Webster Inc., Diamond Bar, California) was performed during sinus rhythm. Right atrial and RV wavefront propagation velocity (WPV), specifically in the direction of propagation, was determined from these maps using a triangulation method. Refractoriness at multiple RA and RV sites, sinus rhythm cycle length, and AH, PR, QRS, and QT intervals were measured. The protocol was repeated 1 h after oral administration of 100 mg of carbenoxolone.

RESULTS
In 11 patients, WPV was reduced from 79.6 ± 13.3 cm/s to 57.2 ± 9.1 cm/s (−27.1 ± 12.8%, p < 0.001) in RA and from 98.7 ± 19.8 cm/s to 76.5 ± 21.7 cm/s (−22.7 ± 14.1%, p < 0.01) in RV after carbenoxolone. Conduction slowing was more marked in 6 older patients with ischemic heart disease compared with younger subjects with normal hearts (RA −35.1 ± 5.5% vs. −17.5 ± 12.7%, p = 0.03; RV −33.8 ± 5.1% vs. −9.3 ± 7.7%, p < 0.001). Refractoriness and electrocardiogram parameters remained unchanged.

CONCLUSIONS
Carbenoxolone causes a 27% reduction in human RA WPV and 23% in the RV without affecting refractoriness. The slowing of myocardial conduction by carbenoxolone demonstrates the significance of GJ in regulating human myocardial conduction and provides a tool for investigating the effects of GJ uncoupling on human arrhythmogenesis. (J Am Coll Cardiol 2006;48:1242–9) © 2006 by the American College of Cardiology Foundation

Gap junctions are regions of membrane specialization between adjacent cells, forming clusters of intercellular communication channels. These are, in turn, formed from connexins, a highly conserved family of membrane proteins (1). Human cardiomyocytes express connexins 40, 43, and 45. Gap junctions are important determinants of action potential propagation in the myocardial syncytium.

Modification of connexin expression and modulation of function has been shown to alter conduction velocity in genetically modified mice and other animal models, but never in the intact heart of a conscious animal (2–7). Alterations in connexin expression and distribution have been documented in human cardiac disease in which there are disturbances of conduction and arrhythmogenesis, such as ischemia, hypertrophy, heart failure, and atrial fibrillation (8–12). We have previously demonstrated an association between connexin levels and human right atrial (RA) conduction velocity (13). The effects of modulating gap junctional coupling in human myocardium have not been determined.

Carbenoxolone is a drug used previously in the treatment of gastrointestinal ulceration and has been shown to reduce gap-junctional coupling in mammalian myocardium without affecting the ion channels responsible for cardiac action potential generation (14,15). We sought to address the hypothesis that carbenoxolone could alter human atrial and ventricular conduction without affecting refractoriness. Such evidence would establish the direct relationship between gap junctional coupling and human myocardial conduction in vivo.

METHODS

Patient selection. Studies were performed in patients undergoing clinically indicated electrophysiology studies (EPS). All antiarrhythmic drugs were stopped 5 half lives before the study. No patient had received amiodarone at any stage. All patients underwent coronary angiography and echocardiography at least 24 h before EPS. The study was approved by the local research and ethics committee.

EPS. The EPS was undertaken in the post-absorptive state with lidocaine used as local anesthesia. Two 7-F venous
sheaths were introduced into the right femoral vein, and a quadripolar catheter with 2-mm interelectrode spacing (Bard EP, Lowell, Massachusetts) was maneuvered to the high RA, distal coronary sinus (CS), and right ventricle (RV) to deliver programmed stimulation, and positioned in a stable position in the CS to provide reference electrograms during electroanatomical mapping.

Surface electrocardiogram (ECG) and bipolar endocardial electrograms were monitored continuously and stored on a digital amplifier/recorder system (Siemens EP, Munich, Germany) for offline analysis. Intracardiac electrograms were filtered from 30 to 500 Hz. The mean values of 10 consecutive measurements of PR, QRS, and QT intervals were taken from lead II with computer-assisted calipers

<table>
<thead>
<tr>
<th>Abbreviations and Acronyms</th>
<th>Description</th>
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<tr>
<td>AERP</td>
<td>absolute effective refractory period</td>
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<tr>
<td>CS</td>
<td>coronary sinus</td>
</tr>
<tr>
<td>EPS</td>
<td>electrophysiology studies</td>
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<td>IHD</td>
<td>ischemic heart disease</td>
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<tr>
<td>LAT</td>
<td>local activation time</td>
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<tr>
<td>RA</td>
<td>right atrial/atrial</td>
</tr>
<tr>
<td>RV</td>
<td>right ventricle/ventricular</td>
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<tr>
<td>WPV</td>
<td>wavefront propagation velocity</td>
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Calculation of wavefront propagation velocity (WPV). Our techniques of measuring the macroscopic rate at which a wavefront travels in the direction of propagation are based on the principles of triangulation and have been previously described (19,20). In brief, points in close proximity and activated by the same wavefront but separated by a LAT of more than 3 ms apart from each chamber were grouped into triads.

A triad of points would define a triangle in a single plane (Fig. 2). The difference in LATs and the distances between these points were used to measure the WPV.

Wavefront propagation velocity (Fig. 2):

\[
\psi = \frac{OA}{tOA \cos \alpha}
\]

The distance between any 2 points in 3-dimensional space could be calculated using the formula:

\[
OA = \sqrt{(x_a - x_o)^2 + (y_a - y_o)^2 + (z_a - z_o)^2},
\]

if O and A had the coordinates \((x_o, y_o, z_o)\) and \((x_a, y_a, z_a)\), respectively.

tOA was the difference in LAT between O and A, and alpha was calculated by the trigonometric principles. In this model, conduction within each triad was assumed to be uniform, and chamber curvature was not taken into account. These potential inaccuracies were minimized by selecting multiple triads of points in regions of uniform isochronal spacing with a mean interpoint distance of 7.4 ± 3.9 mm. Points within the earliest and latest isochronal of activation were not used to avoid the effects of multiple breakthrough and wavefront fusion.

Repeat testing after carbenoxolone. Carbenoxolone (Bioxorex, London, United Kingdom) has been used as a treatment for peptic and oral ulceration since the 1960s at a dose of 100 to 200 mg/day (21). Carbenoxolone is absorbed rapidly and unchanged from the stomach, reaching 90% of their peak (15 µg/ml) within 40 min of oral administration (22). It is highly bound to plasma protein, with a long half-life of about 19 h (23). In this study we aimed to test the effects of a single dose of 100 mg of carbenoxolone (1.42 mg/kg in a 70-kg patient) on cardiac electrophysiology.
Patients were given a 100-mg oral dose of carbenoxolone. After a 60-min waiting period, the RA and RV were re-mapped using the CARTO system as before, and programmed electrical stimulation to measure AERP was repeated.

The same protocol was performed in control patients except carbenoxolone was not administered. All patients had measurement of serum electrolytes at baseline, 1 and 4 h after ingestion of carbenoxolone.

Statistical analysis. Continuous data are presented as mean and SD, and variables were analyzed using paired or unpaired t tests. Data analysis was performed using Prism 4.0 statistical software (GraphPad Software, San Diego, California). Statistical significance was defined as a probability value of <0.05.

RESULTS

Patient population. Carbenoxolone was administered to 11 patients, age 60.8 ± 15.1 years. Patient characteristics are displayed in Table 1. Six patients (Patients #1 to #6) had a history of ischemic heart disease (IHD) with previous revascularization procedures (either angioplasty or coronary artery bypass grafting) and impaired left ventricular systolic function (mean ejection fraction 36.0 ± 11.6%). The other 5 patients were younger and had structurally normal hearts demonstrated by echocardiography (mean ejection fraction 51.8 ± 5.2%).
of 67.7 ± 10.8%) and absence of coronary artery disease as shown by angiography.

**Effects of carbenoxolone on WPVs.** Baseline WPV in the RA was 79.6 ± 13.3 cm/s, which decreased to 57.2 ± 9.1 cm/s (27.1 ± 12.8% reduction, p < 0.001) after administration of carbenoxolone. In the RV, mean WPV decreased from 98.7 ± 19.8 cm/s to 76.5 ± 21.7 m/s (22.7 ± 14.1% reduction, p < 0.01) in the RV after carbenoxolone (Fig. 3).

A greater reduction in WPV after carbenoxolone was seen in the 6 older patients with IHD compared with the 5 patients with structurally normal hearts (Fig. 4). In patients with IHD, mean RA WPV decreased by 35.1 ± 5.5%, from 85.8 ± 15.8 cm/s to 55.0 ± 6.8 cm/s (p < 0.001), whereas in patients without IHD, mean RA WPV fell by 17.5 ± 12.7%, from 72.3 ± 3.0 cm/s to 59.8 ± 11.5 cm/s (p = 0.04). This differential reduction in RA WPV was statistically significant (p = 0.03), although the 2 groups were not matched in age or gender. Similarly, in the RV of IHD subjects, WPV decreased by 33.8 ± 5.1%, from 97.4 ± 24.7 cm/s to 63.8 ± 12.8 cm/s (p < 0.01), whereas in patients without IHD, RV WPV declined by 9.3 ± 7.7%, from

Table 1. Clinical Characteristics of Patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age</th>
<th>Gender</th>
<th>Coronary Artery Disease</th>
<th>EF (%)</th>
<th>LVDD (mm)</th>
<th>LA (mm)</th>
<th>Indications for EPS</th>
<th>Results of EPS</th>
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<tbody>
<tr>
<td>1</td>
<td>71</td>
<td>M</td>
<td>Previous anterior MI, PCI to LAD</td>
<td>51</td>
<td>71.3</td>
<td>44.7</td>
<td>Non-sustained VT</td>
<td>VT induced</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>M</td>
<td>Previous inferior MI, CABG for 3-vessel disease</td>
<td>23</td>
<td>72.0</td>
<td>49.0</td>
<td>Sustained VT</td>
<td>VT induced</td>
</tr>
<tr>
<td>3</td>
<td>71</td>
<td>M</td>
<td>Previous anterior MI, PCI to first diagonal</td>
<td>45</td>
<td>62.2</td>
<td>42.2</td>
<td>Non-sustained VT</td>
<td>Non-inducible study</td>
</tr>
<tr>
<td>4</td>
<td>57</td>
<td>M</td>
<td>Previous anterior and inferior MI, PCI to RCA and LAD</td>
<td>30</td>
<td>67.0</td>
<td>41.3</td>
<td>Syncope</td>
<td>Non-inducible study</td>
</tr>
<tr>
<td>5</td>
<td>77</td>
<td>M</td>
<td>Previous inferior MI, CABG for 3-vessel disease</td>
<td>42</td>
<td>52.0</td>
<td>38.6</td>
<td>Syncope, non-sustained VT</td>
<td>Non-inducible study</td>
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<tr>
<td>6</td>
<td>67</td>
<td>M</td>
<td>Previous anterior MI, CABG for 3-vessel disease</td>
<td>25</td>
<td>68.5</td>
<td>46.3</td>
<td>Syncope, non-sustained VT</td>
<td>VT induced</td>
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<tr>
<td>7</td>
<td>43</td>
<td>F</td>
<td>None</td>
<td>79</td>
<td>48.3</td>
<td>34.5</td>
<td>Syncope, non-sustained VT</td>
<td>Non-inducible study</td>
</tr>
<tr>
<td>8</td>
<td>47</td>
<td>F</td>
<td>None</td>
<td>78</td>
<td>41.0</td>
<td>34.2</td>
<td>Syncope, non-sustained VT</td>
<td>Non-inducible study</td>
</tr>
<tr>
<td>9</td>
<td>55</td>
<td>M</td>
<td>None</td>
<td>66</td>
<td>48.4</td>
<td>36.0</td>
<td>Syncope, non-sustained VT</td>
<td>Non-inducible study</td>
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<tr>
<td>10</td>
<td>83</td>
<td>F</td>
<td>None</td>
<td>60</td>
<td>44.6</td>
<td>40.1</td>
<td>Palpitations, non-sustained VT</td>
<td>Non-inducible study</td>
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<tr>
<td>11</td>
<td>34</td>
<td>F</td>
<td>None</td>
<td>55</td>
<td>41.1</td>
<td>35.3</td>
<td>Aborted SCD</td>
<td>Non-inducible study</td>
</tr>
</tbody>
</table>

CABG = coronary artery bypass graft; EF = left ventricular ejection fraction; EPS = electrophysiological studies; LA = left atrial dimension; LAD = left anterior descending artery; LVDD = left ventricular end-diastolic dimension; MI = myocardial infarction; PCI = percutaneous coronary intervention; RCA = right coronary artery; SCD = sudden cardiac death; VT = ventricular tachycardia.

**Figure 2.** Schematic representation of a triad of 3 endocardial points, marked as O, A, and B. \( v \) represents wavefront propagation velocity.
100.4 ± 14.5 cm/s to 91.8 ± 20.8 cm/s (p < 0.05). This differential reduction in RV WPV was also statistically significant (p < 0.001). Sinus rhythm cycle lengths (832 ± 156 ms at baseline) did not change with carbenoxolone (823 ± 150 ms, p = NS).

In 3 control patients with IHD (mean age of 57 years), RA and RV WPV measured from activation maps taken 60 min apart remained unchanged (RA WPV 77.1 ± 11.0 cm/s, RV WPV 92.4 ± 5.2 cm/s at baseline; RA WPV 74.3 ± 8.5, RV WPV 93.8 ± 7.3 cm/s after 60 min of waiting time, p = NS).

**Effects of carbenoxolone on refractoriness.** There were no significant differences between AERP measurements at high RA, distal CS, RV apex, and RV outflow tract before and after carbenoxolone at both cycle lengths (Fig. 5).

**Effects of carbenoxolone on ECG parameters and nodal conduction.** The PR, AH, and QRS intervals were not affected by the carbenoxolone (pre- vs. post-carbenoxolone: PR interval 183 ± 27 vs. 178 ± 28 ms, AH interval 112 ± 23 vs. 96 ± 41 ms, QRS duration 103 ± 29 vs. 105 ± 31 ms, QT interval 384 ± 26 vs. 389 ± 31 ms; all p = NS).

**Effects of carbenoxolone on serum electrolytes.** Administration of carbenoxolone did not significantly change serum electrolytes. Baseline serum sodium, potassium, and creatinine levels were 137.6 ± 1.3 mEq/l, 4.0 ± 0.3 mEq/l, and 1.15 ± 0.21 mg/dl, respectively. After 4 h, serum sodium, potassium, and creatinine levels were 138.5 ± 1.8 mEq/l, 4.0 ± 0.3 mEq/l, and 1.15 ± 0.21 mg/dl, respectively (all p = NS).

**DISCUSSION**

In the intact human heart, carbenoxolone profoundly slows both atrial and ventricular WPVs, an effect most likely due to gap junctional uncoupling. The degree of slowing was more marked in patients with IHD. Sinus node automaticity, atrioventricular nodal function, refractoriness, and ventricular repolarization appeared unchanged.

Although transgenic murine models of selective connexin deletion provide a body of evidence that gap junctions are important determinants of cardiac conduction, and we have previously demonstrated a relationship between the relative expression of naturally occurring connexins and human atrial conduction velocities, the current study provides evidence that modulation of gap junctional function can modify human cardiac conduction velocity (2,3,13).

**Effects of carbenoxolone in animal models.** Derivatives of glycyrrhetinic acid, a ubiquitous compound in licorice root, were shown to inhibit gap junctional communication between human fibroblasts (24). One such derivative, carbenoxolone, reversibly blocked intercellular dye transfer between Cx43-transfected glioma cells in conjunction with a reduction, but not complete loss of junctional conductance as measured by dual cell voltage clamps (25). In this study, rapid onset and offset of changes in gap junctional conductance occurred without any changes to connexin 43 expression, phosphorylation, or localization.

When applied to isolated rabbit atrial and ventricular myocytes, carbenoxolone did not affect action potential characteristics or ionic currents, namely I_K, I_K1, and I_to1, L-type calcium and sodium currents (14). In a Langerdoff-perfused model of intact rabbit hearts, RA and left ventricular conduction were significantly slowed after perfusion with carbenoxolone, which completely recovered with wash-
out. We have shown that carbenoxolone slows atrial and ventricular conduction in guinea pigs by increasing intercellular resistivity without affecting action potential parameters, an effect that suggests that carbenoxolone specifically uncouples cardiac gap junctions without affecting ion channels responsible for the action potential profile (15).

Although a few experiments on neural tissue have suggested that the effects of carbenoxolone may not solely be due to acute gap junctional closure, but rather through non-specific actions on a variety of ion channels, no changes to action potential profile or major ionic channel currents occur in cardiac tissue (14,15,26–29). Furthermore, the profound differences between neural and cardiac electrophysiology including the complexity of neural activity with spontaneous oscillations occurring simultaneously at varying frequencies and dependence on synaptic chemotransmission for neural action potential propagation limits the extent to which observations in neural tissue are relevant to cardiac electrophysiology.

**Carbenoxolone in human myocardium.** Our current study sought to determine whether carbenoxolone would slow conduction without affecting action potential duration, manifested as the refractory period in the human heart. The absence of an effect of carbenoxolone on ion channel function and action potential properties in cell culture and intact tissue studies, coupled with an absence of change in refractory periods in the intact human heart, provides evidence that carbenoxolone at these doses does not affect the cardiac action potential generation (14,15). In accordance with this, the findings of unchanged maximum velocity upstroke of the action potential in animal models and the universal finding of slowing of conduction in rabbit, guinea pig, and human myocardium strongly implicates gap junctional uncoupling (14,15). The reversibility of these findings coupled with the absence of quantitative changes in connexin protein is supportive of functional changes in gap junctional activity rather than changes in protein expression or transcription (14,25). The absence of any changes in the sinus rhythm cycle length after administration of carbenoxolone in the current study would suggest that carbenoxolone has no effect on sinus node automaticity and that the observed differences in atrial and ventricular conduction were not confounded by a change in sinus rhythm cycle length.

**Preferential effects in patients with IHD.** Ischemia leads to the development of various concurrent biophysical changes such as acidosis and calcium overload, which could individually result in gap junctional uncoupling (30). While these changes may cumulatively confer a physiologically beneficial effect of limiting infarct size by decreasing the intercellular transmission of chemical mediators of injury, it exacerbates electrical uncoupling (31). Computer models suggest that while a decrease in gap junctional uncoupling only modestly affects conduction in regions of high conduction, a similar reduction in uncoupling in regions of initially lower gap junctional conductance would lead to significant conduction slowing and possibly even electrical isolation (32–34). Hence, in the presence of a gap junctional blocker, a greater degree of conduction slowing might be expected in already poorly uncoupled tissue such as previously ischemic myocardium, and the greater reduction of WPV in patients with IHD is consistent with this concept. The validity of our observations of a differential effect of carbenoxolone in IHD patients is confounded by the fact that the IHD and non-IHD groups were not age-matched.

The disparity between significant myocardial slowing but the lack of conduction delay in the specialized conducting tissue needs further clarification. While atrioventricular delay was seen in murine and rabbit models after administration of carbenoxolone, we did not find any such changes in both measures of atrioventricular nodal conduction, namely PR and AH intervals. This discrepancy is most likely dose-related although the influence of interspecies differences in the relative levels of connexins 40, 43, and 45 coexpression cannot be excluded (35). Similarly, slowing of ventricular electrical propagation without altering QRS durations in this cohort could be due to a lack of effect on the specialized conducting His-Purkinje system. Both strains of mice with connexin 40 or 43 deletion demonstrate significant QRS prolongation compared with littermate control mice, despite the absence of connexin 40 expression in ventricular myocardium. Therefore, the observed QRS widening is most likely resultant of slowing...
conduction within the His-Purkinje system, rather than ventricular myocardium (4–7).

**Modulation of gap junctional function as a novel anti-arrhythmic strategy?** Conventional antiarrhythmic drugs act by modifying the ion channels responsible for myocardial action potential generation, but by this action on diseased myocardium, all existing antiarrhythmic drugs are prone to cause ventricular arrhythmias. This renders them largely unsuitable for the long-term treatment of arrhythmias in the diseased heart and provides significant impetus for developing fundamentally different approaches to the pharmacologic suppression of arrhythmias. Commonly used antiarrhythmic drugs have little or no effect on gap junctional conductance (36). Because many disease states that predispose to arrhythmias are known to substantially affect gap junction expression and function, drugs that selectively target gap junctions and preferentially affect diseased myocardium offer the potential for a novel antiarrhythmic strategy. Although enhancement of gap junctional function is likely to be the preferred therapeutic strategy, carbenoxolone provides a useful tool that has not only demonstrated the direct relationship between gap junctional function and conduction and how this may be modified by myocardial disease, but will also help explore the effects of gap junctional modulation on clinical arrhythmogenesis.

**Study limitations.** The IHD group was not age-matched to the non-IHD group. Therefore, it is possible that the greater degree of conduction slowing seen in patients with IHD may be an age-dependent effect. Nevertheless, conduction slowing was demonstrated in all patients, regardless of a history of IHD (Fig. 3). The time course, dose-response relationship, and degree of gap junctional uncoupling in both myocardium and specialized conducting tissues could not be assessed due to the constraints of human clinical research.

**Conclusions.** Carbenoxolone slows atrial and ventricular WPVs without affecting refactoriness or ventricular repolarization. The degree of conduction slowing is more profound in an older group of patients with IHD. Slowing of conduction by carbenoxolone provides direct evidence for the role of gap junctions in regulating human conduction.

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**REFERENCES**