

The Cardiac Gap Junction: A Potential Therapeutic Target in the Treatment of Heart Disease

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Abstract

Cardiac gap junctions have been implicated in maintaining cardiac conduction and function. In cardiac disease, expression of connexin43, the most abundant ventricular gap junction protein, is markedly abnormal, a process termed “gap junction remodeling.” To date, however, the gap junction has not been directly targeted therapeutically in cardiac disease states. Therefore, we have developed novel and complementary experimental models to investigate whether loss of connexin43 expression in the heart can be directly linked to the arrhythmic and functional complications of heart disease. In this article, we discuss how data from connexin43 conditional and chimeric knock-out mice support the hypothesis that gap junction remodeling is a key molecular feature underlying the high incidence of sudden arrhythmic death and exacerbating the ventricular dysfunction associated with acquired heart disease.

Key Words: Gap junction, connexin43, arrhythmia, heart.

A DETAILED UNDERSTANDING of biological function is vital to the process of developing therapeutic breakthroughs. In cardiovascular research, studies related to arrhythmia and heart failure may lead to therapies that will reduce the morbidity and mortality associated with these complications of heart disease. First, however, targets for these therapies must be identified. One such potential target is the cardiac gap junction. Gap junctions have been implicated in maintaining cardiac conduction and function (1–4). However, they have not yet been targeted in the treatment of cardiovascular disease. Therefore, using animal models, we have attempted to establish whether the gap junction could be an important therapeutic target in the diseased heart.

Gap junctions are arrays of intercellular channels that allow for the diffusion of ions and signaling molecules between cells. In the heart, gap junctions also form a low-resistance pathway for cell-to-cell conduction of electrical impulses. Gap junctions are made up of a family of proteins called connexins, of which more than a dozen have been cloned in mammals. While connexins are present in most tissues, the distribution of individual isotypes tends to be more restricted, with substantial overlap of different connexins in many tissues (5, 6). Certain tissues have one predominant connexin subtype, and single-gene mutations in these subtypes tend to have profound phenotypic sequelae. For example, in Schwann cells, which are responsible for forming the myelin sheath around nerves, connexin32 predominates. Mutations in connexin32 cause a demyelinating neuropathy in humans known as X-linked Charcot-Marie-Tooth disease (7). Similarly, connexin43 is the predominant subtype in the cardiac ventricles, and mutations of connexin43 have been implicated in cardiac developmental abnormalities (8).

In the adult heart, abnormalities of connexin43 expression have been described in a variety of cardiac disease states (9–11). Abnormal connexin43 expression in the diseased

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heart, referred to as “gap junction remodeling,” consists of a loss of connexin43 expression from its usual location at the intercalated discs and a global down-regulation in the cardiomyocyte (12). Gap junction remodeling has been described in conditions such as myocardial infarction, hypertrophy, ischemia and hibernating myocardium, which are also associated with arrhythmias and ventricular dysfunction (9–11). In ischemic cardiomyopathies, gap junction remodeling is patchy, affecting areas of hibernating myocardium most profoundly (9). In the peri-infarct zone of experimental myocardial infarction in dogs, both altered electrophysiological properties and connexin43 dysregulation have been described (13, 14). The peri-infarct region, in addition, serves as an important focus for the generation of malignant ventricular arrhythmias, and is also the area involved in infarct expansion (15–17).

As a result of these data regarding gap junction remodeling, we hypothesized that dysregulation of connexin43 expression may be a key molecular feature of arrhythmogenicity and may contribute to ventricular dysfunction. In order to test this hypothesis, we had to develop novel models for the loss of connexin43. We were unable to use germline knock-out mice to study physiologic alterations in the adult heart, since these mice develop right ventricular outflow tract obstruction *in utero* and die perinatally from presumed pulmonary oligemia (18). The connexin43 germline knock-out phenotype was theorized to result from loss of connexin43 in the neural crest cell lineage rather than in the cardiomyocytes themselves (19–21). Thus, we elected to knock out connexin43 specifically in cardiomyocytes, using the Cre-loxP conditional knock-out system (22). For this system, loxP sites, 34 base pair palindromic sequences, are cloned into sites surrounding a gene of interest, which in our case was *connexin43*. The gene of interest is deleted upon exposure to Cre recombinase, an enzyme that catalyzes the recombination of the loxP sites. Cre recombinase can be introduced via a transgene, and its expression is driven in specific tissues by choosing a promoter for the Cre transgene that is active only in specific tissues. In order to generate the connexin43 heart-specific, conditional knock-out mice, we first created a line of mice in which we surrounded the *connexin43* gene with loxP sites (23). Next, we bred these mice with transgenic lines expressing Cre under heart-specific promoters (either alpha-myosin heavy-chain or myosin light-chain 2v in order to create two separate lines of conditional knock-out mice; see Fig. 1) (24, 25).

The connexin43 conditional knock-out mice had significantly lower levels of connexin43 in their hearts than did littermate controls, but did not differ from controls in gross morphology, histology, and echocardiographic dimensions and function. However, all the conditional knock-out mice died suddenly within the first few months of life, which contrasts with the normal murine lifespan of more than two years. To evaluate the cause of sudden death, we implanted miniaturized telemetry transmitters in a subset of conditional knock-out mice and monitored their heart rhythms continuously. All four monitored mice had ventricular tachycardia that degenerated into ventricular fibrillation at the time of death. In separate experiments, conduction velocity was found to be significantly decreased in isolated hearts from connexin43 conditional knock-out mice perfused with a voltage-sensitive dye (23). Thus, we found that heart-specific deletion of *connexin43* led to conduction slowing and sudden arrhythmic death.

Numerous studies (26) have predicted that cellular uncoupling may lead to the initiation of arrhythmias and their propagation. Cellular uncoupling may promote arrhythmogenesis by unmasking ectopic foci or by enhancing the generation of early afterdepolarizations (27). Cellular uncoupling may also lead to increased dispersion of action potential duration, which has been shown to promote reentry and arrhythmia propagation (28, 29). In addition, slow conduction as a result of cellular uncoupling can also provide the substrate for reentry (26). Potential mechanisms of arrhythmia generation and propagation are outlined in Fig. 2.

Interestingly, ventricular function was normal echocardiographically in the connexin43 conditional knock-out mice. However, since gap junction remodeling may be patchy in diseased myocardium (9), we wanted to model a more heterogeneous loss of connexin43. Therefore, we generated chimeric mice from connexin43 knock-out embryonic stem cells, using wildtype recipient blastocysts (see Fig. 3). The chimeric connexin43 knock-out hearts appeared grossly and histologically normal, but immunofluorescent staining showed a patchy loss of connexin43 staining throughout the myocardium. Conduction patterns in isolated chimeric connexin43 knock-out hearts perfused with a voltage-sensitive dye showed distinct areas of conduction delay resulting in scalloped and irregular epicardial conduction wavefronts. These conduction patterns differed markedly from the smooth wildtype con-

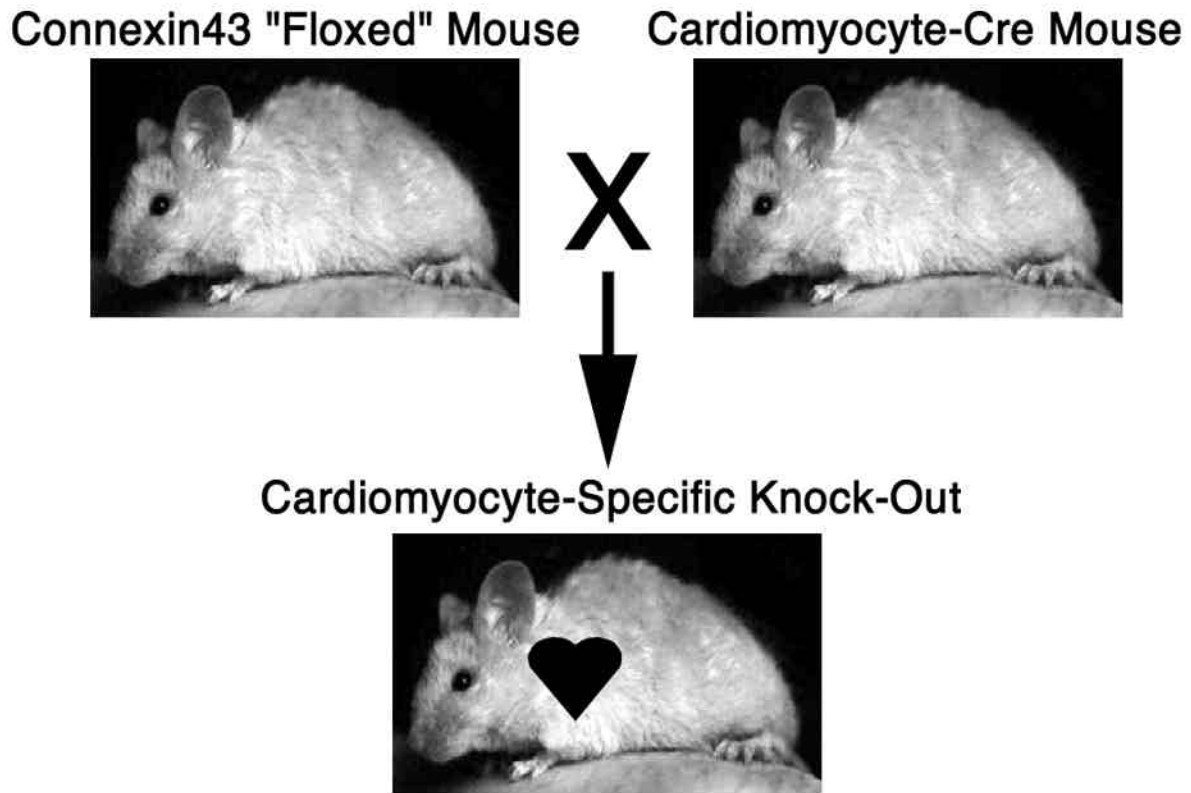


Fig. 1. Schematic illustration of breeding strategy for the generation of cardiomyocyte-specific connexin43 conditional knock-out mice. Genetically altered mice with loxP sites flanking their connexin43 loci (connexin43 “floxed”) were bred with transgenic mice expressing Cre recombinase under the control of heart-specific promoters in order to produce connexin43 conditional knock-out mice.

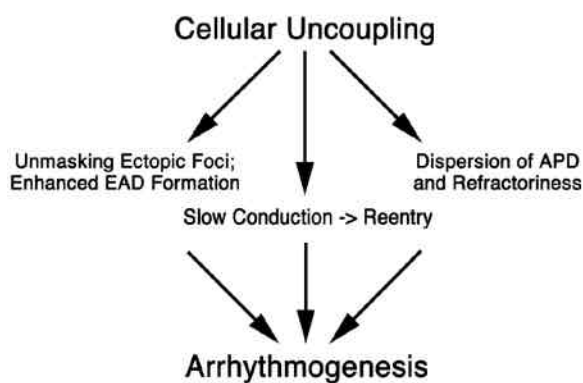


Fig. 2. Potential mechanisms of arrhythmia generation and propagation in the connexin43 conditional knock-out mouse. EAD = early afterdepolarization; APD = action potential duration.

Chimeric Knock-Out Model

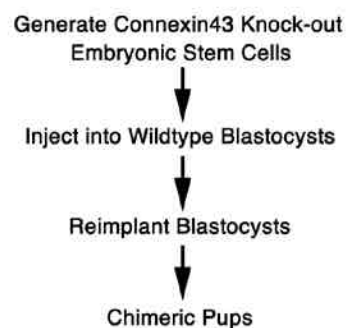


Fig. 3. Strategy for the generation of chimeric connexin43 knock-out mice. Mouse embryonic stem cells with the connexin43 gene loci deleted on both alleles were injected into wildtype blastocysts and implanted into pseudopregnant females. The resulting chimeric embryos developed into chimeric mice that were used for the experiments described in the text.

trol patterns. In addition, ventricular systolic function in the chimeric mice was significantly depressed in comparison to controls (30). These findings suggest that loss of the normal pattern of electrical activation in the heart may result in disordered contraction and mechanical dysfunction.

The conditional and chimeric connexin43 knock-out models have provided important clues regarding the potential role of gap junction remodeling in the diseased heart. For example, heart-specific deletion of connexin43 does not

perturb cardiac development, morphology or function. Loss of connexin43 in the heart, however, does lead to conduction delay and sudden arrhythmic death. On the other hand, patchy loss of connexin43 in the chimeric mouse is associated with discrete conduction defects and ventricular systolic dysfunction. Thus, our studies support the hypothesis that gap junction remodeling is a key molecular feature underlying the high incidence of sudden arrhythmic death, and exacerbating the ventricular dysfunction associated with acquired heart disease. Gap junctions may represent a worthwhile therapeutic target in patients at risk for lethal ventricular arrhythmias.

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References

- Saffitz JE, Yamada KA. Do alterations in intercellular coupling play a role in cardiac contractile dysfunction? *Circulation* 1998; 97:630–632.
- Kirchhoff S, Nelles E, Hagedorff A, et al. Reduced cardiac conduction velocity and predisposition to arrhythmias in connexin40-deficient mice. *Curr Biol* 1998; 8:299–302.
- Simon AM, Goodenough DA, Paul DL. Mice lacking connexin40 have cardiac conduction abnormalities characteristic of atrioventricular block and bundle branch block. *Curr Biol* 1998; 8:295–298.
- Hall DG, Morley GE, Vaidya D, et al. Early onset heart failure in transgenic mice with dilated cardiomyopathy. *Pediatr Res* 2000; 48:36–42.
- Goodenough DA, Goliger JA, Paul DL. Connexins, connexons, and intercellular communication. *Annu Rev Biochem* 1996; 65:475–502.
- Page E. Cardiac gap junctions. In: Fozzard HA, Haber E, Jennings RB, et al., editors. *The heart and cardiovascular system*. New York: Raven Press; 1992. pp. 1003–1048.
- Bergoffen J, Scherer SS, Wang S, et al. Connexin mutations in X-linked Charcot-Marie-Tooth disease. *Science* 1993; 262:2039–2042.
- Britz-Cunningham SH, Shah MM, Zuppan CW, Fletcher WH. Mutations of the connexin43 gap-junction gene in patients with heart malformations and defects of laterality. *N Engl J Med* 1995; 332:1323–1329.
- Kaprielian RR, Gunning M, Dupont E, et al. Downregulation of immunodetectable connexin43 and decreased gap junction size in the pathogenesis of chronic hibernation in the human left ventricle. *Circulation* 1998; 97:651–660.
- Peters NS, Green CR, Poole-Wilson PA, Severs NJ. Reduced content of connexin43 gap junctions in ventricular myocardium from hypertrophied and ischemic human hearts. *Circulation* 1993; 88:864–875.
- Smith JH, Green CR, Peters NS, et al. Altered patterns of gap junction distribution in ischemic heart disease: an immunohistochemical study of human myocardium using laser scanning confocal microscopy. *Am J Pathol* 1991; 139:801–821.
- Jongsma HJ, Wilders R. Gap junctions in cardiovascular disease. *Circ Res* 2000; 86:1193–1197.
- Luke RA, Saffitz JE. Remodeling of ventricular conduction pathways in healed canine infarct border zones. *J Clin Invest* 1991; 87:1594–1602.
- Peters NS, Coromilas J, Severs NJ, Wit AL. Disturbed connexin43 gap junction distribution correlates with the location of reentrant circuits in the epicardial border zone of healing canine infarcts that cause ventricular tachycardia. *Circulation* 1997; 95:988–996.
- Costeas C, Peters NS, Waldecker B, et al. Mechanisms causing sustained ventricular tachycardia with multiple QRS morphologies: results of mapping studies in the infarcted canine heart. *Circulation* 1997; 96:3721–3731.
- Peters NS, Wit AL. Myocardial architecture and ventricular arrhythmogenesis. *Circulation* 1998; 97:1746–1754.
- McKay RG, Pfeffer MA, Pasternak RC, et al. Left ventricular remodeling after myocardial infarction: a corollary to infarct expansion. *Circulation* 1986; 74:693–702.
- Reaume AG, de Sousa PA, Kulkarni S, et al. Cardiac malformation in neonatal mice lacking connexin43. *Science* 1995; 267:1831–1834.
- Sullivan R, Huang GY, Meyer RA, et al. Heart malformations in transgenic mice exhibiting dominant negative inhibition of gap junctional communication in neural crest cells. *Dev Biol* 1998; 204:224–234.
- Huang GY, Wessels A, Smith BR, et al. Alteration in connexin 43 gap junction gene dosage impairs conotruncal heart development. *Dev Biol* 1998; 198:32–44.
- Ewart JL, Cohen MF, Meyer RA, et al. Heart and neural tube defects in transgenic mice overexpressing the Cx43 gap junction gene. *Development* 1997; 124:1281–1292.
- Baubonis W, Sauer B. Genomic targeting with purified Cre recombinase. *Nucleic Acids Res* 1993; 21:2025–2029.
- Gutstein DE, Morley GE, Tamaddon H, et al. Conduction slowing and sudden arrhythmic death in mice with cardiac-restricted inactivation of connexin43. *Circ Res* 2001; 88:333–339.
- Agah R, Frenkel PA, French BA, et al. Gene recombination in postmitotic cells: targeted expression of Cre recombinase provokes cardiac-restricted, site-specific rearrangement in adult ventricular muscle in vivo. *J Clin Invest* 1997; 100:169–179.
- Chen J, Kubalak SW, Chien KR. Ventricular muscle-restricted targeting of the RXRa gene reveals a non-cell-autonomous requirement in cardiac chamber morphogenesis. *Development* 1998; 125:1943–1949.
- Jalife J, Delmar M, Davidenko JM, Amunowo JMB. *Basic cardiac electrophysiology for the clinician*. Armonk (NY): Futura Publishing Company; 1999.
- Saiz J, Ferrero MJ, Monserrat M, et al. Influence of electrical coupling on early afterdepolarizations in ventricular myocytes. *IEEE Trans Biomed Eng* 1999; 46:138–147.
- Viswanathan PC, Shaw RM, Rudy Y. Effects of IKr and IKs heterogeneity on action potential duration and its rate dependence: a simulation study. *Circulation* 1999; 99:2466–2474.
- Viswanathan PC, Rudy Y. Cellular arrhythmogenic effects of congenital and acquired long-QT syndrome in the heterogeneous myocardium. *Circulation* 2000; 101:1192–1198.
- Gutstein DE, Morley GE, Vaidya D, et al. Heterogeneous expression of gap junction channels in the heart leads to conduction defects and ventricular dysfunction. *Circulation* 2001; 104:1194–1199.