Multiple Roles of Connexins in Atherosclerosis- and Restenosis-Induced Vascular Remodelling

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Key Words

Atherosclerosis · Restenosis · Vascular remodelling · Connexins

Abstract

Endothelial dysfunction is the initial step in atherosclerotic plaque development in large- and medium-sized arteries. This progressive disease, which starts during childhood, is characterized by the accumulation of lipids, macrophages, neutrophils, T lymphocytes and smooth muscle cells in the intima of the vessels. Erosion and rupture of the atherosclerotic plaque may induce myocardial infarction and cerebrovascular accidents, which are responsible for a large percentage of sudden deaths. The most common treatment for atherosclerosis is angioplasty and stent implantation, but these surgical interventions favour a vascular reaction called restenosis and the associated de-endothelialization increases the risk of thrombosis. This review provides an overview of the role of connexins, a large family of transmembrane proteins, in vascular remodelling associated with atherosclerosis and restenosis. The connexins expressed in the vascular wall are Cx37, Cx40, Cx43 and Cx45; their expressions vary with vascular territory and species. Connexins form hemichannels or gap junction channels, allowing the exchange of ions and

small metabolites between the cytosol and extracellular space or between neighbouring cells, respectively. Connexins have important roles in vascular physiology; they support radial and longitudinal cell-to-cell communication in the vascular wall, and significant changes in their expression patterns have been described during atherosclerosis and restenosis.

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Introduction

Atherosclerosis is a progressive inflammatory disease of large- and medium-sized arteries whose fatal complications comprise myocardial infarction or stroke [1, 2]. The complications of this pathology are observed in the adult population, but the disease starts already during adolescence and implicates multiple cell types such as endothelial cells (ECs), inflammatory cells and smooth muscle cells (SMCs). The roles of these cells in the formation and progression of atherosclerotic plaque are the subject of many excellent reviews [3–7]. Atherosclerotic disease is usually treated by angioplasty and stenting, but clinical studies have shown that the treatment efficacy may be reduced by early and late thrombosis or restenosis at the site

Table 1. Cx nomenclature

Cx nomenclature based on the gene sequence	Cx nomenclature based on the predicted molecular mass
GJA1	Cx43
GJA3	Cx46
GJA4	Cx37
GJA5	Cx40
GJA6	Cx33 (only in mice)
GJA8	Cx50
GJA9	Cx59 (only in humans)
GJA10	Cx57 (mice)/Cx62 (humans)
GJB1	Cx32
GJB2	Cx26
GJB3	Cx31
GJB4	CX30.3
GJB5	Cx31.1
GJB6	Cx30
GJB7	Cx25 (only in humans)
GJC1	Cx45
GJC2	Cx47
GJC3	Cx29 (mice)/Cx30.2 (humans)
GJD2	Cx36
GJD3	Cx30.2 (mice)/Cx31.9 (humans)
GJD4	Cx39 (mice)/Cx40.1 (humans)
GJE1	Cx23

Cx genes are named according to their sequence: α (GJA), β (GJB), γ (GJC), δ (GJD) and ϵ (GJE), and they receive a number in the order of their discovery. Cx proteins are named according to their predicted molecular mass.

of the intervention [8]. Many investigations are currently focussing on the role of interactions and information exchange between cells during the processes of atherosclerosis, thrombosis and restenosis. In this review, I will focus on the role of intercellular communication provided by connexin (Cx) proteins during vascular remodelling associated with atherogenesis and restenosis.

Cx Channels

Cx are members of a family of 20 proteins in mice and 21 proteins in humans. Cx genes consist of a 5'-untranslated exon, an intron of variable length, an exon harbouring the complete coding region and a 3'-untranslated exon [9]. Cx genes are named according to their sequence: α (GJA), β (GJB), γ (GJC), δ (GJD) and ϵ (GJE), and they receive a number in the order of their discovery [10, 11] (table 1). Cx proteins are synthesized in the endoplasmic reticulum, where they form hexameric connexons (an asso-

ciation of 6 Cx). This process is completed in the Golgi apparatus, after which connexons traffic to the plasma membrane along microtubules [12–14]. The turnover of Cx proteins is relatively fast; their half-lives range from 1 to 5 h [15]. Cx proteins exhibit 4 α -helical transmembrane domains (M1 to M4), 2 extracellular loops (EL1 and EL2) linked by 2 disulphide bonds, a short cytoplasmic loop (CL) and cytoplasmic NH₂ and COOH termini (NT and CT, respectively) (fig. 1a). The most variable part of Cx proteins is the CT, which allows interactions with other proteins such as kinases or structural proteins. In addition, the CT is involved in the modulation of channel activity in response to appropriate biochemical stimuli [16–19]. The highly conserved EL1 and EL2 are involved in the docking and recognition of compatible Cx [12]. Cx proteins are named according to their molecular mass deduced from their cDNA sequences (table 1). As mentioned above, 6 Cx oligomerize to form connexons (fig. 1a). This hemichannel can be composed of 6 identical Cx (homomeric connexon) or multiple Cx types (heteromeric connexon). Non-covalent interactions between the extracellular loops of 2 connexons from 2 neighbouring cells allow the formation of gap junction (GJ) channels (fig. 1a). These channels are composed of identical or different connexons and are thus described as homotypic or heterotypic channels, respectively. Hemichannels and GJ channels allow the passage of ions and small molecules (~1,000 Da) between the cytoplasm and the extracellular space or between the cytosol of 2 neighbouring cells, respectively. Cellular effects of GJ channels, hemichannels and Cx (channel-independent effects) are described in several organs such as the heart, brain, kidney, liver, lens, ear, ovary, bone or testis (fig. 1b).

Exchanges via GJ channels involve ions, small metabolites, second messengers, small linear peptides or small silencing RNA [15, 20–22]. Usually, GJ channels are open to facilitate communication between the cells. Their closure is regulated by modification of the intra-cellular Ca²⁺ concentration, pH and transmembrane voltage and by phosphorylation/dephosphorylation of the CT. GJ intercellular communication is crucial for fast coordinated activities such as contraction of the heart or the transmission of neuronal signals at electrical synapses, but it is also involved in slower physiological processes such as cell growth, differentiation, development and proliferation. In addition, the contribution of GJ channels to cell death has been linked to the exchange of Ca²⁺, inositol 1.4.5-trisphosphate (IP3), cAMP and cGMP through these channels, whereas their contribution to cell survival has been associated with the exchange of ATP, glucose, ascorbic acid or glutathione [23].

Fig. 1. Schematic representation of Cx channels. **a** Cx have 4 transmembrane domains (M1, M2, M3 and M4), 2 extracellular loops (EL1 and EL2), a cytoplasmic loop (CL) and cytoplasmic NH₂- and COOH-termini (NT and CT, respectively). Connexons or hemichannels are formed by the association of 6 Cx, and the docking of 2 connexons from neighbouring cells forms GJ channels. **b** Cx proteins participate in the regulation of important cellular processes via channel-dependent and channel-independent effects.

In contrast to GJ channels, connexons are in a closed configuration under physiological conditions. Their opening can be induced by different stimuli such as the removal of extracellular calcium, dephosphorylation, hypoxic or ischemic stress and mechanical stimulation [24–26]. Hemichannels are implicated in the maintenance of cell homeostasis, paracrine or autocrine signaling and the activation of survival pathways. Compounds that can be released by hemichannels include ions and small molecules such as ATP, glutamate, prostaglandin E2 or nicotinamide adenine dinucleotide (NAD⁺) [27,

28]. ATP released by hemichannels can bind to purinergic receptors and participates in several cellular processes depending on the implicated Cx and the organ. For example: (1) ATP release by Cx37 hemichannels limits monocyte adhesion and thereby prevents atherosclerotic plaque development [29], (2) the Cx26 and Cx30 hemichannels are described to play a crucial role in inner ear calcium signalling by promoting ATP release [30] and (3) in ECs ATP release by Cx43 hemichannels has been proposed to play a role in the initiation of early innate immune responses following activation by peptidoglycan

derived from *Staphylococcus epidermidis* [31]. Glutamate has been described to be secreted by Cx43 hemichannels in astrocytes after the removal of divalent cations [32] and by Cx26 hemichannels in horizontal cells from the retina [33]. Glutamate release in pathological situations leads to overstimulation of post-synaptic glutamate receptors and neuronal death [34]. Prostaglandin E2 release by Cx43 hemichannels has been shown in an osteocyte-like cell line in response to mechanical strain [35]. Paracrine processes implicating Cx43 hemichannels and NAD⁺ have been demonstrated in smooth myocytes, 3T3 murine fibroblasts, hippocampal neurons, and human haemopoietic stem cells [36]. More recently, in mesenchymal stem cells, Cx43 hemichannels were described to be implicated in the secretion of angiogenic factors, e.g. vascular endothelial growth factor and basic fibroblast growth factor, under hypoxic stress [37]. Concerning cell death, hemichannel opening under ischemic stress results in ATP release and thus in necrosis [23]. This opening is induced in part by the dephosphorylation of Cx43 hemichannels. In the context of cardioprotection, it has been shown that pre-conditioning favours the phosphorylation of Cx43 by protein kinase C, which prevents hemichannel opening [38]. Otherwise, Cx43 channels expressed in the mitochondria have been proposed to participate in cell survival by regulating the mitochondrial K_{ATP} channel activity [39] and cytochrome C release [40].

The channel-independent properties of Cx are receiving more and more attention in the context of cell growth, migration, development, signalling and cell death. Evidence of the channel-independent effects of Cx has been highlighted in many studies about tumourigenesis. In this context, it has been shown that usually Cx43, Cx32 and Cx31.1 inhibit cell proliferation, Cx43 and Cx26 inhibit cell growth, Cx43, Cx32 and Cx31.1 inhibit cell migration and Cx43 and Cx32 promote apoptosis [for a review, see 41]. Cx channel-independent effects have also been demonstrated in brain cells. For example, Cx32 is proposed to play a role in Schwann cell proliferation mediated by neuregulin-1 [42], and Cx43 is described to participate in the regulation of polarized cell movement which is essential for the directional migration of neural crest cells [43] and in astrocyte proliferation and wound closure after scratch wound injuries [44]. Channel-independent effects on cell proliferation have been linked to the implication of Cx proteins in the production and activity of several cell cycle regulators [45–47] or to their interactions with growth regulator proteins such as CCN3 [48]. Concerning cell migration, the channel-independent effects appear to be mainly due to the interaction of the CT part of the Cx with

the cytoskeleton but also to effects on purinergic receptors and enzymes (e.g. ERK, PKC, Src and p38) [49–52]. In HL1 cardiomyocytes, it has been shown that Cx43 is implicated in the regulation of transforming growth factor (TGF)- β function by interaction with Smad2/3 and microtubules [53]. Finally, the implication of Cx proteins in cell death has been proposed due to the nuclear localization of Cx in transfected cells [54], alteration of the expression of pro-apoptotic proteins including Bax, caspase-6 and caspase-9 in cells from Cx43 knockout mice [55] and the direct association of Cx with apoptotic proteins (e.g. Bax and Bak) [56].

Cx Expression and Function in Healthy Vessels

Four Cx are present in a healthy vascular wall: Cx37, Cx40, Cx43 and Cx45; their expression varies with the vascular territory and species. Usually, Cx37 and Cx40 are co-expressed in ECs, while Cx43 and Cx45 are mostly present in SMCs (fig. 2a). Of note, Cx43 has also been described in ECs of large vessels in rabbits, hamsters and rats. Homomeric and heteromeric connexons and homocellular and heterocellular GJ channels are found in the vascular wall between ECs, between SMCs and in some cases between ECs and SMCs, allowing the passage of transverse and longitudinal signals in the vessel wall [57–59]. The importance of vascular Cx has been demonstrated by the fact that their deletion alters normal vascular functioning. For example, Cx45 knockout mice die in utero due to the interruption of vessel maturation [60], and Cx43 knockout mice die shortly after birth due to cardiac malformations resulting in obstruction of the outflow tract [61]. Although Cx40 knockout mice are viable, they are hypertensive [62] and display an increased sensitivity for cardiac arrhythmias [63, 64]. The double deletion of Cx37 and Cx40 in mice induces embryonic death due to an excessive dilation of blood vessels [65]. Physiological control of the vascular tone is in part regulated by GJ inter-cellular communication. Indeed, GJ are implicated in the radial transmission of hyperpolarization from ECs to SMCs [the endothelium-derived hyperpolarizing factor (EDHF) phenomenon] and in the longitudinal transmission of electrical signals [66]. Cx37 and Cx40 are co-expressed at the level of the myoendothelial junctions [67]. The role of the different Cx in vascular tone has been investigated in vitro by targeting gap junctional communication and in vivo using transgenic animals. For example, α -glycyrrhetic acid, which inhibits gap junctional communication, blocks the EDHF phe-

Fig. 2. Schematic representation of Cx expression in healthy vessels and during atherosclerosis. In healthy vessels, ECs express Cx37 and Cx40, whereas SMCs express Cx43 and Cx45 (a). Oscillatory shear stress present at the arterial bifurcation induces up- and downregulation of Cx43 and Cx37 expression in ECs, respectively (a). Circulating monocytes and platelets express Cx37. Dur-

ing atherosclerosis, the Cx43 expression in intimal SMCs is sequentially increased (b) and decreased (c), and the ECs that cover the atherosclerotic plaque do not express Cxs at all. In early atheroma, foam cells express only Cx37 (b), whereas in late atheromas, they may co-express Cx37 and Cx43 depending on their location in the plaque (c).

nomenon [68]. The use of specific peptides targeting Cx37, Cx40 or Cx43 in the rat hepatic artery has shown that the inhibition of the EDHF response depends on more than one Cx subtype [69] and that Cx37 and Cx40 are implicated in endothelium-dependent subintimal smooth muscle hyperpolarization whereas Cx43 is involved in the spread of subintimal hyperpolarization through the media [70]. In rat mesenteric arteries, EDHF-mediated dilation seems to depend exclusively on Cx40 [71]. Using arterioles of Cx40-deficient mice, it has been demonstrated that Cx40 plays an important role in the

propagation of acetylcholine- or bradykinin-induced vasodilation [72] and in vasodilation induced by electrical stimulation [73]. The role of Cx40 in vasodilation has been confirmed in Cx40K145 mice in which the expression of Cx45 instead of Cx40 cannot replace the function of Cx40 in the conduction of endothelium-dependent dilations along arterioles [74]. In contrast to Cx40, Cx37 deficiency does not modify vasodilation induced by acetylcholine [75]. Moreover, along the arterial wall, Cx expression is influenced by blood flow. Indeed, oscillatory shear stress present at the branch points of arteries in-

duces Cx43 expression in ECs [76], whereas the Cx37 expression is reduced [77] (fig. 2a). The disturbed shear stress in these regions induces endothelial dysfunction and makes them a starting point for atherosclerosis plaque development [78].

Cx in Atherosclerosis-Induced Vascular Remodelling

Atherosclerosis is a progressive pathology that takes place in the intima of large- and medium-sized arteries. Experimental studies on atherosclerosis are generally performed using mice deficient in apolipoprotein E (ApoE^{-/-}) or low-density lipoprotein-receptor (LDLR^{-/-}) [2]. The additional deletion of Cx37, Cx40 or Cx43 in these atherosclerosis-susceptible mice permits consideration of the implication of each Cx in atherogenesis [29, 79, 80]. The pattern of Cx expression during atherosclerotic plaque development is schematically presented in figure 2.

The initiating step of atherosclerosis is endothelial dysfunction [81] induced by a disturbed blood flow at the arterial bifurcations and by typical cardiovascular risk factors such as dyslipidaemia, hyperglycaemia, hypertension or free radicals. As mentioned above, Cx expression is changed at the arterial branch points; Cx43 appears in ECs whereas Cx37 expression is downregulated [76, 77, 82] (fig. 2a). The Cx37 expression in ECs is regulated by the flow-responsive transcription factor KLF2 [77]. Endothelial dysfunction is associated with altered expression and function of the endothelial nitric oxide synthase (eNOS) that limits the vasoprotective properties of nitric oxide (NO) [83]. It has been highlighted that Cx37 is a direct protein partner of eNOS and that mutual functional regulation exists between Cx37 and eNOS in ECs [84]. In fact, the downregulation of Cx37 in bEnd.3 cells leads to an increase in NO release, and the transfection of N2A cells with eNOS-mimetic peptides modifies Cx37 GJ channel properties. Moreover, eNOS expression has also been shown to be altered in mice deficient in Cx40 [85].

Parallel to this effect on eNOS, the activation of ECs leads to an increase in the expression of different cell adhesion molecules and to the secretion of chemoattractants which induces the recruitment of monocytes, T lymphocytes, neutrophils and platelets [4, 86, 87]. Circulating monocytes express Cx37 in basal conditions [29] (fig. 2a) and Cx43 upon activation by tumour necrosis factor (TNF)- α and interferon (IFN)- γ [88]. At the level of the dysfunctional endothelium, monocytes transmi-

grate between ECs to infiltrate into the arterial intima where they mature into macrophages. These intimal macrophages accumulate lipids and transform into foam cells, creating the earliest atherosclerotic lesion. The development of atherosclerotic lesions in the thoracic-abdominal aorta and in the aortic sinus is accelerated in Cx37^{-/-}ApoE^{-/-} mice compared to controls (Cx37^{+/+}ApoE^{-/-}), suggesting a protective effect of Cx37 against atherosclerosis [29]. Adoptive transfer of Cx37-expressing and Cx37-deficient fluorescent monocytes or macrophages in control and Cx37^{-/-}ApoE^{-/-} mice revealed that the deletion of Cx37 in monocytes/macrophages increased the number of these leucocytes in atherosclerotic plaques [29]. Interestingly, the presence or absence of Cx37 in ECs did not influence the transmigration of monocytes/macrophages. In addition, *in vitro* assays showed that the absence or inhibition of Cx37 hemichannels in the H36.12j mouse peritoneal macrophage cell line reduced the release of ATP by macrophages and increased their adhesion [29]. As it is known that ATP is implicated in inflammation and can pass through GJ channels and hemichannels [89], it has been proposed that Cx37 protects against atherosclerosis by regulating ATP-dependent monocyte adhesion [29]. A similar role was recently proposed for Cx43 [90]. Altogether, these results point to the importance of Cx hemichannels in monocytes during atherosclerotic plaque development and show that Cx37 GJ channels between ECs or between ECs and leucocytes play a relatively minor role in the disease process. Cx expression in T lymphocytes is not consistently reported, but it seems that Cx43 expression in these cells may play a role in their activation [91]. The role of Cx43 in T cells has not yet been described in the context of atherosclerosis. Concerning neutrophils, Cx43 expression has been found by some groups after specific stimulation such as with TNF- α , IFN- γ or lipopolysaccharide [92–94], whereas others have reported the absence of Cx43 [95]. It was recently proposed that Cx43 hemichannels present in neutrophils influence their adhesion by modulating ATP release [96].

During atherosclerotic plaque development, the ECs present in the shoulder part of the atherosclerotic lesion express Cx43 [97] while the ECs covering the atherosclerotic plaque do not express Cx at all [97] (fig. 2b, c). The mechanism implicated in the downregulation of Cx expression in these ECs is not yet known, but it has been described that inflammatory mediators such as TNF- α are able to regulate Cx expression in human umbilical vein ECs [98]. Young mice in which Cx40 was deleted only in ECs (Cx40del mice) developed atherosclerotic

plaques in the aortic sinus spontaneously (i.e. without a high-cholesterol diet) [79]. The progression of these atherosclerotic lesions was increased after 5 or 10 weeks of a high-cholesterol diet, illustrating a protective role of endothelial Cx40 in atherogenesis. The transmigration of monocytes from the blood to the intima depends critically on adhesion molecules such as vascular cell adhesion molecule (VCAM)-1. VCAM-1 expression is regulated by the activity of the 5'-ecto-nucleotidase CD73 at the surface of ECs [99]. Interestingly, Cx40del mice showed a decreased expression of CD73 in en face staining of the aorta and increased VCAM-1 expression [79]. In vitro, it has been confirmed that blocking endothelial Cx40 with anti-sense increases monocyte adhesion on a monolayer of bEnd.3 cells which normally constitutively express CD73 and Cx40. Thus, reducing the Cx40 expression limits the spread of CD73-evoked anti-inflammatory signalling between ECs, leading to increased monocyte adhesion [79].

Activated cells present in the atherosclerotic lesion secrete cytokines, chemokines and growth factors that induce the migration of SMCs from the media to the intima of the atherosclerotic lesions. The Cx43 expression in the SMCs present in the intima temporarily increases and declines later in the process [97, 100] (fig. 2c). The mechanism that regulates Cx43 expression in SMCs during atherosclerosis is not known, but a recent study performed in the human radial artery suggests that the transcription factor NFκB is involved in the regulation of Cx43 expression in SMCs [101]. In the intima, SMCs proliferate and secrete extracellular matrix components which participate in the formation of a strong fibrous cap around the atherosclerotic plaque. This process seems to be associated with the increased expression of Cx43 in SMCs [102, 103] and with modifications in the phosphorylation status of Cx43 [104]. As described by the authors, the phosphorylation of Cx43 on serine 279/282, but not on serine 368, increased the vascular SMC proliferation. Moreover, treatment of human aortic SMCs with TGF-β upregulates Cx43 expression which is correlated with an increased synthetic activity [105]. Overall, these data suggest that atherosclerotic plaque development is influenced not only by the level of Cx43 expression but also by post-translational modifications. Cx37 expression has been observed in medial SMCs of advanced atherosclerotic lesions [97] (fig. 2c). A micro-array analysis showed that, out of >15,000 genes, 106 genes were significantly differentially expressed in Cx37^{-/-}ApoE^{-/-} young mice before diet in comparison with Cx37^{+/+}ApoE^{-/-}; differences mostly involved genes implicated in cell-to-cell signalling

and interactions (e.g. mitogen-activated kinases 1 and 3), cellular compromise (e.g. heat shock proteins HSPA1B and HSPB1) and nutritional disease pathways (e.g. adiponectin and resistin) [106]. This study also showed important changes in the genes and proteins implicated in vascular calcification and matrix degradation in aortas of Cx37^{-/-}ApoE^{-/-} mice after 18 weeks of a cholesterol-rich diet. Thus, Cx37 deficiency seems to alter the global differential gene expression profiles of young mice towards a pro-inflammatory phenotype, which could affect advanced plaque stability.

In the centre of the atherosclerotic plaque, foam cells die and release lipids that form the necrotic core of this lesion. Macrophage foam cells present in the necrotic core co-express Cx43 and Cx37 while foam cells present in the periphery of the necrotic core only express Cx37 (fig. 2c) [97, 107]. Cx43^{+/-}LDLR^{-/-} mice on a high-cholesterol diet showed about a 50% reduction in atherosclerotic plaque development in the thoracic-abdominal aorta and in the aortic sinus in comparison to Cx43^{+/+}LDLR^{-/-} mice [80]. Moreover, the atherosclerotic plaques of these Cx43^{+/-}LDLR^{-/-} mice presented smaller lipid cores, fewer macrophages and more SMCs and interstitial collagen than those of Cx43^{+/+}LDLR^{-/-} mice. In humans, such a plaque phenotype is considered more stable and thus less vulnerable to plaque rupture. The exact role of the different atheroma-associated cell types that express Cx43 (SMCs, ECs and macrophages) is still under investigation. Preliminary data have shown that the endothelial-specific deletion of Cx43 in mice has beneficial effects on both the natural progression and the composition of atherosclerotic lesions [108].

Atherosclerotic plaque may give rise to the formation of a luminal thrombotic occlusion due to the superficial erosion of the endothelial monolayer or to rupture of the plaques' fibrous cap [109, 110]. As a consequence of plaque erosion or rupture, platelets come into contact with extra-cellular matrix, leading to their activation and subsequent thrombus formation. Upon activation, the morphology of the platelets changes and they express molecules to enhance their adhesion to ECs and inflammatory cells. Angelillo-Scherrer et al. [111] recently demonstrated that platelets express Cx37 and that GJ communication between Cx37-expressing platelets provides a mechanism for limiting thrombus propensity. More recent studies seem to implicate the Cx37 and Cx40 hemichannels in thrombus formation as well, even though the authors leave unexplained how hemichannels may open in the presence of a fair amount of extra-cellular calcium needed to induce platelet aggregation [112, 113].

Thrombus formation may lead to myocardial infarction and stroke, which are responsible for a large percentage of sudden deaths worldwide [3]. These clinical complications of atherosclerosis are commonly treated by angioplasty followed by stent implantation [114]. Although angioplasty and stent implantation have tremendously contributed to the increase in survival rates, these procedures induce de-endothelialization which increases the risk of thrombosis shortly or even later after the intervention, and they injure the vessel wall, favouring a vascular reaction which leads to restenosis.

Cx in Restenosis-Induced Vascular Remodelling

Restenosis is a process that occurs generally within 6 months after angioplasty or stent implantation. Restenosis is due to an exaggerated cellular response at the site of the intervention, leading to formation of the neointima and to re-occlusion of the artery [8]. The elaboration of drug-eluting stents has helped tremendously to prevent restenosis by inhibiting neointimal hyperplasia, but drug-eluting stents also delay re-endothelialization, leading to late in-stent thrombosis [115]. The molecular pathways implicated in all of these phenomena remain poorly understood. The development of the model of balloon injury in animals, and certainly in hypercholesterolemic mice [116], allows detailed investigation of the proteins involved in the restenotic process such as Cx [117]. The pattern of Cx expression in restenosis is schematically presented in figure 3.

When an occluded artery is re-opened by angioplasty, the surgical procedure causes damage to the vascular wall, inducing the recruitment and infiltration of leucocytes into the damaged site, a surge in cytokines and growth factors and a phenotypic switch of medial SMCs that become activated, begin to proliferate and migrate towards the intima. Several proteins, including Cx, have been described to regulate SMC phenotypic modulation, proliferation and migration. The possible involvement of Cx in restenosis after a balloon catheter injury was first described by Yeh et al. [118] in 1997. In the rat carotid artery, they carefully followed the Cx43 expression for 14 days after ballooning and showed an upregulation of this expression in medial and intimal SMCs, with a higher expression in the neointima. In addition, the size of the GJ was larger between SMCs in the intima than between SMCs of the media. In this model of the rat carotid artery, restenosis was mainly due to the migration and proliferation of SMCs and, to a lesser extent, to leucocyte infiltra-

tion [118]. In hypercholesterolaemic mice, a reduced expression of Cx43 (Cx43^{+/-}LDLR^{-/-} mice) is associated with the limitation of neointima formation in comparison to mice with a normal expression of Cx43 (Cx43^{+/+}LDLR^{-/-}) [117]. In addition, the *in vivo* macrophage infiltration is lower in the carotid intima of Cx43^{+/-}LDLR^{-/-} mice than in Cx43^{+/+}LDLR^{-/-} mice, and *in vitro* experiments have shown that macrophages from Cx43^{+/-}LDLR^{-/-} mice have a reduced migration capacity compared to macrophages from Cx43^{+/+}LDLR^{-/-} mice. Interestingly, conditioned medium obtained from Cx43^{+/+}LDLR^{-/-} macrophages induced, *in vitro*, a higher migration of SMCs than conditioned medium obtained from Cx43^{+/-}LDLR^{-/-} macrophages, suggesting that these macrophages secrete fewer chemoattractant factors than control macrophages. Two types of SMCs have been described in rat aortas [119] and in porcine coronary arteries [120]. The differentiated SMCs, called spindle-shaped SMCs (S-SMCs), present the classical 'hills-and-valleys' growth pattern and the rhomboid SMCs (R-SMCs) have high proliferative, migratory and proteolytic activities [120]. Interestingly, R-SMCs are more present in the intimal thickening induced by stent implantation in porcine coronary arteries than S-SMCs [120], and Cx43 expression is strongly upregulated in this intimal thickening whereas Cx40 is absent [103]. *In vitro* experiments have confirmed that Cx43 and Cx40 are co-expressed in S-SMCs whereas R-SMCs expressed only Cx43 [103]. In addition, R-SMC migration induced by platelet-derived growth factor (PDGF)-BB is highly reduced in the presence of Cx43 anti-sense or Cx43 blocking peptide [103], suggesting that Cx43 expression and channel function regulate the migration process of R-SMCs. Furthermore, Cx43 anti-sense prevents PDGF-BB-induced deleterious phenotypic changes of porcine S-SMCs to R-SMCs [103]. In the rat model of a balloon injury, the downregulation of Cx43 expression also inhibits SMC proliferation [121]. To specifically study the role of Cx43 in SMCs, Liao et al. [122] generated mice in which the deletion of the Cx43 gene was confined to SMCs, and they performed carotid artery denudation using a guide wire. Seven days after the surgery, the neointima in these mice was larger than the neointima in control mice. The opposite results obtained between the studies have not yet been explained but are likely due to differences in mouse models (deletion of Cx43 in SMCs vs. half of Cx43 in all cells) and methodologies (balloon injury with hypercholesterolaemia vs. wire injury) used. Finally, in New Zealand white rabbits, balloon injuries induced an increase in Cx40 mRNA and protein expression in neointimal SMCs [123, 124]. Inter-

Fig. 3. Schematic representation of Cx expression during restenosis. Angioplasty allows re-opening of an occluded artery (a). However, this surgical procedure may also induce the recruitment and infiltration of leucocytes into the damaged site, the proliferation and migration of SMCs from the media towards the intima and platelet aggregation at the site of the intervention (b). During this process, the main modification observed in Cx expression is an upregulation of Cx43 and Cx40 in SMCs.

estingly, in the presence of statins, a molecule with anti-inflammatory and anti-proliferative properties, the formation of the neointima induced by the balloon injury is reduced and the expression of Cx43 and Cx40 in the rabbit iliac artery is less important [123]. Treatment of New Zealand white rabbits with ramipril, an angiotensin-converting enzyme inhibitor, also seems to inhibit neointimal formation after a balloon injury and it appears to downregulate the expression of Cx43 mRNA and protein [124].

The progression of restenosis after vascular surgery may also be influenced by thrombosis at the site of the injury. Indeed, increased platelet activation at the site of the injury results in increased PDGF-BB secretion, which then has additional effects on SMCs. The role of platelet Cx37 in this respect has not yet been investigated.

Although it has been shown that the expression of Cx is modified after vascular injury, their roles in restenosis are not yet fully clear at the molecular level. Studies of Cx as modulators of migration or proliferation suggest that they may play a role in these processes via either channel-dependent (hemichannel or GJ channel) or channel-independent interactions with cytoskeletal proteins, junctional proteins or enzymes [51, 125].

Conclusion

This review provides an overview of the role of Cx in vascular remodelling associated with atherosclerosis and restenosis. The importance of each Cx is revealed by the use of transgenic mice, transfected cells, specific blocking

peptides and anti-sense. Further studies are needed to more fully understand the exact role of each Cx expressed in the different cell types (ECs, SMCs and inflammatory cells) implicated in these processes. The development of treatments targeting Cx might hold promise, particularly for limiting the deleterious consequences of angioplasty and stent implantation.

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