**TRPC channels as prospective targets in atherosclerosis: *terra incognita***

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**TABLE OF CONTENTS**



## 1. ABSTRACT

Transient Receptor Potential Canonical (TRPC) proteins are non-selective cation channels ubiquitously expressed throughout the cardiovascular system, where they participate as $Ca^{2+}/Na^{+}$-permeable channels and/or signaling platforms in various physiological and pathophysiological mechanisms. TRPCs have been implicated in essential hypertension, cardiac hypertrophy and endothelial dysfunction. Despite these pathologies being related, directly or indirectly to development of atherosclerotic lesions, the potential role of TRPCs in the pathogenesis of atherosclerosis remains unexplored. Recent studies from our laboratory showing an obligatory requirement of TRPC3 in the inflammatory signaling linked to monocycle recruitment to coronary endothelium, suggest for the first time potential pathophysiological relevance of a member of the TRPC group in atherogenesis. This brings about the question whether we can envision TRPCs as potential targets for diagnosis, prognosis and/or treatment of atherosclerosis. Here we revisit some of the existing knowledge on TRPCs and cardiovascular pathology and discuss it within the context of cellular/molecular processes related to atherogenesis. Potential limitations and advantages of TRPCs as prospectives targets in atherosclerosis are discussed and confronted against those of channel blockers currently in use.

## 2. TRANSIENT RECEPTOR POTENTIAL CANONICAL (TRPC) PROTEINS

Without question TRPC proteins stand up amongst the most important channel forming proteins in the vasculature, where they are expressed in all cell types composing the heart and vessel's wall, and also in hematopoietic cells, and participate, directly or indirectly, in $Ca^{2+}$ signaling events required for modulation of a myriad of vascular functions (1; 2). The TRPC family belongs to the larger TRP superfamily of channel forming proteins and contains those TRP members most closely related to the original *Drosophila melanogaster* TRP protein (3; 4). TRPC proteins can be subgrouped on the basis of structural, pharmacological and functional properties: TRPC1, TRPC2 (a pseudogene in humans, old world monkeys and apes), TRPC3/6/7, and TRPC4/5 (4). Functional TRPC channels are made of homo- or hetero-tetrameric arrangements of four TRPC proteins with each subunit exhibiting a membrane topology which includes six transmembrane domains (TM1-TM6) separating cytoplasmic N- and C-termini, and a re-entry loop between TM5 and TM6 which lines the wall of the pore ( (4) and references therein). The presence of ankyrin repeats at the N-terminus and coiled-coil regions both at N- and C-ends -among other structural features that vary within individual members- enable TRPCs to interact with a diversity of signaling proteins, although this strongly depends on the particular TRPC subunit, cell type and expression conditions. Ectopic expression of TRPC proteins in homo- or heterologous expression systems renders non-voltage gated, non-selective $Ca^{2+}$-permeable cation channels characteristically activated downstream receptor-stimulated phospholipases (*v.g.*, PLC and/or PLD; see for instance (5)). This is also the situation in native conditions, as TRPC channels expressed at endogenous cellular levels take part in the action of several phospholipase-coupled receptors for peptides and growth factors that contribute to various aspects of vascular physiology, such as regulation of arterial tone, endothelial permeability, secretion, endothelial cell proliferation, apoptosis and survival, among others (2). It is important to keep in mind that in terms of permeability properties all TRPCs are non-selective for cations, which confers them the ability to also alter $Ca^{2+}$ signaling by mediating $Na^{+}$ influx, *i.e.*, by altering membrane potential and modifying the driving force for $Ca^{2+}$ entry (see (6) and references therein). The mechanisms underlying activation of TRPC channels are diverse, and is still controversial whether lessons learned from heterologous expression systems truly reflect mechanisms involved in TRPC regulation under native conditions (reviewed by us in (7; 8)). Despite this uncertainty, there is a great deal of *in vitro* and *in vivo* data documenting the participation of TRPCs in $Ca^{2+}/Na^{+}$ influx associated to vascular physiology (2; 9). Most importantly,

accumulating evidence shows that TRPCs are indisputable players in the pathogenesis of cardiovascular disease (10; 11). Notably, it was not until we began to pay attention to which cellular and/or molecular events rely upon appropriate TRPC expression/function –regardless of the particular mechanism/s driving channel activation- that we started to move forward in better understanding the role of TRPCs in cardiovascular physiology and disease.

**3. TRPC PROTEINS AND CARDIOVASCULAR DISEASE: *QUID NOVIS?***

For more than a decade now we have witnessed an increasing enthusiasm to learn more about the role of TRPC proteins in cardiovascular pathology. Because all members of the TRPC family are ubiquitously expressed along the vascular tree, it is not surprising that altered TRPC function and/or expression is frequently associated to a variety of vascular disease states (reviewed by us in (12); see also (3; 13)). For example, TRPC1, 4 and 6 participate in regulation of vascular tone and play a role in hypertension . TRPC1 and 6 modulate proliferation of vascular smooth muscle cells and may have implications in the development of both idiopathic and hypoxia-induced –acute and chronic- pulmonary hypertension (14; 15; 16; 17; 18; 19) as well as in the pathogenesis of intima hyperplasia (20; 21). More recently, the pro-proliferative role of TRPC6 in endothelium underscores a potential role for this TRPC member in angiogenesis and neovascularization (see section 4.4. below). Regulated $Ca^{2+}$ entry through TRPC3 and 6 activates Nuclear Factor of Activated T cells (NFAT), a critical event in development of cardiac hypertrophy (22; 23). A significant amount of information on regulatory aspects of TRPC3 and 7 in lymphocytes has been derived from studies on both human and avian cells, and is likely that at least these closely related TRPC members may take part in immune responses mediated by B and T cells (24; 25; 26). Because those studies were conducted on cell lines, it remains to be explored if those findings represent relevant TRPC functions in intracellular signaling associated to the pathogenesis of inflammatory vascular disease in *in vivo* models. TRPC3, by yet to be known mechanisms, is upregulated in circulating monocytes from spontaneously hypertensive rats (27; 28) and that strongly correlates with increased regulated $Ca^{2+}$ influx. Similarly, increased store-operated and diacylglycerol-regulated $Ca^{2+}$ influx accompany augmented expression of TRPC3 and 5 in circulating monocytes from patients with essential hypertension (29; 30). The speculation has been made that this may account for the increased monocyte activation observed in hypertensive individuals with potential implications in promoting vascular disease in these patients – *v.g.*, atherosclerosis- (30) but this notion has not been further explored. Expression of TRPC3 and 6 is increased in pre-glomerular arterioles from patients with malignant hypertension (31) and TRPC3 is also elevated in kidney endothelium obtained from renal cell carcinoma patients who had a history of hypertension, but not in those with normal blood pressure (32). Because TRPC3 is endowed with high constitutive, non-regulated activity (4; 33) it is plausible to speculate that upregulated channel expression may derive in increased constitutive $Ca^{2+}$ and/or $Na^{+}$ influx into endothelium and/or smooth muscle, and thus affect the basal activity of $Ca^{2+}/Na^{+}$ dependent signaling processes underlying vascular function. The best example that such phenomenon does indeed take place *in vivo*, is provided by the observation that constitutively active TRPC3 is upregulated in TRPC6 knockout mice and is responsible for enhanced contractility of aorta and cerebral arteries and elevated blood pressure (34). More recently, we showed that upregulated expression of TRPC3 in coronary endothelium promotes inflammatory signaling and supports regulated expression of cell adhesion molecules ( (35; 36); discussed in section 4.1 below).

**4. TRPC PROTEINS AND ATHEROSCLEROSIS: *QUO VADIS*?**

Atherosclerosis is a chronic disease of the arterial wall with a dominant inflammatory component that is present throughout all stages of the disease (37). Atherosclerosis is perhaps the best example of an inflammatory vascular disease arising as the result of a complex interplay between mechanisms that are intrinsic to the endothelium, such as endothelial dysfunction, cell adhesion-mediated recruitment of inflammatory cells, oxidative stress, and factors related to the individual genetics and its environment, such as metabolic disorders or lifestyle, among others (38; 39). Also, atherosclerotic lesions are the major vascular complication of diabetes, obesity and metabolic syndrome. It is not surprising then that atherosclerosis still constitutes the major cause of death in western societies, where in the United States only, one in five annual deaths are due to coronary artery disease, a manifestation of atherosclerosis of the coronary arteries, in both men and women (40). Most frequently, atherosclerotic lesions are present in epicardial coronaries, aorta, carotid or peripheral arteries, and the narrowing of the arterial lumen caused by lesion growth can lead to ischemia of the heart, brain, or extremities and eventually, infarction, stroke and/or complications derived from ischemic or thromboembolic events that follow plaque rupture (39; 41). The inflammatory response that takes place in the subintima plays a central role not only in the initiation of the atherosclerotic lesion, but also in its progression and the fate of the plaque at more advanced stages (reviewed in (41; 42)). At the cellular and molecular level, inflammatory signaling in the endothelial cell, the endothelial repertoire of constitutive and inducible cell adhesion molecules (CAMs) and the recruitment of circulating monocytes to the inflammation sites in the subintima, all constitute salient features that somehow determine progression of initial fatty streaks –lipid laden subendothelial macrophages- towards complex plaques that may later become unstable, life-threatening lesions.

Recent studies from our laboratory aimed at understanding the role of TRPC3 in regulated expression of CAMs and monocyte recruitment to coronary artery endothelium (*vide infra*) provided, for the first time, experimental support to the novel notion that TRPC3 - and perhaps other members of the TRPC group, *vide infra*- might play a signaling role in atherogenesis. Our findings and those from other groups -although the latter not directly aimed at studying atherosclerosis- suggest the existence of a novel relationship between expression and function of native TRPC proteins and cellular and molecular events that contribute to atherogenesis, suggesting pathophysiological relevance for TRPCs in this disease. The available evidence is briefly revisited in

the following subsections and it was grouped, for a didactic purpose only, on the basis of observations that, although not originally intended to examine a role of TRPCs in atherogenesis, provide a potential link between TRPC function to molecular/cellular events and/or risk factors known to play a role in the pathogenesis of atherosclerosis. The sections below by no means intend to represent a thorough revision of existing literature in the field; rather, only those examples most representative in terms of their relevance to atherosclerosis have been chosen in order to illustrate our viewpoint. For further reading on TRPCs in cardiovascular disease the reader is referred to some recent reviews on the topic (see for instance, (2; 6; 11; 13; 43; 44)).

### 4.1. TRPCS IN REGULATED EXPRESSION OF CELL ADHESION MOLECULES AND INFLAMMATORY CELL RECRUITMENT

Recruitment of circulating monocytes to the endothelium and their migration to the subendothelial milieu where they differentiate into macrophages is one of the earliest cellular events in atherogenesis (37; 45). Nevertheless, monocyte infiltration takes place throughout all stages of lesion development and this perpetuation of monocyte/macrophage replenishment at the lesion site has a prominent role in the mechanisms that lead to plaque instability and rupture (46). Monocyte recruitment to the endothelium entails interaction of integrins on the monocyte surface with CAMs expressed on the endothelial cell, particularly selectins (*v.g.*, E-selectin) and CAMs from the immunoglobulin superfamily such as intercellular cell adhesion-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Both ICAM-1 and VCAM-1 have a central function not only in mediating attachment and firm monocyte adhesion to the endothelial layer but also in the endothelial intracellular signaling that supports transmigration of the bound monocyte (47; 48; 49). Whereas it is clear that ICAM-1 and E-selectin contribute to monocyte recruitment, the role of VCAM-1 in this process is unique in that while ICAM-1 or E-selectin are mostly constitutively expressed in non-activated endothelium, VCAM-1 is absent or undetectable, but its expression is drastically upregulated when the endothelium is exposed to pro-atherogenic stimuli (50). For instance, in hypercholesterolemic animals both ICAM-1 and VCAM-1 are induced in early lesions. However, VCAM-1 expression is largely restricted to lesions, or to sites prone to lesion formation, and can also be detected even before the onset of visible fatty streaks, while ICAM-1 also extends to uninvolved aorta and lesion-protected regions (51). This dissimilar pattern strongly suggested different functions for ICAM-1 and VCAM-1, at least in lesion initiation. Pro-atherogenic factors such as tumor necrosis factor-α (TNFα), locally released nucleotides or oxidized low density lipoprotein (oxLDL) among others, are potent inducers of VCAM-1 expression (50). Oxidized LDL is of most relevance to atherogenesis, as endothelial permeability to apolipoprotein B-containing lipoproteins (*i.e.*, LDL) and their retention and oxidation in the subendothelial space is decisive in triggering endothelial activation and monocyte recruitment. TNFα and nucleotides can be released abluminally or to the subendothelial milieu in response to ischemia, hypoxia, chemical or mechanical stress, and amplify the inflammatory response by promoting release of other inflammatory mediators (*v.g.*, interleukin 6, monocyte chemoattractant protein-1 (52; 53). In human coronary artery endothelial cells (HCAECs) ATP is a potent inducer of VCAM-1 through a mechanism that requires activation of $P2Y_2$ purinergic receptors and changes in intracellular $Ca^{2+}$ ( (36) and references therein). In a recent study, we showed that in HCAECs native TRPC3 protein forms $Ca^{2+}$-permeable channels that contribute to constitutive, non-regulated $Ca^{2+}$ influx and also to receptor-regulated $Ca^{2+}$ entry (36). Those studies showed that TRPC3 expression and its characteristic constitutive function are obligatory for ATP-induced VCAM-1 and monocyte adhesion. In a follow up work, we showed that TRPC3 constitutive function is also required for TNFα-induced expression of VCAM-1 in HCAECs (35). Using knockdown and overepxression approaches, it was demonstrated that it is indeed the constitutive, non-regulated function of TRPC3 rather than regulated channel activity, what is required for the signaling underlying VCAM-1 expression in these cells, through a mechanism that involves constitutive TRPC3-mediated $Ca^{2+}$ influx for proper activation of NFκB, presumably through $Ca^{2+}$-dependent activation of a Calmodulin/Calmodulin-dependent kinase axis (35). Notably, knockdown of TRPC3 in HCAECs also caused a significant reduction in TNFα-induced ICAM-1 expression and interleukin-1β-induced VCAM-1, suggesting that TRPC3 may exert a more general role in NFκB-dependent regulated-expression of CAMs by different pro-atherogenic stimuli, rather than a specific function in TNFα-induced VCAM-1. These findings represented the first direct indication that TRPC-mediated $Ca^{2+}$ influx plays a role in the signaling driving expression of endothelial VCAM-1, underscoring for the first time a potential novel function of TRPC3 in atherogenesis and coronary artery disease.

### 4.2. Oxidative stress

Available evidence indicates that TRPC3 contributes not only to normal redox-dependent endothelial functions but to the signaling associated to oxidative stress, a critical component of endothelial dysfunction associated to atherogenesis (54; 55; 56). In porcine pulmonary artery endothelial cells native TRPC3 heteromultimerizes with TRPC4 to make non-selective cation channels that are sensitive to the redox status of the cell (54; 55). In addition, molecular and electrophysiological evidence suggests that TRPC3 is likely the peroxide-activated non-selective cation channel that mediates $Na^+$ influx and membrane potential breakdown in calf pulmonary endothelium (57; 58). The mechanism by which TRPC-based channels sense redox status in the membrane microenvironment remains unknown. In HCAECs, TNFα-dependent generation of reactive oxygen species occurs mainly through activation of membrane associated NADPH oxidases (Nox2/Nox4A; (59)) and this contributes to TNFα-induced activation of the NFκB pathway. We recently showed that this process has a mandatory requirement for constitutive $Ca^{2+}$ influx, presumably through native TRPC3 channels (35). Notably, in HCAECs NADPH oxidase-dependent generation of reactive oxygen species is necessary but not sufficient for full activation of NFκB by TNFα, and constitutive $Ca^{2+}$ influx is required in order for the system to be fully operational (35).

### 4.3. Endothelial permeability

The ability of endothelial cells to undergo retraction under certain local or systemic conditions confers the endothelium with the ability to adjust its permeability to a variety of molecules and/or cells (*v.g.*, during monocyte transendothelial migration). This also has pathophysiological relevance, as altered transcellular and/or paracellular endothelial cell permeability is now well recognized as a contributing factor to the pathogenesis of atherosclerosis (60). Notably, members of the TRPC family have been involved in the processes that regulate endothelial barrier function (61; 62). Of relevance to atherogenesis, TRPC6-mediated $Ca^{2+}$ influx is obligatory for the inhibitory actions of lysophosphatidylcholine (LysoPC) on migration of bovine aortic endothelial cells (63; 64). TRPC6 is expressed in endothelium, macrophages and medial smooth muscle cells, and therefore it is conceivable that LysoPC, which is an important component of oxLDL at the lesion site, can act as a signaling mediator for LysoPC pro-inflammatory actions. The role of TRPC1, 4 and to some extent, TRPC6, in receptor-regulated lung endothelial permeability is well supported by both *in vivo* and *in vitro* studies (65; 66), but their participation in endothelial permeability in atherosclerosis prone arterial beds remains to be explored. Recently, endothelial hyperpermeability in Klotho deficient mice has been shown to be related to inefficient internalization of TRPC1, which results in augmented vascular endothelial growth factor (VEGF)-dependent $Ca^{2+}$ influx and hyperactivity of $Ca^{2+}$-dependent proteases (67).

**4.4. Adventitial angiogenesis and plaque neovascularization**

As the atherosclerotic lesion grows, new vessels can sprout from the adventitial *vassa vasorum* and neovascularize the growing plaque. Plaque neovascularization provides an additional entering route for inflammatory cell recruitment into the plaque, what derives into exacerbation of the inflammatory state and speeds up plaque growth. Most importantly, plaque neovascularization is an important contributing factor to the instability of the plaque and ultimately, its rupture and subsequent thromboembolic complications (46). VEGF is a central player in adventitial angiogenesis and neovascularization and whether its role in atherosclerotic lesion progression is beneficial of detrimental is still a matter of debate (68). Although not derived from vascular beds prone to atherosclerotic lesion development, human microvascular and umbilical vein endothelial cells migrate, sprout and proliferate in response to VEGF by a mechanism that requires TRPC6-mediated $Ca^{2+}$ influx (69; 70). These results can be interpreted as suggestive of a potential role of TRPC6 in the signaling events that drive angiogenesis during plaque neovascularization. However, the impact of different cellular and molecular processes on lesion progression might also be significantly dependent upon the overall inflammatory/necrotic status of the plaque, and therefore it is possible that the function of TRPC6 in plaque neovascularization, if any at all, might have differential impacts depending on the plaque being at early or advanced stage. *In vivo* studies using mouse models of plaque destabilization –*v.g.*, brachiocephalic artery in $ApoE^{-/-}$ mice (71; 72)- in mice lacking or overexpressing TRPC6 in particular vascular tissues will provide valuable information on this potentially critical role of TRPC6 in atherosclerosis.

**4.5. Blood pressure**

A significant amount of experimental evidence supports a role for TRPC proteins in smooth muscle cells of the systemic and pulmonary circulation where they seem to contribute to events related to systemic blood pressure and hypoxia-induced and idiopathic pulmonary hypertension ( (13; 73) and references therein). Monocytes isolated from patients with essential hypertension exhibit a significant increase in TRPC3 levels compared to cells from normotensive individuals, and that correlates with increased regulated $Ca^{2+}$ influx (29; 30). TRPC3 was also found to be significantly higher in renal endothelium from patients with malignant hypertension (31) and patients with renal cell carcinoma with a history of elevated systolic blood pressure (32). This evidence however, only provides "guilt by association" and it does not answer a more fundamental question, *i.e.*, whether increased levels of TRPC3 play a causative role in the pathogenesis of human hypertension. Evidence on the modulation of nitric oxide (NO) bioavailability through TRPC function as a potential mechanism linking channel activity to vasorelaxation and blood pressure is not yet available. However, NO levels seem to affect TRPC activity, and recent work points for a role of the NO/cGMP axis as a potential strong modulator of TRPC3 and 6 functions. Increasing NO levels either through guanylyl cyclase-linked agonists or by inhibition of cGMP-specific type 5 phosphodiesterase (PDE5; *i.e.*, with sildenafil) inhibits the activities of TRPC3 and more robustly, TRPC6, resulting in a more vigorous and sustained PKG activity which is known to exert phosphorylation-mediated negative feedback of these TRPC proteins (see (74) and references therein). This mechanism has been proposed to be of relevance in PKG mediated modulation of vascular relaxation, and therefore blood pressure (75).

**5. ATHEROSCLEROSIS, CHANNEL BLOCKERS AND TRPC PROTEINS AS PROSPECTIVE TARGETS: DO WE NEED MORE CHANNELS TO BLOCK?**

The notion of $Ca^{2+}$ influx exerting a permissive effect in atherogenesis has been recognized long before the concept of atherosclerosis as an inflammatory disease would fully develop (76; 77) and even before having available the significant amount of information that exists today regarding signaling pathways that directly or indirectly depend on $Ca^{2+}$ influx in endothelium, vascular smooth muscle or macrophages. The realization that traditional L-type channel blockers in clinical use for other cardiovascular situations –*v.g.*, hypertension, arrhythmias- have a beneficial effect on atherosclerosis, somehow centered the attention on their role on L-type channels in smooth muscle. On this ground, any action of L-type blockers on cells other than smooth muscle, and not expressing L-type channels –*v.g.*, endothelium - were systematically attributed to "non-channel related" effects. This somewhat may explain the lack of a systematic exploration of the nature of other $Ca^{2+}$-influx channels that may be involved in atherogenesis or of the mechanism/s linking $Ca^{2+}$ entry to atherogenic processes. Our recent studies on TRPC3 in inflammatory signaling in coronary endothelium and those from others (*vide supra*) provide the first experimental support to the notion that non-L-type channels, in this case members of the TRPC group, might play a signaling role in atherogenesis. This concept immediately opens a spectrum of possibilities regarding potential exploitation of TRPCs -once their individual roles are

elucidated- as novel molecular targets in diagnosis, prognosis and/or treatment of atherosclerosis. But how realistic is to think of TRPCs as prospective targets for this disease? This is a fundamental question particularly if we consider the following. First, TRPC proteins are ubiquitously expressed throughout tissues in the body, what imposes a limitation when strategies for selective vascular targeting are to be designed - *v.g.*, endothelial *vs.* smooth muscle targeting- unless cell specific delivery is achieved. Second, provided this issue is resolved, targeting TRPCs will most likely be aimed at blocking channel function. This immediately raises the question whether targeting TRPCs would provide any additional benefit over what is currently available from clinical use of traditional CCBs whose anti-atherogenic effects have been known for quite some time (78; 79; 80; 81). Indeed, several randomized control clinical trials have shown that CCBs of the phenylalkylamine, benzothiazepine and dihydropyridine groups, historically used in the treatment of hypertension, arrythmias, cardiac hypertrophy, cardiac failure and angina pectoris (82; 83), reduce progression of atherosclerosis (reviewed in (81; 84; 85; 86). However, two critical aspects of CCBs in clinical use are worth mentioning. First, several vascular effects of CCBs –with exception of their action on smooth muscle cells and cardiomyocytes- are unrelated to their channel blocking properties and the underlying mechanism remains, for the most part, unknown. This is well exemplified by the effects of verapamil – a phenylalkylamine- or amlodipine – a dihydropyridine- on expression of endothelial CAMs. It has been shown that long term therapy with these CCBs results in a reduction in expression levels of VCAM-1 with the concomitant benefit derived from reduced recruitment of inflammatory cells (87; 88); these actions are not dependent on channel blockade –endothelium does not rely upon L-type channels for $Ca^{2+}$ influx- but rather seem to be, at least in part, subsequent to an anti-oxidant effect of these CCBs by a poorly understood mechanism which may involve a scavenging action and/or preservation of superoxide dismutase activity (reviewed in (89)). That actions of this nature are not subsequent to channel blockade is better exemplified by the anti-oxidant and anti-apoptotic effects of the dihydropyridine nifedipine on endothelium, which can be completely recapitulated by BayW9798, a dihydropyridine structurally related to nifedipine but with no channel blocker activity (see (90) and references therein). Second, in those few instances where the vascular actions of CCBs do indeed reflect a $Ca^{2+}$-influx blockade effect – *v.g.*, nifedipine and verapamil blockade of L-type $Ca^{2+}$ channels in arteriolar smooth muscle or cardiomyocytes, respectively, with the concomitant reduction in peripheral resistance and cardiac output- several side effects occur. Indeed, the most significant toxic effects of CCBs are direct extensions of their therapeutic actions. For example, persistent $Ca^{2+}$ influx blockade can derive in serious cardiac depression, including cardiac arrest, bradycardia, atrioventricular block and heart failure; although these extreme effects have not been frequent in clinical use, side effects of intermediate caliber are observed in an important number of individuals undergoing CCB therapy. In addition, all prototypical CCBs –verapamil, nifedipine and diltiazem- interfere not only with L-type channels but also some non-selective cation channels including TRPCs. For example, verapamil suppresses both constitutive and regulated TRPC3 activity (36; 91). Verapamil's anti-inflammatory actions on endothelium, including a reduction in VCAM-1 expression and leukocyte adhesion (87; 92), have been historically thought to be unrelated to its channel blocker properties. Whereas verapamil is not specific for TRPC3, our recent observation that in coronary endothelial cells verapamil reduces VCAM-1 expression and monocyte binding to the same extent as knockdown of TRPC3 does (36), raises the question whether at least part of the anti-inflammatory actions of this CCB might be related to a non-L type channel blocker effect. Although additional studies are required to specifically address this notion, it is possible that actions of CCBs on non-L type channels may interfere, *in vivo*, with a myriad of signaling functions that depend upon proper operation of those channels. Based on these considerations, an immediate benefit can be envisaged from targeting TRPCs over current calcium channels, and that is the possibility of achieving efficient suppression of signaling events downstream TRPC-mediated $Ca^{2+}$ influx with no interference on pathways usually affected by traditional L-type CCBs. Therefore, typical CCBs side effects are expected to be avoided or at least minimized. Crucial to achieve this goal is the development of subtype-specific inhibitors for TRPCs. As of this writing, no specific pharmacological inhibitors of TRPCs have been developed so far and none of the existing blockers that affect TRPC function – *v.g.*, verapamil, SKF96365, flufenamate, hyperforin, among others; see for instance (93) and references therein- are selective enough to be expected to discriminate, *in vivo*, between TRPCs and other channels. In this regard, some progress has been recently made with pyrazole compounds (94), but their efficacy *in vivo* awaits further examination. An alternative approach that illustrates the possibility of specific TRPC targeting in the vasculature is provided by work showing that antibody-mediated targeting of extracellular domains of TRPC proteins may have therapeutic potential (21; 95). This strategy relies upon using antibodies to target the third extracellular loop of TRPCs –"E3 targeting"- which lies next to the pore region; this may impose both conformational and steric constraints to the pore which then results in abrogation of channel activity. The E3-targeting strategy was successfully applied to block TRPC1 (21) and TRPC5 (95) channel activities. Importantly, because of the exquisitely high selectivity and specificity that can be attained with antibodies, this strategy provides an opportunity to discriminate even between closely related members within the TRPC group –*v.g.*, TRPC5 *vs.* TRPC4, see for instance (95) – with the potential benefit of minimizing side effects derived from incomplete selectivity of pharmacological blockers.

## 6. CONCLUDING REMARKS

The recognition of atherosclerosis as an inflammatory disease revolutionized the field regarding the way basic and clinical research efforts were conceived. For example, advances on the knowledge of molecular and cellular components involved in lesion development, such as the role of endothelial CAMs in monocyte recruitment, and appreciation of the fundamental role of the macrophage as a central player in lesion formation and progression, were rapidly and enthusiastically envisaged as promising new opportunities to develop anti-inflammatory therapies for the disease. However, evidence accumulated so far indicates that interfering at the level of one single target may render limited success (see (86; 96; 97)) and that dual or multiple targeting might be necessary to achieve results of clinical significance. This is not surprising considering the

multifactorial nature of atherosclerosis and the diverse repertoire of receptors, signaling molecules and cell types that contribute to its pathogenesis. It is easy then to understand why characterization of new components of signaling events that contribute to monocyte recruitment, their migration to the subintima, factors involved in their differentiation to macrophage and events that modulate macrophage survival and/or apoptosis at the lesion site, is of fundamental importance to move forward in the development of new therapeutic and/or prognostic/diagnostic strategies for effective management of this disease. Within this context, studies aimed at understanding the role of TRPC proteins in atherogenesis have an unequivocal potential to make a major contribution into that direction. An invaluable step forward to achieve such goal will be the generation of genetically modified mouse models with conditional overexpression or knockout of one or more TRPC members in atherosclerosis relevant vascular cells in order to allow examination of the *in vivo* contribution of that particular TRPC in atherogenesis.

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**Abbreviations:** CAM: Cell adhesion molecule, CCB: Calcium channel blocker, DHP: Dihydropyridine, HCAEC: Human coronary artery endothelial cells, HUVEC: human umbilical vein endothelial cells, ICAM-1: Intercellular cell adhesion molecule-1, NFAT: Nuclear factor of activated T cells, NFkB: Nuclear factor kappa B, NO: Nitric oxide, oxLDL: Oxidized low density lipoprotein, PLC: Phospholipase C, PLD: Phospholipase D, TNFa: Tumor necrosis factor alpha, TRPC: Transient Receptor Potential Canonical, VCAM-1 : Vascular cell adhesion molecule-1, VEGF: Vascular endothelial growth factor

**Key words:** Transient Receptor Potential Canonical Channels, Atherosclerosis, Cardiovascular Disease, Endothelial Dysfunction, Calcium Channels, Inflammatory Signaling, Review

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