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Caveolae Disassembly using Methyl-β-cyclodextrin Causes the Abolition of Coupling of the Caveolae and the Sarcoplasmic Reticulum in the Rat Femoral Artery

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ABSTRACT

Background: Caveolae are essential in regulating signal transduction mechanisms of ion channels in vascular tissue, including BK_{Ca} channels (maxi-K). The current study investigated the localization of maxi-K channels within caveolae.

Methods: Sixteen rats were divided into two groups: A control group and a treated group, where arteries in the treated group were incubated with methyl-β-cyclodextrin (MβCD) to disassemble caveolae from artery tissue. Immunohistochemistry (IHC), immunocytochemistry (ICC), transmission electron microscopy (TEM) and Western blot techniques were used in this study.

Result: IHC of intact arteries showed colocalization of maxi-K channels with caveolin-1 in smooth muscle and endothelial cells and colocalized of maxi-K channels with caveolin-3 in smooth muscle cells only. These findings were also corroborated with ICC in a single smooth muscle cell. TEM revealed caveolae covering most plasma membranes of smooth muscle and endothelial cells and showed that caveolae sit close to the sarcoplasmic reticulum only in smooth muscle cells. TEM showed incubating arteries with MβCD led to the disassembly of caveolae from artery tissue. This study concluded that maxi-K channels localize to caveolae and that caveolae abolishment by MβCD led to the abolition of the coupling of caveolae and the sarcoplasmic reticulum.

Key words: Caveolae, Femoral artery, Maxi-K channel, MβCD, Smooth muscle cells.

INTRODUCTION

Caveolae cover most plasma membranes of the vascular tissue (Parton and Del Pozo, 2013). Many vital ion channels and proteins with functions related to cellular signaling are located in caveolae (Isshiki and Anderson, 1999). These structures are instrumental in modulating various functions, including the critical process of vasoconstriction in blood vessels. Vasoconstriction in muscular arteries initiates through smooth muscle contraction due to increased intracellular Ca^{2+} concentration (Brozovich *et al*., 2016).

Maxi-K channels, also known as Slo1 or BK_{Ca}, are one type of calcium-activated potassium channel (Marty, 1981). The maxi-K channel can be activated by several factors, such as Ca^{2+} transients released from the peripheral sarcoplasmic reticulum (Bolton and Imaizumi, 1996). In the blood vessels, maxi-K channel activation act to induce membrane hyperpolarization, leading to the L-type channel switching off (Baruah and Deka, 2022). Thus, reducing intracellular Ca^{2+} concentration and hence vascular relaxation (Lohn *et al*., 2000). The caveolins regulate intracellular calcium ions via the effective influence of maxi-K channels on L-type Ca^{2+} channels in smooth muscle cells to maintain vascular tone (Pereira da Silva *et al*., 2022). Several recent studies point out the colocalization of maxi-K channels with caveolin-1/caveolin-3 in various vascular tissues to understand the association between caveolin and maxi-K channels (Alioua *et al*., 2008; Riddle *et al*., 2011).

Endothelial BK_{Ca} channels and eNOS work in harmony to control vascular function together. BK_{Ca} channels activate membrane hyperpolarization and vasodilation, whereas eNOS generates NO to increase it. In this regard, caveolae house endothelial nitric oxide synthase (eNOS), an enzyme that produces NO (Ramadoss *et al*., 2013). At the same time, caveolae integrity is essential for regulating NO production from the endothelial cells (Razani *et al*., 2002). In contrast, caveolae disruption can lead to endothelial dysfunction, which is a common sign for many cardiovascular diseases, including hypertension and heart failure (Xu *et al*., 2007).

Caveolae in blood vessels have significant clinical importance due to their involvement in vascular functions such as regulating endothelial function, smooth muscle contraction and signaling pathways. In this regards, cholesterol is essential for the integrity of caveolae structure (Matthaeus *et al*., 2019). In addition, several ultrastructural studies indicate that caveolae are located close to the

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peripheral sarcoplasmic reticulum in smooth muscle cells (Sweeney *et al*., 2006). Caveolae disruption using a cholesterol-depleted agent such as MβCD has many effects on arteries, involving impairment of endothelium-dependent relaxation and close link between voltage-gated calcium channels and the sarcoplasmic reticulum (Xu *et al*., 2008). Accordingly, this causes a decrease in local Ca^{2+} spark frequency released from the sarcoplasmic reticulum, which leads to the deactivation of the maxi-K channel and hence induces smooth muscle cell contraction. In contrast, opening the maxi-K channel inhibits the L-type Ca^{2+} channel, reducing Ca^{2+} influx into smooth muscle cells, thus vasodilation (Taggart, 2001).

Understanding the function of caveolae in blood arteries is essential in improving targeted medicines to treat and prevent different diseases. So, caveolae disruption using a cholesterol-depleted agent such as MβCD represents a useful model for understanding the relationship between the maxi-K channels and caveolins in the caveolar domain and investigating the juxtaposition of maxi-K channels with the sarcoplasmic reticulum (Dreja *et al*., 2002). However, the relationship between the maxi-K channels within the caveolae and the sarcoplasmic reticulum has not been studied yet in rat femoral arteries. Therefore, the current study was designed to investigate the localization of maxi-K channels with caveolin-1 and/or caveolin-3 in smooth muscle and endothelial cells of the rat femoral artery using IHC and ICC. Also, to assess the topological relationship between caveolae and sarcoplasmic reticulum before and after caveolae disassembly using MâCD inhibitors by TEM.

MATERIALS AND METHODS

Animal groups and experimental procedures

In this study, sixteen adults male Wistar rats were divided into two groups; eight rats in each group (n= 8) and only one femoral artery was collected from each animal. All rats in both groups were killed by cervical dislocation, and femoral arteries were quickly dissected and collected for IHC, ICC, TEM and Western blot.

Research period

The current study was achieved between 2021-2022.

Control group

In this group, rat femoral arteries were incubated with normal saline and used as controls.

Treated group

In this group, rat femoral arteries (5-mm) were incubated with 5 mM MβCD for one hour to disassemble the caveolae from the plasma membrane of smooth muscle and endothelial cells.

Primary and secondary antibodies

Mouse anti-α-actin (1:300) and rabbit anti-von Willebrand factor (1:500). Mouse monoclonal anti-caveolin-1 (1:200); caveolin-1-scaffolding domain peptide (SDP) was mapped to amino acids 82-101 (DGIWKASFTTFTVTKYWFYR). Mouse monoclonal anti-caveolin-3 (1:500); caveolin-3 SDP was mapped to amino acids 55-74, (DGVWRVS YTTFTVSKYWCYR). Rabbit polyclonal anti-maxi-K channels (1:500) were also utilized. All primary antibodies were purchased from BD Biosciences, San Jose, USA, except Rabbit polyclonal anti-maxi-K channels were purchased from Alomone Labs, Jerusalem, Israel. All primary antibodies were diluted with Tris-buffered saline mixed with Tween 20 (TBST; 1% milk). Anti-mouse Alexa Fluor 488/Alexa Fluor 594 and anti-rabbit Alexa Fluor 488/ Alexia Fluor 594 secondary antibodies (dilution 1:500) were purchased from Molecular Probes, Inc., Eugene, USA).

Immunohistochemical preparation of rat femoral arteries

A small piece of the femoral arteries (5 mm in length) was embedded on a cork disk and then frozen in isopentane and cooled in liquid nitrogen. The blocks were cut into 12 µm-thick tissue sections and mounted on glass slides. The sections were fixed in 2% paraformaldehyde in phosphate-buffered saline (PBS; pH 7.4). The sections were then permeabilized using 0.1% Triton ×100 in PBS. Next, the blocking solution (1% BSA) was applied to block the non-specific binding of antibodies for 30 min at room temperature. The slides were incubated overnight at four °C with primary antibodies, followed by incubation with secondary antibodies at room temperature for 1 hour. After mounting, slides were examined under a confocal microscope (Leica SP5 white laser, Leica, Germany).

Preparation of individual smooth muscle cells isolated from the rat femoral artery

A small piece of the femoral artery (2–4 mm) was incubated in a low-concentration Ca^{2+} buffer for 40 min at 35°C. After the artery was digested, single cells were dispersed in this solution by trituration of the digested tissue using a fire-polished Pasteur pipette, as previously reported.

Immunofluorescence confocal microscopy

For immunofluorescence confocal microscopy, cryosections (12 µm-thick) of rat femoral arteries were fixed and permeabilized as described previously by Allen (2007). Double-immunofluorescence labeling was applied in this study to assess the colocalization of maxi-K channels with caveolin-1 and caveolin-3 in smooth muscle and endothelial cells of rat femoral tissue using two different methods: (i) double labeling of primary antibodies from different host species; and (ii) double labeling of primary antibodies from the same host species as described . Coverslips were mounted with an antifade mounting medium (Biomeda, Foster City, CA) and imaged using a confocal laser-scanning microscope (Leica).

Transmission electron microscopy

Small segment (0.5-mm) was collected from each rat from both groups. Artery segment from treated group treatment with 5 mM MβCD for one hour. Whereas artery segments

from control group were collected and prepared without treatment. The segments were fixed in PBS containing 2% glutaraldehyde and 1% paraformaldehyde for two hours. Samples were stored in PBS at 4°C until post-fixation for 1 hour with 1% (w/v) osmium tetroxide (OsO4) in 0.1 M sodium cacodylate for another hour. This was followed by a dehydration step in graded ethanol and embedded in resin. Ultrathin sections were cut with a thickness of 80 nm from each group and mounted on copper grids. Sections were then stained with 2% aqueous uranyl acetate for 5 min, then with 0.1 M lead citrate for 5 min and left to dry at room temperature. The grids were examined using TEM (JEOL-JEM-1010, Liverpool, UK) .

Western blot

Western blotting was performed to confirm the protein expression of caveolin-1, caveolin-3, maxi-K channels and á-actin in rat artery tissues. A total of 1 gram of the arterial tissue was mixed in lysis buffer and protease inhibitors (Santa Cruz Biotechnology, Dallas, TX). After that, the protein mixture was homogenized on ice using a blender for 8 min. The samples were then centrifuged at 12000 rpm for 10 min at four °C. The supernatant was stored at -80°C for further use. Briefly, 20 µg of femoral arterial protein extracts were separated on 10% polyacrylamide gels by SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were incubated with mouse monoclonal anti-caveolin-1 (dilution 1:200), mouse monoclonal anti-caveolin-3 (dilution1:500), rabbit polyclonal anti-maxi-K channels and (dilution 1:300) mouse monoclonal anti-α-actin (dilution1:300). The membranes were incubated with the designated secondary antibodies for two hours at room temperature. α-actin was used as a positive control for smooth muscle cells. The developed protein signal was detected using auto-radiographic Hyperfilm.

The fluorescence intensity of α-actin, von Willebrand factor, caveolin-1, caveolin-3 and maxi-K channels

The mean fluorescence intensity of all proteins (α-actin, von Willebrand factor, caveolin-, caveolin-3 and maxi-K channels) in endothelial and smooth muscle cells of the rat femoral artery and individual smooth muscle cells isolated was determined by ImageJ as described by Scott *et al.* (2018).

Approval ethics

This study and all experimental procedures were performed according to the principles of the Ethics Committee of Taif University, Taif, Saudi Arabia (Approval No. T-2023-523) which are in line with the Declaration of Helsinki.

Statistical analysis

All results were expressed as the means ±SD. For comparison of caveolae per micrometer in smooth muscle and endothelial cells, the mean was calculated from 8 independent samples. For comparison of fluorescence intensity of α-actin, von Willebrand factor, caveolin-1, caveolin-3 and maxi-K channels, the mean was calculated from 8 independent samples. Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA), followed by Tukey HSD post-hoc test, using the statistical package SPSS. The acceptable level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Effect of the luteolin treatment against the oxidant/ antioxidant status following acetamiprid exposure of the cerebral cortex

The stained sections with anti-α-actin showed an unequivocal red signal localized in the smooth muscle cell layer but completely absent in the endothelial cell layer (Fig 1A “a”). The stained sections with the von Willebrand factor showed a strong green signal observed only in the endothelium cell layer (Fig 1A “b”). As expected, rat artery tissues had no overlapping between α-actin and von Willebrand factor (Fig 1A “c”). In addition, the fluorescence intensity of the α-actin and von Willebrand factors was analyzed in smooth muscle and endothelial cells. The results revealed a significant expression of von Willebrand factor in the endothelial cells compared to smooth muscle cells. In contrast, the fluorescence intensity showed a significant expression of á-actin in the smooth muscle cells compared to á-actin in the endothelial cells (Fig 1A “d”).

Western blotting was performed to examine the specific expression of caveolin-1, caveolin -3, maxi-K channels and á-actin in protein lysates extracted from rat femoral arteries. As expected, specific single bands of caveolin-1, caveolin-3 and maxi-K channels were detected at the expected protein size (Fig 1B). When the membrane was stripped and probed with anti-α-actin, it exerted another band of α-actin. These findings confirmed the specificity of antibodies and the expression of the three proteins in the smooth muscle cells.

Expression of caveolins-1 and caveolins-3 in endothelial cells of rat femoral arteries

Endothelial cells had positive red staining for von Willebrand factor, whereas smooth muscle and endothelial cells stained green for caveolin-1 (Fig 2A-B). The yellow color represents the colocalization of caveolin-1 and von Willebrand factor in endothelium cells (Fig 2C). The fluorescence intensity of caveolin-1 was analyzed in smooth muscle and endothelial cells. The results revealed a non-significant expression of caveolin-1 in the endothelial cells compared to smooth muscle cells (Fig 2D). The same pattern was identified in other tissue sections stained with von Willebrand factor and caveolin-3 (Fig 2E, F and G). Interestingly, caveolin-3 and von Willebrand factor were not overlapped in endothelial cells. The results of the fluorescence intensity showed that caveolin-3 was significantly expressed in smooth muscle compared to endothelial cells (Fig 2H). This suggests that caveolin-3 is expressed in smooth muscle cells but not in endothelial cells.

Expression of maxi-K channels in the endothelial and smooth muscle cells of rat femoral arteries

The endothelial cells were stained red with von Willebrand factor and stained green with maxi-K channels (Fig 3A and B). The staining of maxi-K channels was colocalized with von Willebrand factor in endothelial cells (Fig 3C). The stained sections with anti-α-actin and anti-maxi-K channels showed strong green and red signals, respectively, in the smooth muscle cell layer (Fig 3E and F). The two proteins colocalized in the smooth muscle cell layers (Fig 3G). Analysis of the fluorescence intensity showed a non-significant expression of maxi-K channels in smooth muscle cells compared to endothelial cells. The results also showed that maxi-K channels were significantly expressed in endothelial cells compared to á-actin. They found the same pattern, showing that maxi-K channels were significantly expressed in smooth muscle cells compared to the von Willebrand factor (Fig 3D and H).

Colocalization of maxi-K channels with caveolins-1 and caveolins-3 in rat femoral arteries

Staining sections with anti-caveolins-1 and anti-caveolins-3 exerted a homogenous expression of the two caveolins in smooth muscle cells and only caveolin-1 in the endothelial layer as a green signal (Fig 4A and F). When the sections were stained with anti-maxi-K channels, an unequivocal red signal was observed in smooth muscle

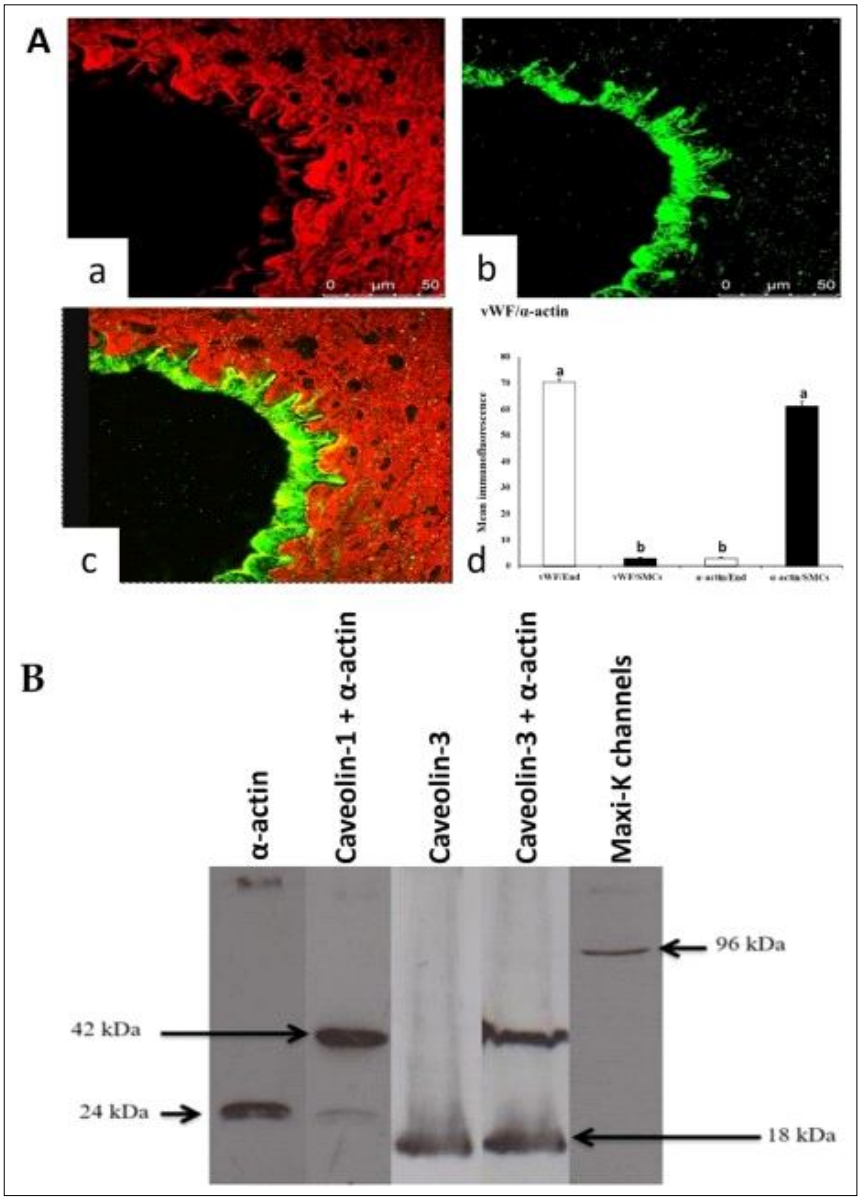

Fig 1 A: Confocal images show frozen sections of the femoral artery stained with α-actin and von Willebrand factor. **B:** Western blot analysis results of the femoral artery.

cells and endothelial cells (Fig 4B and F). Overlaying the two colors exhibits yellow color, which represents colocalization of caveolin-1 and 3 with maxi-K channels, as shown in Fig 4C and G.

Analysis of the fluorescence intensity showed a non-significant expression of maxi-K channels compared to caveolin-1 in the smooth muscle cells or the endothelial cells. The results also showed that maxi-K channels were significantly expressed in endothelial cells compared to caveolin-3. In contrast, maxi-K channels were not significantly expressed in smooth muscle cells compared to caveolin-3 (Fig 4D and H).

Colocalization of maxi-K channels with caveolin-1 and caveolins-3 in individual smooth muscle cells isolated from rat femoral arteries

The isolated smooth muscle cells showed a homogenous expression of maxi-K channels on the plasma membrane and cytosol of cells as a red signal (Fig 5A and E). Caveolins-1 and caveolins-3 expressions were observed as a green signal spread throughout the cytosol and plasma membrane of the smooth muscle cell (Fig 5B and F). Staining of maxi-K channels was overlapped with caveolin-1 and 3, as indicated by the yellow color in the cytosol and plasma membrane (Fig 5C and G). Analysis of the

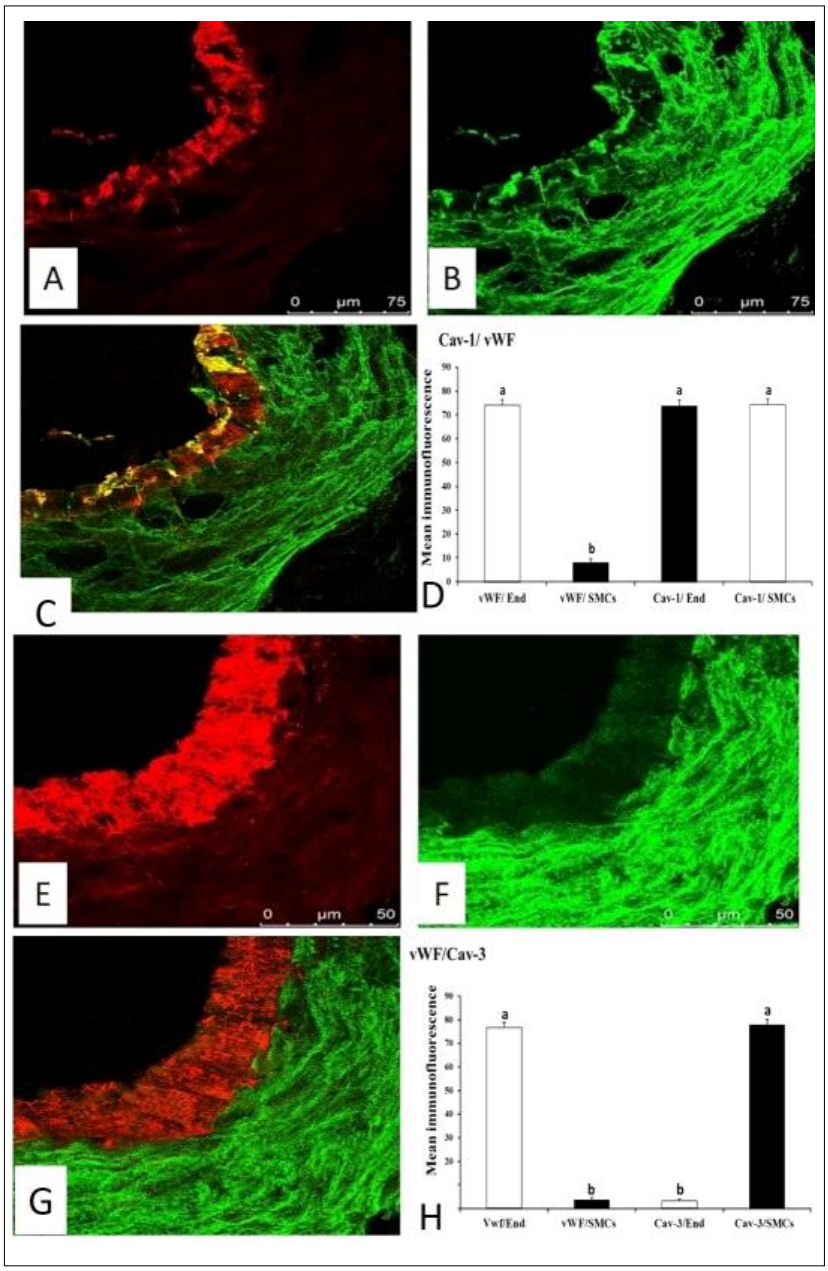

Fig 2: Confocal images shows frozen sections of the femoral artery stained with caveolin-1, caveolin-3 and von Willebrand factor.

fluorescence intensity showed a non-significant expression of maxi-K channels compared to caveolin-1 or caveolin-3 in the isolated single smooth muscle cell (Fig 5H).

Caveolae in the cellular membrane of the smooth muscle and endothelial cells of rat femoral arteries before and after MβCD treatment

TEM examination of the smooth muscle and endothelial cells showed that the caveolae appear as omega-shaped or tubular invaginations within the plasma membrane. The cells also revealed nanocontacts between the caveolae and sarcoplasmic reticulum. The sarcoplasmic reticulum was concentrated at the periphery of the cell close to the cell membrane (Fig 6A, a, B and b). When the arteries were treated with MâCD, the smooth muscle and endothelial cells showed the removal of caveolae from the plasma membrane. TEM also showed that caveolae abolishment by MâCD led to the abolition of the coupling of caveolae and the sarcoplasmic reticulum as shown in Fig 6C, c, D and d.

The current study presents the relationship of maxi-K channels with caveolin-1 and caveolin-3. Also, to examine the relationship between the coupling of maxi-K channels within the caveolar domain and the sarcoplasmic reticulum in rat femoral arteries. These results were corroborated by four different methods, including HC, ICC, TEM and Western blotting.

The smooth muscle layer is the primary determinant of blood flow in the conducting arteries. In blood vessel tissue, proteins of the maxi-K channel interact with caveolin-1 and caveolin-3 in the caveolar domain, which leads to

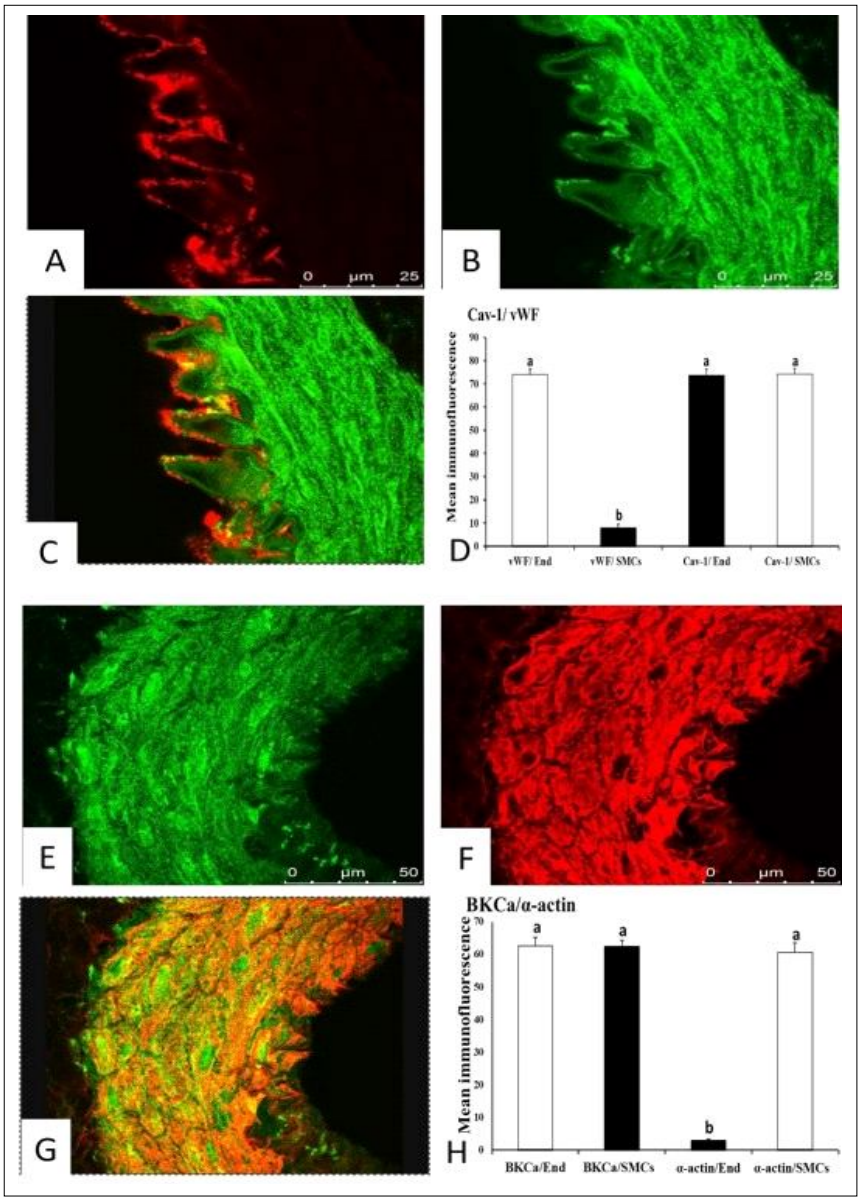

Fig 3: Confocal images shows frozen sections of the femoral artery stained with von Willebrand factor, maxi-K channels and α-actin.

regulating cellular Ca^{2+} homeostasis in conducting arteries (Riddle *et al*., 2011).

Results of the IHC of the current study showed a cellular colocalization of maxi-K channels with caveolin-1 in smooth muscle cells and endothelial cells and with caveolin-3 exclusively in the smooth muscle cell layer. Additionally, ICC showed colocalization of maxi-K channels and caveolin-1 at the freshly-isolated smooth muscle cells. These results suggest that the caveolin-1 scaffolding domain peptide (DGIWKASFTTFTVTKYWFYR, amino acid residues 82–101) may interact with binding motifs, C terminates (1007YNMLCFGIY1015) in the maxi-K channels. These results confirm that the caveolin-1 scaffolding domains serve as anchor sites to maxi-K channels within caveolae and thus might regulate maxi-K channels. The results of this study agree with (Feher *et al*., 2010) , who showed that the maxi-K channels interact with caveolin-1 in smooth muscle and endothelial cells of frozen sections of intact gracilis artery using IHC. Moreover, Alioua *et al*. (Alioua *et al*., 2002), showed expression of caveolin-1 and maxi-K channels in intact aortic myocytes using IHC. Also, these results decide with Brainard *et al*. (2005), who showed that the maxi-K channels interact with caveolin-1 in human myometrial smooth muscle cells (cultured cells) using ICC.

Several prior studies have reported that the expression of caveolin-3 is muscle-specific and found in cardiac and skeletal muscle, as well as smooth muscle cells

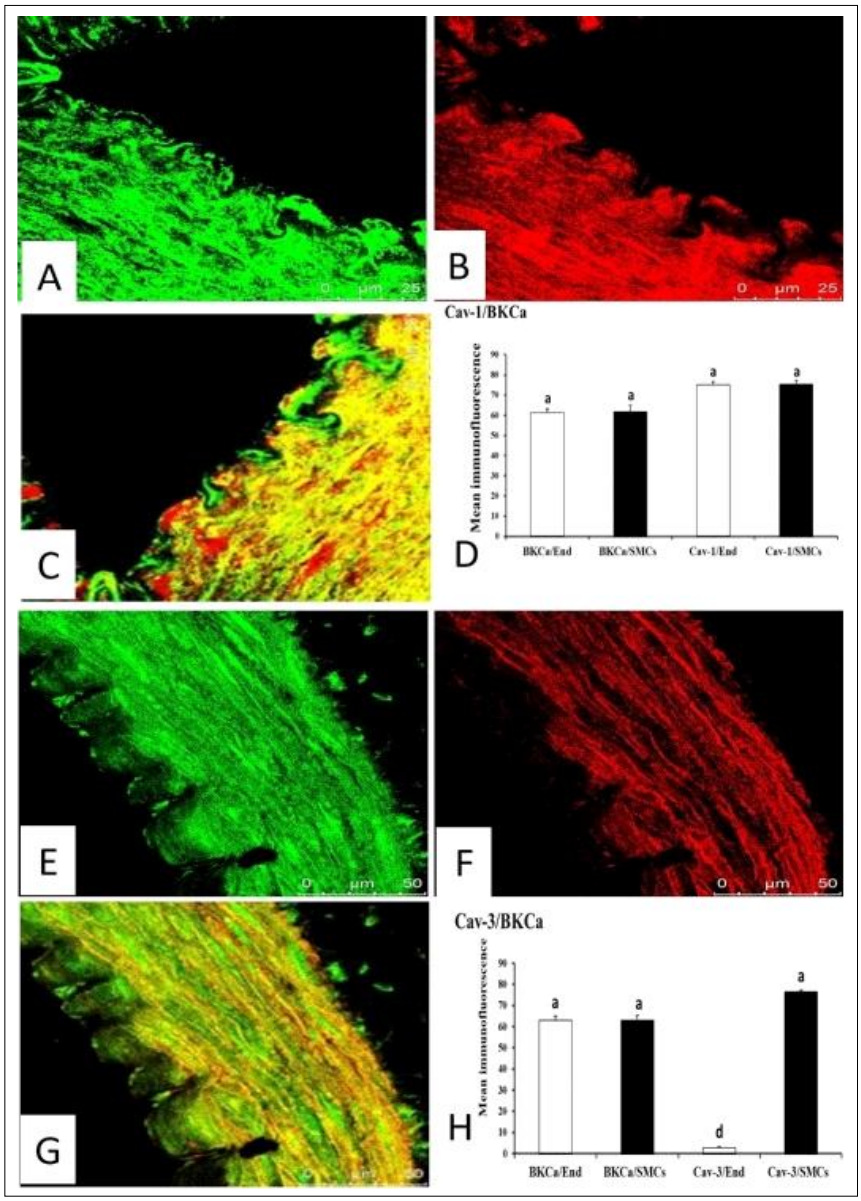

Fig 4: Confocal images shows frozen sections of the femoral artery stained with caveolin-1 and maxi-K channels.

(Engelman *et al*., 1998). Additionally, Kamishima *et al*. (2007) suggested that caveolin-3 is essential in the Ca^{2+} removal of conducting artery's smooth muscle cells. However, the role of caveolin-3 in regulating other ion channels in smooth muscle cells is still controversial. Results of IHC and ICC showed the colocalization of maxi-K channels with caveolin-3 in the smooth muscle cells layer and at the individual smooth muscle cells. These findings suggested that caveolin-3 scaffolding-domain peptide (DGVWRVSYTTFTVSKYWCYR, amino acid residues 55–74) may interact with binding motifs at the C terminus (1007YNMLCFGIY1015) in maxi-K channels of rat femoral artery smooth muscle cells. Therefore, these results may confirm that the caveolin-3 scaffolding domains serve as anchor sites to anchor several proteins within caveolae, thus regulating many signaling molecules. These results agree with Jiang *et al.* (2005), who showed colocalization of maxi-K channels with caveolin-1in bovine aortic endothelial cells (cultured cells) using ICC.

In vascular smooth muscle, the opening of the maxi-K channel inhibits the voltage-gated calcium channels, reducing Ca^{2+} influx into smooth muscle cells, thus vasodilation (Kaczorowski *et al*., 1996). Recent studies have focused on the ultrastructure of vascular smooth muscle cells to examine the relationship between caveolae and sarcoplasmic reticulum contacts in the smooth muscle cells (Popescu *et al*., 2006). The caveolae's proximity to the superficial sarcoplasmic reticulum, about 20 nm in smooth muscle cells, may explain the importance of sarcoplasmic reticulum to maxi-K channels in caveolar membranes.

TEM showed caveolae covering most of the plasma membrane smooth muscle cells. Also, caveolae were close to the peripheral sarcoplasmic reticulum in intact smooth muscle cells of the femoral arteries. These results agree with Popescu *et al.* (2006), who stated caveolae are within a few nanometers of the sarcoplasmic reticulum in smooth muscle cells of the vascular artery. Moreover, these results agree with (Gherghiceanu and Popescu, 2007). Saliez *et al.* (2008) demonstrated the caveola juxtaposition of the sarcoplasmic reticulum in smooth muscle cells of the rat urinary bladder. In addition, This finding also agrees with other studies conducted by , who reported that the caveolae were localized in juxtaposition to the sarcoplasmic reticulum in the vascular smooth muscle cells of different arterial beds.

TEM examination of endothelial cells in intact rat femoral arteries showed caveolae covering the endothelial

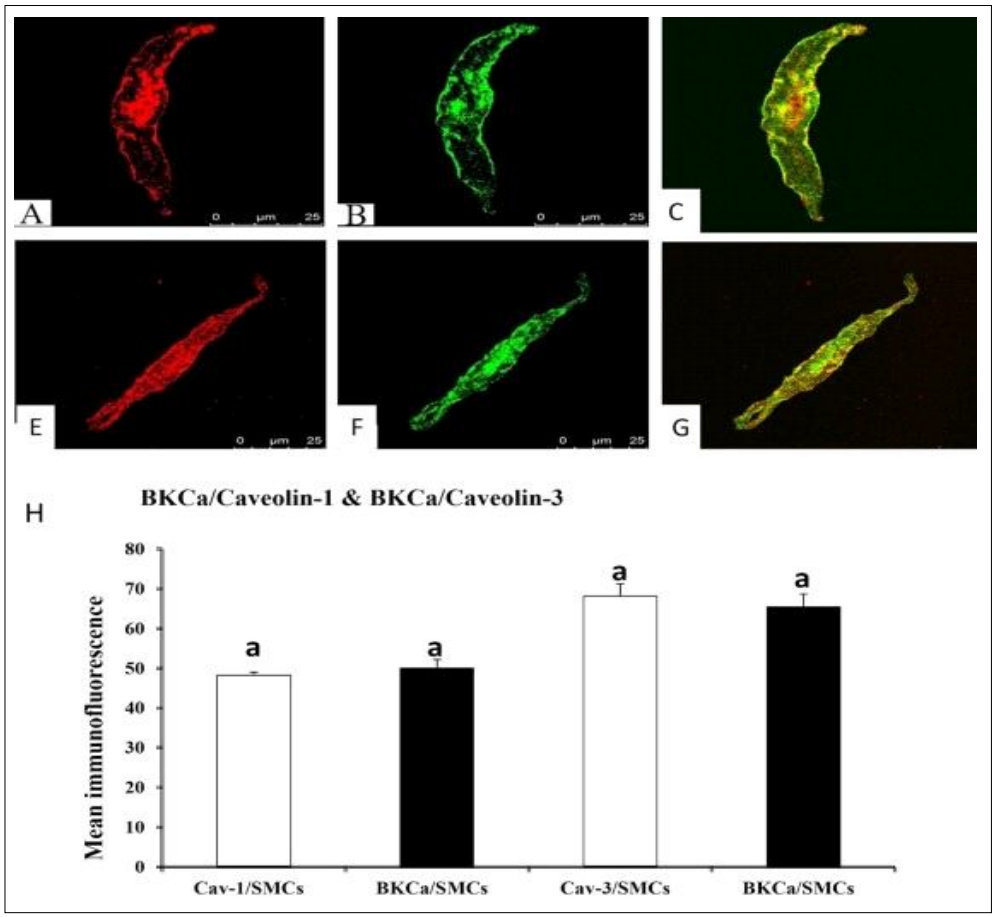

Fig 5: Confocal images shows frozen sections of the femoral artery stained with maxi-K channels, caveolin-1 and caveolin-1.

cells' plasma membrane. These findings were consistent with Razani *et al.* (2002). This study showed that caveolae are present in the plasma membranes of the endothelial cells of rat aorta. The results of this study agree with Saliez *et al.* (2008), who demonstrated the presence of caveolae in the endothelial cells of rat mesenteric arteries. Moreover, the current study also did not observe any sarcoplasmic reticulum in the endothelial cells of the rat femoral artery. A similar result was shown by Linder *et al.* (2005), who did not observe any sarcoplasmic reticulum in the endothelial cells of rat aorta.

Multiple prior studies have reported that the integrity of caveolae is essential for nitric oxide synthesis in the vascular endothelial cells of rat mesenteric arteries (Shaul, 2002; Cohen *et al*., 2004). TEM revealed the disassembling of caveolae in the plasma membranes of smooth muscle and endothelial cells following MβCD treatment. These findings aligned with studies on smooth muscle cells, where MβCD disassembled caveolae from the plasma membrane (Dreja *et al*., 2002; Bergdahl *et al*., 2003). Also, they reported that the disproportion of the caveolae by MβCD might lead to disproportion and/or redistribution of the caveolae in the plasma membrane, which increases the gap between the caveolae and sarcoplasmic reticulum (Gherghiceanu and Popescu, 2007).

The Western blot results of the current study showed the expression of caveolin-1, caveolin-3, α-actin and maxi-K channels in rat femoral artery lysates. These results were partially decided by Feher *et al.* (2010), who showed the expression of caveolin-1, Maxi-K channels and á-actin in the coronary arteries using Western blot. Our findings also agreed with Sampson *et al*. (2004), who reported the expression of caveolin-1 and caveolin-3 in rat arterial tissues using Western blot.

The current study is essential for understanding the caveolae-sarcoplasmic reticulum relationship and its importance in regulating calcium ion concentration in

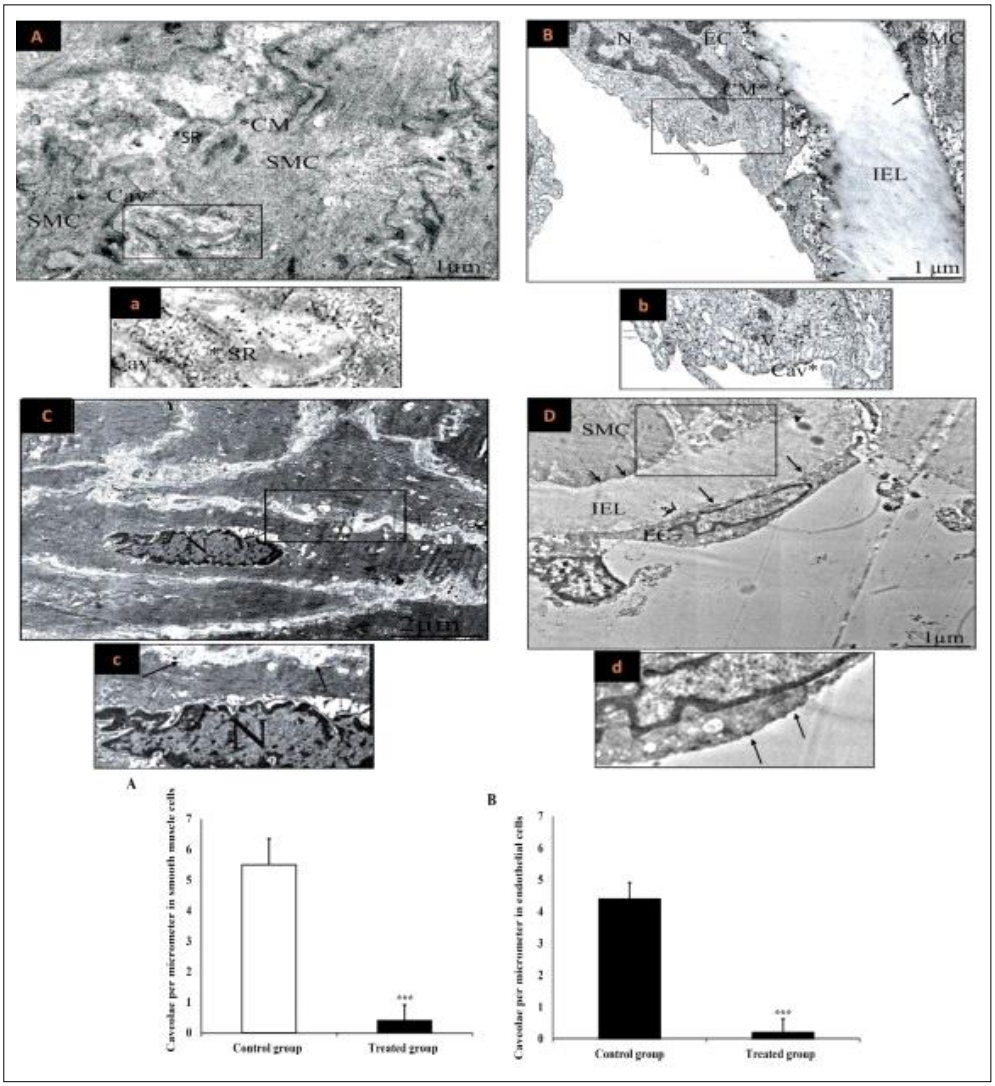

Fig 6: Transmission electron micrographs of the rat femoral artery.

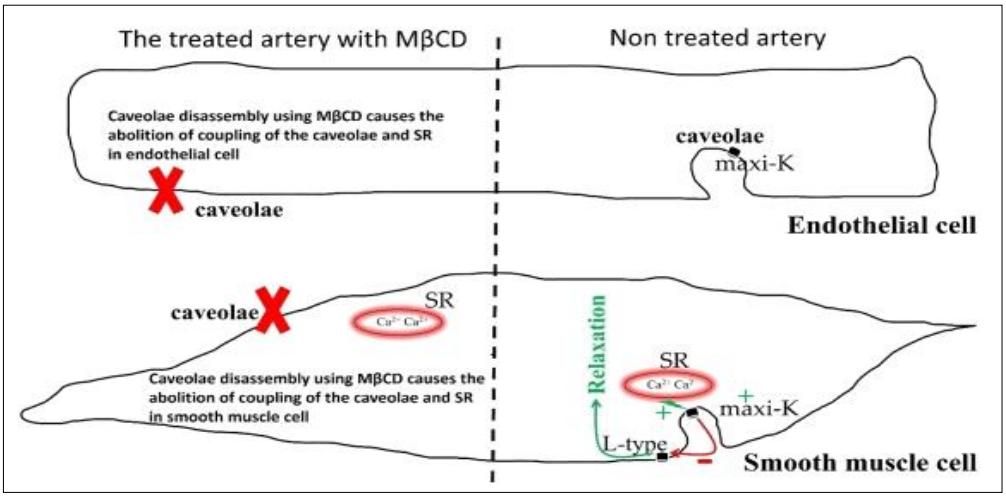

Fig 7: Summary figure of the rat femoral artery before and after treatment with MβCD.

smooth muscle and endothelial cells (Shaw *et al*., 2006). However, these results may reveal the role of caveolae in regulating signaling pathways in smooth muscle cells and, hence, the contractility of the blood vessels, as suggested in the summary figure (Fig 7).

CONCLUSION

This study concluded that maxi-K channels are localized to caveolae of rat femoral arteries, whereas caveolin-1 scaffolding domains serve as anchor sites to anchor maxi-K channels in smooth muscle cells and endothelial cells and caveolin-3 serves as anchor sites to anchor maxi-K channels in smooth muscle cells only. On the other hand, disassembling the caveolae by MβCD abolish the coupling of the caveolar domain and the sarcoplasmic reticulum, due to the disruption of the caveolae.

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Conflict of interest

The author has no conflicts of interest to declare. The author has seen and agreed with the manuscript's contents, and there is no financial interest to report.

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