Role of cathepsin Kinhibitor and atorvastatin in protection against atherosclerosis in high fat diet-fed rats

Accepted 12th June, 2020

ABSTRACT

Cathepsin K (CatK) is a member of Cathepsins, a family of Cysteine protease enzyme in Lysosomes. CatK plays a role in atherosclerosis leads to reduce the ability of lipid-free apoA-I to induce cholesterol efflux from macrophages, promoting the progress of foam cells in atherosclerosis. To explore whether a combination of cholesterol lowering drug (atorvastatin) and CatK inhibitor may offer more improvement in derangement of lipid profile in atherosclerotic lesion in rats fed high fat diet. The rats were divided randomly into 5 experimental groups (normal diet group; normal diet (ND), while the other 4 groups were fed high fat diet for 8 weeks. The HFD rats group (32 rats) was subdivided as follows: (Group I; HFD) in which rats received HFD only, (Group II; Atorvastatin treated group) in which rats received Atorvastatin (2 mg/kg/day), (Group III; Balecatib -CatK inhibitor treated), (Group IV; combination treated group of atorvastatin and CatK inhibitor). Serum TC was reduced in Atorvastatin- and CatK inhibitor treated groups showed less remarkable degeneration and fewer foam cells and mononuclear cells. Balecatib in the current study is exploring an effect on CatK biochemically and histopathologically leading to attenuation of the inflammatory atherosclerotic process.

Key words: Cathepsin Kinhibitors, atorvastatin, atherosclerosis, high fat diet-fed rats.

INTRODUCTION

Cathepsin K (CatK) is a member of Cathepsins, a family of Cysteine protease enzymes that is localized in Lysosomes under physiological conditions (Lutgens et al., 2007). However, under pathological conditions Lysosomal leakage could occur releasing them into the cytoplasm with their strong Elastase and Collagenase properties (Barascuk et al., 2010). Previous studies have reported new roles for CatK in pathological conditions, such as arthritis, metabolic disorders and atherosclerosis, and further substantial observations have added CatK to the list of newly emerged factors involved in the onset and progression of the atherosclerotic lesion (Lutgens et al., 2006; Chatzizisis et al., 2012). Also, a role of CatK in abnormalities of lipid profile has been postulated. Specifically, CatK is shown to reduce the ability of lipid-free apoA-I to induce cholesterol efflux from macrophages, thus promoting the onset and development of foam cells in atherosclerotic lesions (Lindstedt et al., 2003).

On the other hand, the use of Statins as low density lipoproteins (LDL) – lowering drugs is not aiming only at
reducing the retention of LDL in intima and atherosclerotic plaque but also on their anti-inflammatory effect. However, it is argued that such anti-inflammatory properties of Statins are not complete to offer complete clearance at the plaque level leading to imbalance between pro-inflammatory and anti-inflammatory mediators (Yang et al., 2008). Thus, the aim of the present study is to explore whether a combination of LDL-C lowering drug (atorvastatin) and CatK inhibitor may offer more improvement in derangement of lipid profile and in the development of atherosclerotic lesion in rats fed high fat diet.

MATERIALS AND METHODS

Animals and experimental groups

Experiments were done according to the Guide for Care and Use of Laboratory Animals approved by our local committee of Animal Care and Use. Forty Sprague Dawely male rats aged 4 weeks (weight about 100 gm) were obtained from and housed at the animal house, Medical Experimental Research Centre (MERC), Mansoura Faculty of Medicine, Mansoura University. Rats were maintained on a standard 12-h light-dark cycle in a temperature controlled environment (about 22-24°C) with access to water and standard rat chow for one week before the start of experimental protocol. The rats were divided randomly into 5 experimental groups (each = 8 rats) as follows: One group (B) of rats were fed standard rat chow (normal diet group; normal diet (ND), while the other 4 groups were fed high fat diet for 8 weeks (thus the rats grew up from young age to adult while on high fat diet). High fat diet comprised of 58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal respectively (Srinivasan et al., 2005). The HFD rats group (32 rats) was further subdivided as follows: (Group I; HFD) rats received HFD only, (Group II; Ator-treated group) in which rats received Atorvastatin (2 mg/kg/day, Lunder et al., 2013), (Group III; CatK inhibitor-treated) in which rats received Balicatib - CatK inhibitor (3 mg/kg/day, Fujii et al., 2015) (Group IV; combination treated group) in which rats received a combination of atorvastatin and CatK inhibitor. This protocol was scheduled for the following 8 weeks. All procedures were performed according to the guidelines of Research Ethics Committee of Mansoura Faculty of Medicine.

Blood sampling and tissue collection

At the end of experimental protocol, and after 12 h fasting, the rats were weighed and then killed by overdose of sodium thiopental (50 mg/kg body weight). Blood samples were drawn from the heart on dry vials and to left to clot. Then, serum was centrifuged, divided into aliquots and frozen at -20 °C for subsequent biochemical assay. The thoracic and abdominal aorta was dissected, weighed and divided into 2 parts. One part was snapped in liquid nitrogen for RNA extraction, while the other part was placed in 10% buffered neutral formalin for subsequent histopathological assessment.

Biochemical measurements

Assay of serum total cholesterol, LDL, HDL and triglycerides:

Serum lipid profile was assessed by diagnostic kits purchased from Spinreact Company (Spain). Measuring of Serum triglycerides (TGs) was done by enzymatic colorimetric GPO-POD method (Fossat, 1982), while total cholesterol was done by enzymatic colorimetric CHOD-POD method (Meiattini et al., 1987) Serum HDL-cholesterol concentration was measured by colometric phosphotungstic precipitation method (Burstein et al., 1970) whereas LDL levels were calculated using the Friedewald equation (Friedewald et al., 1972).

Determination of CRP

Serum CRP levels were measured using CRP (rat) ELISA Kit (Biovision, Milpitas, CA, USA). This assay employs a quantitative sandwich enzyme immunoassay technique that measures rat CRP. The final absorbance of the samples in a microplate reader is at 450 nm (BioTek ELx800, USA). The serum concentrations of CRP are determined by using the standard curve and multiply the value by the dilution factor.

Study of monocyte chemoattractant protein 1 (MCP-1) and intercellular adhesion molecule 1 (ICAM-1) mRNA expression in aortic segments

The transcription of ICAM-1 and MCP-1 mRNA in rat thoracic aorta was determined through the use of real-time PCR. Total RNA was extracted from rat thoracic aorta homogenate with RNeasy mini kit (Qiagen, Hilden, Germany) according to manufacturer’s protocol. RNA quality and concentrations were assessed using the Nanophotometer P-Class (Implen, Gmbh, Munich, Germany). RNA concentrations ranged from 0.04-0.15 μg/μl and an optical density at wave length 260/280 nm range of 1.8-2.2 were chosen for this study. The extracted RNA was then stored in liquid nitrogen until reverse transcription. Two micrograms of total RNA were
reverse transcribed to complementary DNA (cDNA). This was achieved using Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, Massachusetts, USA) according to manufacturer's guidelines. The reaction was set up in the thermal cycler Block 5020 (Thermo Fisher Scientific, Ratakse, Vantaa, Finland). The reaction was set up incubation for 60 min at 42°C followed by heating to 70°C for 5 min for termination.

Reverse Transcriptase Semi-Quantitative Real-time PCR (RT-qPCR). Briefly, RT-qPCR reactions were carried out using Maxima SYBR Green/ROX qPCR Master Mix (2X) (Thermo Scientific) on a Real time thermal cycler (Thermo Fisher Scientific, Ratakse, Vantaa, Finland). The final volume for each reaction was 25 μl with 0.3 μM of corresponding gene specific primers, and 5 μl of total cDNA. Primers for ICAM-1 and MCP-1 were selected based on published sequences (Wu et al., 2018; Tohru et al., 2003). The primer sequences for ICAM-1 were as follows: F: 5’-GGTGAGCAGACAGCTACCTATTGACAT-3′ and R: 5’- CAGGCGGCT CAGTGTCTCATT -3′, which give 103 bp product size. While, the primer sequences for MCP-1 were: F: 5’- CAGATCCTCTTCTCCACACATT -3′ and R: 5’- CAGGCAAGAATGTGAACAC -3′, giving 73 bp product size. The expression of ICAM-1 and MCP-1 were normalized to the expression of the internal reference (GAPDH) and the primers used were: F: 5’- TTGTGCACTGCCAGCTCGT-3′ and R: 5’- TGGCTTTGACACTGCGGTGG -3′, which give 201 bp product size.

The thermal cycling was initiated at 95°C for 10 min followed by 40 cycles of 5 s at 95°C and 60 s at the optimal annealing temperature 60°C. Dissociation curve analyses were carried out at the end of each run for PCR product verification. The baseline and threshold were adjusted then data from amplification plot were obtained and analyzed. The data were presented as relative quantification (RQ) of target mRNA, normalized in respect to housekeeping gene (GAPDH) and relative to control sample (calibrator). RQ was performed using the ΔΔCt method (Livak and Schmittgen, 2001).

### Histopathological examination

Aortic tissues were harvested, fixed in 10% buffered formaline, and embedded in paraffin. Paraffin-embedded tissues were cut into 5 μm-thick cross sections and deparaffinized prior to staining using a standard protocol. 

Hematoxylin and Eosin staining was used for examining the morphology and detecting the lesion area. Sirius red staining was used for detecting collagen content. Sections were examined using light microscopy (Olympus BX-50, Tokyo, Japan) and photographed with a high-resolution digital camera (Olympus LC20- Japan). Statistical Package for Social Science software computer program version 23 (SPSS, Inc., Chicago, IL, USA) was used for quantification and analysis.

### Chemicals

**Atorvastatin** (powder, 10 mg supplied by Sigma chemical co. St. Louis, USA). **Balicatib** (powder, 10 mg was supplied by Clini lab company, Egypt). The chemicals and reagents were purchased from Sigma (Sigma Chemical Co., St Louis, MO, USA). **Balicatib** and **Atorvastatin** were dissolved in corresponding solvent according to manufacture guidelines. **Balicatib** aliquots were preserved at 20°C to be used daily, whereas **Atorvastatin** was freshly prepared daily.

### Statistical analysis

All results are expressed as mean ± SD. One-way ANOVA test was used to compare the means of groups. P < 0.05 was considered significant. Data analysis was performed using the SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA).

### RESULTS

**Effect of HFD on body weight (BW) and lipid profile**

Table 1 shows change in BW induced by feeding HFD. As expected HFD increased final BW as compared to BW of rats fed the standard chow-diet. Feeding rats HFD increased significantly serum level of TC, LDL and TG while

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ND</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (gm)</td>
<td>263±5.2</td>
<td>395***±9.08</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>83.16±5.29</td>
<td>118.16***±8.81</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>37.03±4.89</td>
<td>75.46***±9.34</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>47.03±6.42</td>
<td>26.13***±3.9</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>51.03±6.06</td>
<td>103.45***±9.51</td>
</tr>
</tbody>
</table>

Table 1: Comparison of body weight and Lipid profile in normal diet fed rats (ND) and high fat diet fed rats (HFD) (n= 8 rats each). All results are mean ±SD.
Table 2: Serum level of total cholesterol (TC), low density lipoproteins (LDL), high density lipoproteins (HDL) and triglycerides (TG) in high fat diet (HFD), HFD fed rats receiving Atorvastatin (HFD+ Ator), HFD fed rats receiving Cathepsin K inhibitor (HFD+ CatKI) and in HFD rats receiving both drugs (n=8 rats each).

<table>
<thead>
<tr>
<th></th>
<th>HFD</th>
<th>HFD + Ator</th>
<th>HFD + CatKI</th>
<th>HFD+ Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dL)</td>
<td>118.16±8.81</td>
<td>94.89***±9.59</td>
<td>90.48***±8.69</td>
<td>91.09***±6.37</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>75.46±9.34</td>
<td>50.07***±6.92</td>
<td>45.44***±8.04</td>
<td>44.36***#±5.58</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>26.13±3.9</td>
<td>38.06**±7.29</td>
<td>39.30**±6.94</td>
<td>43.87***±8.27</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>103.45±9.51</td>
<td>60.18***$$±7.26</td>
<td>78.12***±2.39</td>
<td>56.32***$$±7.09</td>
</tr>
</tbody>
</table>

Figure 1: Serum level of C-Reactive Protein (CRP) in high fat diet fed rats (HFD), HFD fed rats receiving Atorvastatin (HFD+ Ator), HFD fed rats receiving Cathepsin K inhibitor (HFD+ CatKI) and in HFD rats receiving both drugs (n=8 each). All values are mean ±SD. **, ***p< 0.001, 0.0001 respectively each group vs. HFD group.

HDL was significantly reduced.

Lipid profile; total cholesterol, LDL, HDL and TG

Table 2 shows that serum TC was significantly reduced in Ator-treated group or CatKI or combined one, when compared to HFD group. There was no significant difference between the three treated groups. The same also was noticed regarding LDL level as it was reduced significantly in the three treated group; Ator, CatKI and combined. However, its level was significantly reduced in combined when compared with Ator- treated rats. For HDL, both Ator and CatKI increased its serum level as compared to HFD group. However, the combination induced greater improvement in HDL-c level. On the other hand, Ator has greatly reduced TG serum level as compared to HFD group (60.18 ±7.26 vs. 103.45 ±9.51 mg/dL). Also, in rats received CatKI and in combination group, TG level was significantly reduced to 78.12 ±2.39 and 56.32 ±7.09 mg/dL respectively. Comparison of the three treated groups showed that Ator and combination has induced greater reduction in TG.

Calculation of atherogenic Index of plasma (AIP)

AIP was measured with formula: \( \log_{10} \left( \frac{\text{TG}}{\text{HDL}-\text{C}} \right) \) (Dobiásová and Frohlich 2001). The plasma parameter \( \log \left( \frac{\text{TG}}{\text{HDL}-\text{C}} \right) \) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FER(HDL). Comparing the different groups studied, AIP was significantly higher in HFD as compared to treated groups while it was almost zero in ND group.

Circulating inflammatory marker; serum C-reactive protein (CRP)

Regarding serum level of inflammatory marker CRP, its level was reduced greatly in HFD+ Ator group as compared to HFD group (0.674** ±0.119 vs. 0.878 ±0.049 respectively). Its level in HFD + CatKI group was also significantly reduced to 0.716±0.051** and in HFD + combination group it was 0.655** ±0.052. Comparing the serum level of CRP in the three treated rats group, there was no significant difference (Figure 1).
**Expression of ICAM-1**

Relative quantification of mRNA level of ICAM-1 in aortic tissue using RT-qPCR showed great elevation in HFD rats compared to normal diet fed rats (mean ±SD vs. mean ±SD respectively, p<0.0001). On the other hand, treated rat groups fed on HFD showed a significant reduction in ICAM-1 mRNA levels compared to HFD rats (p<0.0001). Comparison of the three treated groups showed that combination group has induced greater reduction in ICAM-1 mRNA levels compared to CatKI group (p<0.01). Also, there was more decrease in Ator group compared to CatKI group even though it did not reach statistical significance (p>0.05) (Figure 2a and b).

**Expression of MCP-1**

Regarding the expression of MCP-1 mRNA in aortic tissue, it also showed a great elevation in HFD rats compared to ND rats (mean ±SD vs. mean ±SD respectively, p<0.0001). A great reduction was found in treated groups compared to HFD rats (p<0.0001). In particular, treatment with CatKI decreased MCP-1 transcription in HFD rats as well as in
combination rats compared to Ator group (p < 0.01) (Figure 3a and b).

**Histopathological examination of aortic segments**

H&E staining showed the atherosclerotic lesion area at the aortic wall. No pathologic changes had been observed in normal diet group. However, numerous foam cells and mononuclear cell infiltration together with nuclear condensation in medial smooth muscle cells were observed in the aortas of HFD (Figure 4a). In comparison to HFD group, the three treated groups; group III, IV, and V showed less remarkable degenerative changes and fewer foam cells and mononuclear cells. The combined administration of Atorvastatin and CatK inhibitor resulted in much stronger effects than the use of Atorvastatin or CatK inhibitor alone. The average plaque lesion area was 461.6±115µm² in HFD group, 94.09±23.5 µm² in Atorvastatin treated group (P<0.001 relative to HFD group), 214.1±53.53 µm² in CatK inhibitor treated group (P<0.001* relative to HFD group, P=0.01 relative to Ator group), and 18.04±4.50 µm² in the combined treatment group (P<0.001* relative to HFD group, P=0.18 relative to Ator group, P<0.001 relative to CatK inhibitor group) (Figure 4b). Sirius-red staining, revealed an increased amount of interstitial fibrosis in the aortic media of the HFD group as compared to the ND group with significant difference (P<0.001). Collagen content was significantly lower in groups IV, and V as compared to HFD group (P<0.001) (Figure 4c).

Figure 3: (a) Expression of MCP-1 in thoracic aortic tissues. 3% agarose gel electrophoresis of RT PCR products of MCP-1 (73bp). Lane 1: normal diet fed rats, Lane 2: HFD rats, Lane 3: HFD+CatKI group, Lane 4: HFD+Ator group, Lane 5: HFD + combination group, M:50bp DNA ladder. (b) MCP-1 expression in aortic tissue +++p<0.0001 HFD vs. ND group; **p<0.0001 each group vs. HFD group; #p<0.01 each group vs. Ator group.
DISCUSSION

One of the major adverse effects of obesity is the deranged lipids metabolism that is linked to development of Atherosclerosis. Cathepsin k (CTSK) family is a papain-like Cysteine peptidase that is expressed in high levels in Osteoclasts. Its physiological role primarily was identified as degradation of type I collagen. Several CTSK inhibitors have been developed to reduce the excessive bone matrix loss associated with osteoporosis. Besides Osteoclasts, CTSK was also expressed in visceral adipose tissue of obese patients and CTSK inhibitor were found to exert its anti-obesity effects through regulating adipocyte differentiation in high-fat diet induced obese mice. However, the role of CatK and its pathophysiological role in cardiovascular disease has not received much attention until recently. CatK and Cathepsin S were the first cathepsins found to be expressed in human atherosclerotic lesions (Cheng et al., 2012). Therefore, the present work has tried to be a step in more understanding whether Catk inhibition would improve the deranged lipid milieu and the progress of atherosclerotic lesions following 8 weeks of high fat diet in rats. Another aim was to explore whether a combination of it with lipid lowering drug (Statin) would offer better result or not.

The high-fat diet induced the expected overweight model. As shown in Table 1, final body weights of the rats fed on high-fat diet were statistically greater in comparison with the standard chow-fed rats. As expected also, the effect of feeding HFD on lipid profile is evidenced by the increase in TC, LDL and TG with the decrease in HDL-c level. Regarding histopathology, lipid accumulation both within and outside cells is a pathological feature of atherosclerotic plaques. Hypercholesterolemia induces vascular smooth muscle cells (VSMCs) proliferation and migration to intima and eventual fibrosis (Tousoulis et al., 2011). In the present
Figure 4b: Quantification of the plaque lesion area. (C) Quantification of collagen content in SR-stained sections. One-way ANOVA followed by post-hoc Tukey tests were used. P1: (significance relative to ND Group) = *, P2: (significance relative to HFD Group) = #, P3: (significance relative to Ator Group) = ¶, P4: (significance relative to CatK I Group) = π.

In the study, H&E staining of aortic rings showed that high fat diet for 8 weeks induced atherosclerotic pathology with the appearance of foam cells resulting from intracellular lipid accumulation. Furthermore, it resulted in excess collagen deposition and interstitial fibrosis observed on SR staining. Notably, pathologic changes characteristic of atherosclerosis appeared significantly less severe in rats fed similar diet but treated with Atorvastatin and/or CatK inhibitor with best protective effect being obtained by the combined treatment.

Either CatKI or Ator has improved the derangement in lipid parameters seen with HFD only. Thus, both have reduced TC, LDL and TG and increased the level of HDL (Table 2). Such effect of either drug was comparable regarding TC, LDL and HDL in that there was no significant difference although the level of LDL was slightly less with CatKI but that difference did not reach significance. However, for TG, the effect of Ator was more pronounced than that of CatKI. The use of both drugs, in combination group, shows greater improvement only regarding LDL and TG, but for LDL it was against Ator while for TG it was more effective when compared with CatKI group. The present results are expected as far as Ator is concerned, being an established lipid lowering drug. However, for CatKI, it shows that it has a comparable effect in improving the lipid profile derangement in rats fed high fat diet. Indeed, the study of Han et al. (2015) has indicated a role of CatK in adipocytes differentiation and that the level of CatK was elevated in HFD mice with a positive correlation to both visceral adipose tissue and subcutaneous adipose tissue. Such finding highlighted the possible use of CatKI as anti-obesity factor which is partially outlined by the present experiments. The greater improvement in LDL and TG noticed in combination group highlights the benefit of such
combination. Previous reports have indicated that long-term treatment with Statins reduced plasma and tissues CatK levels preventing cardiovascular and renal injury in animal models (Cheng et al., 2011). Thus, in the present experiments, the greater improvement in LDL and TG in combination group could be the result of further inhibition of CatK synthesis by Ator. This point needs further studies in which CatK level to be measured, a limitation that should be addressed for these experiments.

Regarding CRP, its level was reduced in either Ator, CatK inhibitor or combined group (Figure 2). However, that reduction was not significantly different between the 3 groups although the combination has induced greater reduction when compared with HFD group. CRP signifies the presence of systemic inflammation and reports (Drakapolou et al., 2009) have implicated it as a valuable predictive tool for cardiovascular disease even in healthy subjects. The current finding shows that the inflammatory process mediated by HFD (obesity) seems to be counteracted by either Ator or CatK and that their effect is more or less comparable. A positive correlation between CRP and CatK serum level was reported previously indicating the relationship between CatK and inflammation. Knowing that the inflammatory process represents a major mechanism in atherosclerotic plaque rupture and subsequent acute coronary syndrome (Weber et al., 2011) highlights the importance of the determination of CatK serum level as inflammatory biomarker in CVD. Aortic tissue mRNA expression of ICAM-1 in the present experiment showed a significant elevation in HFD group compared to ND group (Figure 2). This is similar to the findings of a study that revealed that the ICAM-1 expression was greatly decreased in normal fed rats compared to high fat diet (Heransyah et al., 2018). This is in line with theory that high fat diet causes an ectopic vascular fat deposition, which in turn induces the production of oxidized LDL (OxLDL), one of stimulators of expression of cell adhesion molecules including ICAM-1 (Kim et al., 2012). ICAM-1 is an immunoglobulin superfamily that plays important role in the adhesion of leukocytes to vascular endothelium (Hansson, 2011). It has been shown to be present in and involved in the progression of atherosclerotic lesions (Poston et al., 1992). The involvement of ICAM-1 in atherosclerosis has been reported in apoE-/− mice deficient in ICAM-1, which showed a reduced lesion size. Time course analysis revealed that ICAM-1 is involved in both the initial plaque formation and subsequent progression in mice. In the present study, HFD rats treated with CatK and Statin as a potential anti-atherosclerotic drugs showed a significant reduction in the enhanced ICAM-1 mRNA expression (Figure 2a and b) and their combination showed further reduction compared to CatK alone.

The present experiments have shown that MCP-1 mRNA expression is highly enhanced in aortic tissue in HFD group compared to ND group. Previous reports have shown that MCP-1 is linked to obesity and CVD pathophysiology. An early study by Higa et al. (2011) has found high plasma MCP-1 in obese mice in comparison to lean controls. In the study by Omar et al. (2015) MCP-1 expression was enhanced in retroperitoneal adipose tissue, aorta and heart tissues in HFD rats. Regarding atherosclerosis, MCP-1, a CC chemokine, has been found to be involved in induction of local inflammation, recruitment of immune cells, acceleration of atherosclerosis and has shown an increased expression in atherosclerotic lesions (Lin et al., 2014; Aiello et al., 1999). The new element in the current study was exploring effect of CatKI and Statin on such enhanced expression. As observed, either Ator or CatKI has significantly reduced expression of MCP-1 (Figure 3a and b), however, that reduction is more evident with CatKI. The combination, again, has not induced further change. There is little evidence regarding the role of CatK as inflammatory biomarker although some studies have indicated that the presence of strong anti-inflammatory effects following CatK inhibition in a rat model of autoimmune arthritis, while CatK deficient mice were resistant to experimental autoimmune encephalomyelitis (Asagiri et al., 2008; Burstein et al., 1970). In addition, the pathologic changes characteristic of atherosclerosis appeared less severe in rats fed similar diet but treated with atorvastatin and/or CatK inhibitor with best protective effect being achieved by the combination treatment (Lunder et al., 2011). Some recent findings show that measurement of CatK represents a promising, novel and accurate tool to document and monitor the atherosclerotic processes. This is one of limitation of the present work that should be addressed as measuring CatK level would provide more depth to analysis of results. Indeed there is limited available information regarding these inhibitors in treating cardiovascular diseases (CVD). Thus, exploring the role of CatK in atherosclerotic processes can add new insights into CV physiopathology. Moreover, if lowering or inhibiting CatK can directly affect atherosclerotic processes, targeting CatK may be therapeutically useful in CVD management.

CONCLUSION

The present results suggest that the administration of CatKI (Balicatib) either alone or in combination with the LDL-c lowering drug (atorvastatin) attenuate the derangement in lipid profile, expression of atherogenic ICAM-1 and MCP-1 and progression of atherosclerotic aortic lesion indicating its possible antiatherosclerotic and anti-inflammatory role.

ETHICAL APPROVAL

All authors have declared that all experiments have been examined and approved by our local ethics committee and
have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

**ACKNOWLEDGMENT**

The authors are grateful to the Medical Research Center - Mansoura University for their support.

**REFERENCES**


