

## Format for ANSWERING REVIEWERS

6<sup>th</sup> July, 2014

Dear Editor,



Please find enclosed the edited manuscript in Word format (file name: 11325-review.doc).

**Title:** Mitochondria-targeted agents: Future perspectives of mitochondrial pharmaceuticals in cardiovascular diseases

**Author:** Thekkuttuparambil Ananthanarayanan Ajith, Thankamani Gopinathan Jayakumar

**Name of Journal:** *World Journal of Cardiology*

**ESPS Manuscript NO:** 11325

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated: yes

2 Revision has been made according to the suggestions of the reviewer: yes

### **Reviewer # 1**

In their manuscript, Ajith and Jayakumar review a controversial field of mitochondrial antioxidant therapies for CVDs. While authors provide in their review some useful information, some statements are inaccurate or outdated, and therefore are misleading to the reader.

1. On p.4 statement that "Energy for the cardiomyocytes as well as endothelial cells (EC), even though relatively little dependence, is solely met from the mitochondrial OXPHOS". This statement is inaccurate, since endothelial cells derive about 50% of their ATP from glycolysis and are among cell types with the LOWEST reliance on OXPHOS (reviewed in [1]). **Authors' response:** The statement has been modified as 'Energy for the cardiomyocytes is solely met from the mitochondrial OXPHOS for the contractile function'.
2. P.5, contains a statement "where as, 1-5 % of O<sub>2</sub> can give rise to potentially cytotoxic ROS such as superoxide anion radical (O<sub>2</sub><sup>-</sup>), hydroxyl radical (.OH) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), indicating that mitochondria itself is the source of ROS". This statement is not sourced (no reference provided) and contains two factual inaccuracies: a) less than 0.2% of oxygen is converted to ROS by the ETC under physiological conditions in vitro [2,3], and this percentage in vivo remains to be determined b) actively respiring mitochondria can CONSUME extramitochondrial ROS [4], and therefore it is unclear whether mitochondria in vivo are net producers

or net consumers of ROS. **Authors' response:** There are various reports regarding the % of O<sub>2</sub> converted to super oxide anion in mt, ranges from 0.2% [ref. cited by this reviewer] to 0.4–4.0% [PNAS. 1994; 91: 10771 –10778]. We agree the suggestion of the reviewer. Hence, the statement has been modified as 'Major part of cellular oxygen (O<sub>2</sub>) that entered into mitochondria is reduced to water in the mitochondrial respiratory chain, whereas a fraction of all O<sub>2</sub> consumed can converted to potentially cytotoxic ROS such as superoxide anion radical (O<sub>2</sub><sup>-</sup>),.....'

3. P.5 "The O<sub>2</sub><sup>-</sup> is a primary radical that could produce other ROS, such as H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub><sup>-</sup>?" This statement makes no sense. **Authors' response:** The statement has been modified as 'The O<sub>2</sub><sup>-</sup> is a primary radical that could produce other ROS, such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl radicals (·OH) in the failing myocardium'
4. P.5 "The ·OH is generated by the reduction of H<sub>2</sub>O<sub>2</sub> in the presence of endogenous iron by means of the Fenton reaction." Fenton reaction can also be catalyzed by other transition metals such as copper **Authors' response:** The modification incorporated in the revised MS as 'The ·OH is generated by the reduction of H<sub>2</sub>O<sub>2</sub> in the presence of endogenous iron and copper by means of the Fenton reaction. Copper and iron are found to be mobilized following the myocardial ischemia. Chevion et al. [7] reported that 8- to 9-fold higher level of copper and iron in the first coronary flow fraction of reperfusion after the 35 min of ischemia than the pre-ischemic value in isolated rat heart. This was further supported by the observation of Reddy et al. [8] that early treatment with deferoxamine, a potent iron chelator, limits the injury related to myocardial ischemic/reperfusion in dogs probably due to the lesser availability of iron for Fenton reaction'.
5. P.5 "Further, the declined antioxidant status in the mitochondria, during ageing, can provoke the generation of ROS especially in cardiomyocytes as well [6]". Declined antioxidant status can not change ROS generation (it can only affect ROS detoxification), but can induce oxidative stress. **Authors' response:** The statement has been modified as 'Further, the declined antioxidant status in the mitochondria, during aging, can provoke the mitochondrial dysfunctions in cardiomyocytes as well [6]
6. P.6 "Among the damage induced by generated ROS at the cellular level, mtDNA remains the major target". Whether mtDNA can be damaged by physiologically produced ROS is highly controversial [3,5]. **Authors' response** Since the first sentence of that paragraph begins with 'The generated ROS, under oxidative stress,.....' The authors' had given the next sentence as 'under the oxidative stress situation'
7. P.6 "Since 1988, when the first mutation in mtDNA has described". This is not accurate. In 1988 a link between mtDNA mutations and human disease was established for the first time. mtDNA mutations were known before that. **Authors' response:** The statement has been modified as 'Since 1988, when the first mutation in mtDNA has established'
8. P.6 "The mutations described are either being typically 50 to 60 % for single, large-scale deletions or 80 to 90 % for point mutations". What authors apparently mean, are thresholds for phenotypic manifestation of mtDNA mutations, not typical mutation loads **Authors' response:** In the revised MS, the statement has been modified and further strengthened as 'The mutations described are either being

typically 50 to 60 % for single, large-scale deletions or 80 to 90 % for point mutations in patient with mitochondrial myopathy and encephalomyopathy [12]. In general, majority of the pathogenic point mutations are maternally transmitted, whereas large-scale deletions of mtDNA are mostly sporadic. More than 10 different types of deletions have been identified in the mtDNA among these; the 4977-bp deletion is the most prevalent in skeletal muscle, whereas the 7436-bp deletion was detected in the heart of human subjects in their late thirties with no apparent sex difference [13].

9. P.7 "Further more, it is believed that a mammalian DNA recombinase is involved in repairing recombination processes". The statement is not sourced. Most experts in the field agree that mammalian mitochondria lack homologous recombination system Questions: **Authors' response:** The statement has been deleted since the suitable ref. could not be selected
10. Is it possible that the effect of the isoproterenol on ROS production is direct, and not mediated by increased cardiac output? **Authors' response:** The statement has been modified as 'Sudheesh et al.[9] recently reported that isoproterenol-induced acute MI in rat affected the respiratory chain complexes I-IV, mediated through an increase in the ROS level in the cardiomyocytes'
11. It would be useful to provide references for the following statements found in the manuscript: 1. Around 2% of the adults suffer from HF; the prevalence is found to be increased up to approximately 6–10% over the age of 65. 2. HF from IHD is found to be ~62%. **Authors' response:** Both statements were modified slightly and ref has given for 1 ie. 'In developing countries, around 2% of the adults suffer from HF; the prevalence is found to be increased up to ~ 6–10% over the age of 65 [1].' Similarly, for 2 ie 'Study of healthy adults in the United States reported that IHD increases the risk factors of HF ~62% [2].'

#### Reviewer # 2

1. This is an EXCELLENT paper but with HORRIBLE, ATROCIOUS grammar and typos. These MUST all be fixed and then this fine paper can be and should be published. **Authors' response:** Grammar and typos of this MS has been corrected

#### Reviewer # 3

The authors have thoroughly discussed the ROS production in mitochondria and its damaging effect on mtDNA, protein and lipid. The antioxidant and protection are also well documented. It is a well written review paper, which will give the readers of the journal good information on mitochondria ROS production and cardiovascular diseases.

#### Reviewer # 4

This article is a concise review illustrating mitochondrial dysfunction by reactive oxygen species associated with CVD and beneficial mitochondria-targeted antioxidants. However, this article should be corrected according to the following suggestions before publication.

1. Page 2, line 7, line 1 up, Check the spelling. dialatation is dilation? **Authors' response:** corrected as 'dilations'
2. Page 5, line 5, Check the follow sentence. The O<sub>2</sub> – is a primary radical that could produce other ROS, such as H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub>–· in the failing myocardium. The .OH is generated.... I think that "such as H<sub>2</sub>O<sub>2</sub> and OH" instead of "such as H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub>–". **Authors' response:** The O<sub>2</sub><sup>-</sup> is a primary radical that could produce other

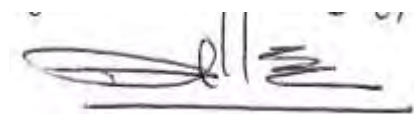
- ROS, such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl radicals (·OH) in the failing myocardium.
3. Page 5, line 6, ETC; Give the full spelling. **Authors' response:** given as 'electron transport chain (ETC)'
  4. Page 6, line 9, Check the follow sentence. Further, the oxidized LDL abrogated the oxidative stress by binding to the lectin-like oxidized low-density lipoprotein scavenger receptor-1 (LOX-1) on the arterial wall. The oxidized LDL abrogated the oxidative stress, or the oxidized LDL is abrogated by LOX-1? **Authors' response:** Modified as 'The oxidized LDL is abrogated by binding to the lectin-like oxidized LDL scavenger receptor-1 (LOX-1) on the arterial wall [14].
  5. Page 10, line 10, Check the follow sentence. Co-enzyme Q10 (CoQ10) and L-acetyl-carnitine can be considered to be a safe adjunct to standard therapies in CVD [18]. Although the authors mentioned L-acetyl-carnitine, there is no description about it. The authors should also refer to L-acetyl-carnitine. **Authors' response:** L-carnitine therapy in HF patients (2 g/day, orally) showed improved survival [37]. A recent study in patients with mild diastolic HF treated with L-carnitine (1.5 g/day, p.o for three months) showed improvement in diastolic function [38]. Therapy with L-carnitine 9 g/day, intravenously for five days followed by 6 g/day orally for 12 months along with the standard medical therapy may limit adverse effects of acute MI on the heart muscle [39,40]. Tolerance to exercise was significantly improved in patients with higher left ventricular ejection fraction volume (greater than 30%), when treated with the propionyl-L-carnitine adjunct to appropriate medical therapy [41].
  6. Page 10, line 1 up, Check the follow sentence. In another study, fifty consecutive new patients 11 discontinued 28 months of statin therapy due to side effects and began CoQ10 supplementation at an average of 240 mg/day [24]. The authors should describe the result of this study. Was the CoQ10 supplementation beneficial? **Authors' response:** Beneficial effect has included in the revised MS as 'CoQ10 supplementation (100 mg/day) for 30-day is found to decrease the muscle pain associated with statin treatment [35]. In another study, fifty consecutive new patients discontinued 28 months of statin therapy due to side effects and began CoQ10 supplementation at an average of 240 mg/day [36] and they have been followed for an average of 22 months (84% of the patients for more than 12 months). The prevalence of fatigue from 84% on initial visit decreased to 16%, whereas the decreased rate of myalgia from 64% to 6%, dyspnea from 58% to 12%, memory loss from 8% to 4% and peripheral neuropathy from 10% to 2%. Moreover, statin-induced cardiomyopathy was found to be reversed with the combination of statin discontinuation and supplementation with CoQ10'
  7. Page 12, Table 1 The structures of mitochondria-targeted antioxidants listed in Table 1 should be depicted for general readers. **Authors' response:** We could not incorporate the structures due to the non availability of software to draw the structures

1. It is a very interesting topic. In this review, the authors discussed mtDNA point mutations. It would be helpful if the authors cover the review on the other effects of oxygen free radicals on mtDNA deletions, mutations or loss of large fragments.  
**Authors' response:** In the revised Ms included more about the mtDNA mutations ie. 'In general, majority of the pathogenic point mutations are maternally transmitted, whereas large-scale deletions of mtDNA are mostly sporadic. More than 10 different types of deletions have been identified in the mtDNA among these; the 4977-bp deletion is the most prevalent in skeletal muscle, whereas the 7436-bp deletion was detected in the heart of human subjects in their late thirties with no apparent sex difference [17]. However, the clinical severity of the disease is correlated usually with the presence of > 80% of the mutated mtDNA in the target tissues [18]. Furthermore, at the same level, large-scale deletions cause much more severe pathologies than do point mutations. The patterns of distribution of the mutated mtDNA as well as the energy demand of the target tissues are two important factors that determine the pathological outcome of the mutation'
2. In this paper, the authors described mtDNA injury affected by the O<sub>2</sub><sup>-</sup>, more discussions on the mechanisms are encouraged. Ca<sup>2+</sup> overload occurs when the hearts are exposed to an excess amount of oxygen free radicals, and Ca<sup>2+</sup> can increase the generation of oxygen free radicals in turn. The relationship between Ca<sup>2+</sup> and oxygen free radicals is not entirely clear  
**Authors' response:** Cross talk between Ca<sup>2+</sup> and ROS has been incorporated ie. 'Ca<sup>2+</sup> overload to the mitochondrial matrix can further enhance the generation of ROS. Though the exact mechanism of ROS production is debatable, the effect may probably mediated through Ca<sup>2+</sup> mediated inhibition on the complex I [22], III [23], and IV [23] of ETC (Fig. 4). Ca<sup>2+</sup> can stimulate the TCA cycle dehydrogenases to increase the production of reduced substrate for OXPHOS [24] and further increased the rate of respiration as well. Ca<sup>2+</sup> can also activate the mitochondrial nitric oxide synthase to produce the NO which in turn inhibits the complex IV [25]. The simultaneous generation of NO with O<sub>2</sub><sup>-</sup> favors the formation of peroxynitrite, one of the major agents to induce conformational change in many proteins [26].'

3 References and typesetting were corrected: **Authors' response:** Corrected as per the style of the WJC

Thank you again for publishing our manuscript in the *World Journal of Cardiology*.

Sincerely yours,



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