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A research update: Significance of cytokine storm and diaphragm in COVID-19

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Abstract

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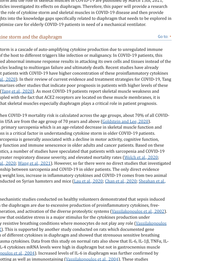
Emerging research on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) shows that it is spreading to multiple organs in addition to the respiratory system. Though the SARS-CoV2 enters the human body by binding to ACE2 receptors on pulmonary alveolar cells, recent studies indicate that it is spreading to the central nervous system, cardiac and skeletal muscles leading to multiple complications and long-term sequelae of the disease. The SARS-CoV-2 also has a strong tropism for cytokine storm and its consequential effects on skeletal muscles has generated a lot of discussion. The effects of this virus on muscular function especially in susceptible elderly populations is still being explored. However, its effects on diaphragm, a respiratory muscle which plays an important role in determining lung capacity are not completely explored. Currently, we have evidence on using lung ultrasounds to confirm COVID-19 diagnosis is gaining traction, it is necessary to explore the role of diaphragm in treating COVID-19 patients. This article will review the current literature on the role of diaphragm in COVID-19 patients and provide a research update with a specific focus on the diaphragm in order to identify knowledge gaps in understanding severe COVID-19 patients, especially those who are on a mechanical ventilator.

Keywords: COVID-19, cytokine storm, Skeletal muscle, Diaphragm

Graphical abstract



1. Introduction

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Coronavirus disease-2019 (COVID-19) which began in December 2019 in China has caused an unprecedented global pandemic and is the most serious threat to global health. Though not much was known about COVID-19 in the beginning, the past year provided critical insights in understanding the pathophysiology of this SARS-CoV-2 virus. According to the World Health Organization (WHO), this virus has affected 115 million people that resulted in more than 2.6 million deaths around the world by March 15th, 2021 (Coronavirus, 2021). It is now established that SARS-CoV-2 uses the angiotensin converting enzyme 2 (ACE2) as a functional receptor to bind and enter host cells through endocytosis. Angiotensin converting enzyme 2 (ACE2), a life-threatening condition characterized by poor oxygenation and pulmonary infiltration (Siddiqui et al., 2020) is a membrane protein ACE2 is widely distributed in multiple organs including the skeletal muscles (Clarke, 2021). Several studies on mouse limb muscle and human diaphragm have clearly shown the ACE2 expression on their cell membranes (Gianchetti et al., 2019; Qi et al., 2020). As the virus enters the human body, skeletal muscle has the ability to produce and release a number of cytokines known as myokines. When the SARS-CoV-2 enters the circulation it triggers a cytokine storm that is characterized by a surge in pro-inflammatory cytokines. Numerous studies have shown that myokines like TNFα, IL-1, IL-6, IL-18, and IL-8 are released in response to diverse physiological and pathophysiological conditions (Giovanna et al., 2015; Hulmi et al., 2018; Pedersen et al., 2007). These myokines then act in endocrine, paracrine or autocrine manner on multiple organs which may in turn initiate immune response (Pedersen and Febbraio, 2008; Pedersen, 2009).

Skeletal muscles are rich in proteins and mitochondria and therefore more prone to undergo loss of their mass or function in response to different metabolic stress or pathological conditions. Diaphragm, one of the essential skeletal muscles of the respiratory system, plays a key role in maintaining lung capacity and any decline in its function can lead to decreased pulmonary function. With increasing age, sarcopenia is more evident with changes in morphological features that make these muscles less elastic with a lower number of fibers. Diaphragm also undergoes this age-related decline in its structure and function. Data from animal models and clinical studies show that the diaphragm is more susceptible to the effects of mechanical ventilation, which results in a loss of muscle fibers and product respiratory pressure (Supinski et al., 2019). Clinical studies showed that ventilated patients with sepsis conditions often need mechanical ventilation for their survival. Available literature also illustrates that mechanical ventilation acts as a two-edged sword associated with sepsis leading to development of diaphragm atrophy and ventilator-induced diaphragmatic dysfunction (VIDD) while providing the much needed mechanical ventilation (Maes et al., 2011; Supinski et al., 2019). From the ARDS condition, the preserved diaphragmatic activity is associated with a reduced risk of VIDD and better outcomes in ventilated patients. Therefore, it is critical to understand the most appropriate physiological mode of respiratory support (Supinski et al., 2019).

Surprisingly, a time-dependent decrease of diaphragm strength after initiation of mechanical ventilation, has gained much research interest while diaphragm dysfunction at the onset of critical illness did not receive due attention (Supinski et al., 2019). Though more than 3000 articles on cytokine storm and the role of skeletal muscles in COVID-19 are published by March 15th, 2021, there is a lack of research on the role of diaphragm in COVID-19 patients. This review will provide an update on the role of cytokine storm and skeletal muscles in COVID-19 patients and then provide some insights into the knowledge gaps specifically related to diaphragm that needs to be explored in order to optimize care for COVID-19 patients in need of a mechanical ventilator.

2. Cytokine storm and the diaphragm

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Cytokine storm is a cascade of auto-amplifying cytokine production due to unregulated immune response of the host to different triggers like infection or malignancy. In COVID-19 patients, this uncontrolled abnormal immune response results in attacking its own cells and tissues instead of the virus particles leading to multiorgan failure and ultimately death. Recent research data shows that patients with COVID-19 have higher concentration of these proinflammatory cytokines (Gandhi et al., 2020) in comparison to other of current evidence and treatment strategies (Yi, Lagniton, Ye, et al., summarize other studies that indicate poor prognosis in patients with higher levels of these cytokines (Zaim et al., 2020). In severe COVID-19 patients respiratory failure is a major concern. Intriguge coupled with the fact that ACE2 receptors are located on these muscle myokinesis, it is possible that skeletal muscle and specifically diaphragm plays a critical role in patient prognosis.

Further, when COVID-19 mortality risk is calculated across the age groups, about 70% of all COVID-19 deaths in USA are from the age group of 75 years and above (Mortality Analyses, 2021). Therefore, primary sarcopenia which is an age-related decrease in skeletal muscle function and strength is a major concern in the elderly population affected by COVID-19 pandemic. Primary sarcopenia is generally associated with a decline in motor activity, cognitive function, respiratory function and immune senescence in older adults and cancer patients. Based on these characteristics, a number of studies have speculated that patients with sarcopenia and COVID-19 will have greater respiratory disease severity, and elevated mortality rates (Ehlich et al., 2020; Barlas et al., 2020; Bonetti, 2020). However, so far there seem no direct studies that investigated the relationship between sarcopenia and COVID-19 in older patients. The only direct evidence connecting weight loss, increase in inflammatory cytokines and mortality risk comes from recent studies conducted on Syrian hamsters and mice (Sun et al., 2020; Chan et al., 2020; Shoulder et al., 2020).

Previous mechanistic studies conducted on healthy volunteers demonstrated that sepsis induced changes in the diaphragm are due to excessive production of proinflammatory cytokines, free radical generation, and activation of the diverse proteolytic systems (Supinski, 2020). Recent research data shows that patients with severe COVID-19 have higher concentration of these respiratory enzymes/ biomarkers of cytokines which measures the out play on the diaphragm (Gandhi et al., 2020). This is supported by another study conducted in rats which documented gene expression of different cytokines in diaphragm and showed that cytokines accumulation in diaphragm induces plasma cytokines. Data from this study on normal rats also show that IL-6, IL-1β, TNFα, IL-10, IFNγ, IL-4 cytokines which were high in diaphragm but not in gastrocnemius muscle (Vassilakopoulos et al., 2011). Increased levels of IL-6 diaphragm was further confirmed by immunohistochemical analysis (Vassilakopoulos et al., 2011). Recent data also demonstrate the role of diaphragm in producing cytokines during strenuous breathing. Similar to animal studies, increases in the pro-inflammatory cytokine expressions (TNFα, IL-1β, and IL-6) were observed in the serum of patients with COPD patients as well (Gayan-Ramirez et al., 2013).

In contrast to primary sarcopenia, Duchenne muscular dystrophy (DMD) is a severe, progressive, muscle-wasting genetic disorder of the younger patients. Studies conducted on DMD patients and mouse models of DMD have shown that cytokines TNFα, IL-1β, IL-6, IL-10, and IFNγ in the diaphragm reach of both DMD patients and dystrophic mice (Porichis et al., 2017). More specifically Porichis et al., has demonstrated that IL-6 overexpression in transgenic mouse model parallels the amelioration in muscle pathology and the decrease in inflammatory cytokines (Porichis et al., 2017). Data from dystrophic mice show that deregulated levels of IL-6 alters the local and systemic inflammatory markers. In addition, IL-6 also acts as a central player in disturbing the redox balance in the diaphragm (Pelosi et al., 2017). In support of these findings, another study demonstrated that overexpression of interleukin 13 enhances the generation and accumulation of free radicals in the diaphragm that leads to skeletal muscle atrophy in adult transgenic mice (Gazzerro et al., 2010).

Overall, these studies indicate that oxidative stress, inflammation and cytokines are closely related to each other and consist in a muscle environment under distinct clinical settings for example, a study conducted in rats demonstrated that during resistive breathing the production of nitric oxide increases in the diaphragm and that this increase is associated with the production of inflammatory cytokines (IL-6, IL-1β, IL-10, and IL-1β). However, when nitric oxide is suppressed this response is downregulated. Available data also demonstrate that cytokines are differentially regulated by different signaling pathways (c-fl, JAK-1, IL-7, TNFα, and IL-1β) and that NF-κB dependent pathways (muscle atrophy-related transcription factor) while IL-6 is regulated by a pathway independent of NF-κB (Supinski et al., 2021).

3. Skeletal muscle function in COVID-19

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From the available COVID-19 related literature, we found two ideas that need further investigation. The first idea requires exploring the cytokine storm to identify potential targets for intervention. For example, IL-6 blockade has been reported to be use in China for COVID-19 patients as an increase in the level of IL-6 has been reported to be associated with severe respiratory failure in the cytokine storm (Huang et al., 2020; Liu et al., 2020). Another approach is to target the sensitive stress pathway. An extracellular heat shock protein domain, an antimicrobial peptide released by cells in response to regular exercise, may reduce the risk of acute respiratory distress syndrome (ARDS) in COVID-19 patients (Kouzaki et al., 2020). The second idea is to explore the role of skeletal muscle in the cytokine storm initiation and tissue damage for COVID-19 patients (Kouzaki et al., 2020; Clarke, 2021). A recent study on COVID-19 patients has confirmed skeletal muscle injury in these patients that correlated with an increase in creatinine kinase levels in their blood (Mao et al., 2020). Therefore, specific studies on using creatinine kinase levels in blood as an early biomarker for COVID-19 could be very helpful in long term care for the patients.

The second idea requires optimizing the conditions for using mechanical ventilation. Prolonged immune cases are COVID-19 patients required for stabilization of COVID-19 patients and may itself directly impact loss of skeletal muscle mass and function which may further add on to human morbidity (Hopkinson et al., 2020). Available clinical reports clearly indicate that prolonged mechanical ventilation is associated with gradual development of diaphragm atrophy and ventilator-induced diaphragm dysfunction (VIDD) (Vassilakopoulos and Petrof, 2004). To prevent or reverse VIDD, United States FDA approved long-term diaphragm pacing therapy (Hopkinson et al., 2020). Data from animal models and human studies have provided a detailed clear biological evidence for ACE2 expression and SARS-CoV2 infection in the diaphragm muscle that ultimately leads to development of fibrosis (Dixit et al., 2021). In addition, diagnostic tests like lung ultrasound are currently used to assess diaphragm function in COVID-19 patients and manage their weaning process (Guarracino et al., 2021).

These observations strongly emphasize the significance of skeletal muscles in the treatment of ARDS associated with COVID-19. The SARS-CoV-2 enters the body through the respiratory system and then gradually enters the circulation system. As ACE2 receptors are widely distributed in all the tissues, the virus enters other organs especially like skeletal muscles that are likely to trigger the cytokine storm (Fig. 1). Based on the current knowledge of SARS-CoV-2 pathogenesis and its clinical manifestations, the search for specific antivirals along with immune-modulating drugs must include supplemental drugs that protect the skeletal muscles especially the diaphragm. This two-pronged approach will not only minimize the multiorgan failure by controlling the cytokine storm but also enhance the recovery of patients on mechanical ventilation by improving diaphragm function.

Graphical abstract showing the interaction between SARS-CoV2, cytokine storm and the diaphragm.

4. Conclusion

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The current literature and clinical data on the role of diaphragm in COVID-19 patients indicates a complex interplay between SARS-CoV-2 mediated lung injury, cytokine storm, ARDS, ventilator, and aging which eventually becomes fatal to the susceptible patient. Diaphragm not only is the center of such ventilation but is critically important during ARDS and ventilation. Based on the current evidence, Based on our review of available COVID-19 literature, we identified the following knowledge gaps that require further research:

- Direct studies in COVID-19 patients investigating the effects of cytokine storm on diaphragm function are lacking. Studies conducted on identifying signaling pathways of the cytokines in diaphragm and mechanisms of their regulation are also not known. These studies are critical as they will help in identifying potential drug targets by differentiating between the NF-dependent and NF-κB independent pathways.
- Studies on the effectiveness of the mechanical ventilators by considering effects of sarcopenia when using them in elderly COVID-19 patients. Since sarcopenia is a common in these patients, studies on chest radiography in addition to cardiac and vascular ultrasounds must be explored to have a comprehensive understanding on clinical management of COVID-19 patients on ventilators. Though the effects of nitric oxide on cytokines levels in rat diaphragm were studied before the COVID-19 pandemic, further studies starting then to the ongoing COVID-19 patients especially those on ventilator are warranted.

Completion of the above research studies will provide a foundational basis to identify therapeutic targets and develop a potential treatment plan for COVID-19 patients. This is necessary as the currently available COVID-19 vaccines will protect people from the effects of SARS-CoV-2 but not helpful in treating COVID-19 patients.

CRediT authorship contribution statement

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Adriana Bibbo: Conceptualization, Writing – original draft. **Anita Dua:** Data curation, Visualization. **Sanjeev Gupta:** Resources, Project administration. **Elisha Sapoh:** Investigation, Conceptualization, Writing – editing.

Declaration of competing interest

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Efficient and modified 2-NBDG assay to measure glucose uptake in cultured myotubes

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Abstract

Under type-2 diabetes, insulin resistance develops in skeletal muscles as a key defect and to study the disorder, its manifestation, and possible solution, measurement of glucose uptake is a fundamental necessity. Of various approaches (i.e. scintillation counting, flow cytometry, fluorometry and spectrophotometry) fluorescent labelled glucose analogue, 2-NBDG solution is the most popular one. Although 2-NBDG based assay is the most widely used approach in various cells including skeletal muscle, even then all available protocols possess huge variability which impacts the overall data reproducibility. Moreover, starvation (use of glucose/serum free medium), one of the prerequisite condition for glucose uptake assay, itself induces stress specifically during longer pre-incubation periods and alters muscle cell metabolism and morphology, but the fact has not been duly considered. Therefore in the present article, using specific skeletal muscle cells i.e. C2C12 myotubes, we have re-established the conditions like pre-incubation time period, concentrations of insulin, glucose and serum/BSA while maintaining the cultured myotubes in morphologically healthy state. Our lab standardized protocols were observed to be effective in studying insulin resistance condition induced by diverse stresses (oxidative & inflammation) in myotubes. Comparative study conducted with already established protocols demonstrates that the present method is more efficient, effective and better improvised for studying glucose uptake in C2C12.

Keywords: 2-NBDG assay; C2C12 myotubes; Insulin resistance.

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- Deoxyglucose
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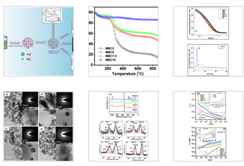

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Ceramics International
Volume 47, Issue 3, 1 February 2021, Pages 3586-3596

Mesoporous electroactive silver doped calcium borosilicates: Structural, antibacterial and myogenic potential relationship of improved bio-ceramics

Alesh Kumar [a], Ashwani Mittal [b], Avik Das [c, d], Debasis Sen [c, d], C.R. Mariappan [a]

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Abstract

In this work improved electroactive mesoporous Ag-doped bio-ceramics for medical usages are developed, examining their structural, electrical, in-vitro bioactivity, cell cultures and antibacterial properties against various classical pathogenic bacteria. Ag-containing mesoporous bio-ceramics (MBCs): xmol%Ag_2O - (100-x)[45.8CaO-8.4B_2O_3-45.8SiO_2] where x = 2, 5, 7.5 and 10 were synthesized through a sol-gel method. The small angle X-ray scattering and electron microscopy studies reveal the embedment of silver nanoparticles in the samples. Existence of silver as Ag^+/Ag^0 forms in the samples is confirmed by X-ray photoelectron spectroscopy. The N_2 adsorption-desorption analysis evidence the mesoporous structure of the samples. The electrical conductivity of samples increases from 5.4 x 10^{-8} S cm^{-1} for x = 2 to 1.9 x 10^{-6} S cm^{-1} for x = 7.5 and then decreases to 0.9 x 10^{-6} S cm^{-1} for x = 10 at 110 °C. In vitro bioactivity studies revealed that Ag-containing MBCs hold the bone-like hydroxyapatite formation after immersion in human blood plasma like-solution such as Dulbecco's Modified Eagle's Medium. The antibacterial effect of samples against pathogenic bacteria (*S. aureus, E. coli, P. monas aeruginosa, and B. cereus*) increases with Ag concentration (x = 7.5) and then decreases with Ag content (x = 10). Antibacterial effect is greater for the sample with high electrical conductivity. The cell culture studies evidence not considerable cytotoxic effects for Ag-containing MBCs. Finally, the C2C12 myoblast cell culture studies reveal the significant cell growths and differentiation (myogenesis) for high electrical conducting Ag-containing MBCs.

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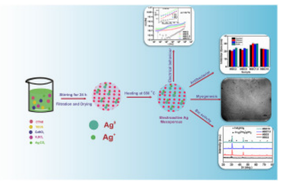

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Keywords

Mesoporous bio-ceramics; Silver nanoparticle; Sol-gel process; Electrical property; In-vitro bioactivity; Antibacterial effect; Myogenesis

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Antimicrobial potential and *in vitro* cytotoxicity study of polyvinyl pyrollidone-stabilised silver nanoparticles synthesised from *Lysinibacillus boronitolerans*

Divya Bhatia, Ashwani Mittal, Deepak Kumar Malik

First published: 04 June 2021 | https://doi.org/10.1049/nbt2.12054

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Abstract

The main emphasis herein is on the eco-friendly synthesis and assessment of the antimicrobial potential of silver nanoparticles (AgNPs) and a cytotoxicity study. Silver nanoparticles were synthesised by an extracellular method using bacterial supernatant. Biosynthesised silver nanoparticles were characterised by UV-vis spectroscopy,

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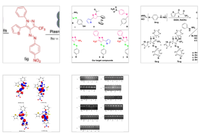

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Document

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Journal of Fluorine Chemistry
Volume 236, August 2020, 109573

Synthesis, characterization, *in vitro* DNA photocleavage and cytotoxicity studies of 4-arylazo-1-phenyl-3-(2-thienyl)-5-hydroxy-5-trifluoromethylpyrazolines and regioisomeric 4-arylazo-1-phenyl-5(3)-(2-thienyl)-3(5)-trifluoromethylpyrazoles

Ranjana Aggarwal [a], Suresh Kumar [b], Ashwani Mittal [c], Rachna Sadana [d], Vikas Dutt [c]

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Highlights

- Synthesis of 4-arylazo-1-phenyl-3-(2-thienyl)-5-hydroxy-5-trifluoromethylpyrazolines and regioisomeric 4-arylazo-1-phenyl-5(3)-(2-thienyl)-3(5)-trifluoromethylpyrazoles was accomplished.
- All the synthesized compounds were screened for their DNA photocleavage on suercoiled pBr322 plasmoid.
- Seventeen compounds were evaluated against MCF7, BT474, SBALL and MOLT4 cancer cell lines.
- 1-Phenyl-5-(2-thienyl)-4-(4-nitrophenylazo)-3-trifluoromethylpyrazole was found to be the most promising compound as photocleavage and anticancer agent.

Abstract

1-(2-Thienyl)-2-arylazo-4,4,4-trifluorobutane-1,3-diones (**4**), generated by the condensation of aryldiazonium salts (**2**) with 4,4,4-trifluoro-1-(2-thienyl)butane-1,3-dione (**3**), were used as common intermediates to synthesize regioisomeric 4-arylazo-1-phenyl-5(3)-(2-thienyl)-3(5)-trifluoromethylpyrazoles (**5** and **6**) and 4-arylazo-1-phenyl-3-(2-thienyl)-5-hydroxy-5-trifluoromethylpyrazolines (**7**) by reacting with phenylhydrazine in neutral conditions. All the synthesized compounds **4**, **5**, **6** and **7** were screened for their DNA photocleavage activity on pBR322 supercoiled DNA plasmid under UV radiation at λ_{max} 312 nm without any additive. The results indicated that 3-trifluoromethyl isomers (**5**) photocleaves plasmid DNA better than the corresponding 5-trifluoromethylpyrazoles (**6**) and 5-hydroxy5-trifluoromethylpyrazolines (**7**). 4-(4-nitrophenylazo)-1-phenyl-5-(2-thienyl)-3-trifluoromethylpyrazole **5g**, exhibited the best potential as DNA photocleaver, cleaving the supercoiled plasmid (Form-I) into smaller fragments at 20 μM of compound with 60 μM of DNA plasmid in DMSO. Selected seventeen compounds were further screened for their cytotoxicity against four cancer cell lines namely MCF-7, BT-474, SBALL and MOLT-4 and again compound **5g** was identified as the most potent compound with an IC_{50} value of 8.6±3.8 μM against MCF-7 cell line.

Graphical abstract

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Keywords

DNA photocleavage; Cytotoxicity; 4-Arylazo-3-trifluoromethylpyrazoles; 4-Arylazo-5-trifluoromethylpyrazole; 4-Arylazo-5-hydroxy-5-trifluoromethylpyrazolines

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Biochim Biophys Acta Gen Subj. 2020 Oct;1864(10):129676. doi: 10.1016/j.bbagen.2020.129676. Epub 2020 Jul 7.

S-allyl cysteine: A potential compound against skeletal muscle atrophy

Prachi Gupta [1], Vikas Dutt [1], Nirmaljeet Kaur [1], Priya Kalra [2], Sanjeev Gupta [1], Anita Dua [1], Rajesh Dabur [3], Vikram Saini [2], Ashwani Mittal [4]

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PMID: 32649980 DOI: 10.1016/j.bbagen.2020.129676

Abstract

Background: Oxidative stress is crucial player in skeletal muscle atrophy pathogenesis. S-allyl cysteine (SAC), an organosulfur compound of Allium sativum, possesses broad-spectrum properties including immuno- and redox-modulatory impact. Considering the role of SAC in regulating redox balance, we hypothesize that SAC may have a protective role in oxidative-stress induced atrophy.

Methods: C2C12 myotubes were treated with H_2O_2 (100 μM) in the presence or absence of SAC (200 μM) to study morphology, redox status, inflammatory cytokines and proteolytic systems using fluorescence microscopy, biochemical analysis, real-time PCR and immunoblotting approaches. The anti-atrophic potential of SAC was confirmed in denervation-induced atrophy model.

Results: SAC pre-incubation (4 h) could protect the myotube morphology (i.e. length/diameter/fusion index) from atrophic effects of H_2O_2. Lower levels of ROS, lipid peroxidation, oxidized glutathione and altered antioxidant enzymes were observed in H_2O_2-exposed cells upon pre-treatment with SAC. SAC supplementation also suppressed the rise in cytokines levels (TWEAK/IL6/myostatin) caused by H_2O_2. SAC treatment also moderated the degradation of muscle-specific proteins (MHCf) in the H_2O_2-treated myotubes supported by lower induction of diverse proteolytic systems (i.e. cathepsin, calpain, ubiquitin-proteasome E3-ligases, caspase-3, autophagy). Denervation-induced atrophy in mice illustrates that SAC administration alleviates the negative effects (i.e. mass loss, decreased cross-sectional area, up-regulation of proteolytic systems, and degradation of total/specific protein) of denervation on muscles.

Conclusions: SAC exerts significant anti-atrophic effects to protect myotubes from H_2O_2-induced protein loss and myofibers from denervation-induced muscle loss, due to the prevention of elevated proteolytic systems and inflammatory/oxidative molecules.

General significance: The results signify the potential of SAC against muscle atrophy.

Keywords: Atrophy; C2C12; Denervation; Oxidative stress; SAC; Skeletal muscle.

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Conflict of interest statement

Declaration of Competing Interest All authors (PG, VD, NK, PK, SG, AD, RD, VS, AM) declare that they have no conflicts of interest involving this work.

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