Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print), ISSN:2521-3520 (online) Volume 34, Issue 3, 2023 DOI: http://doi.org/10.23851/mjs.v34i3.1326

Research Article **Open Access**

The Measurement of Activity of the Ca^{2+}_Mg^{2+} ATPase in Membranous Vesicles Isolated from Smooth Muscle of Ileum in Rats Treated with Ultraviolet Radiation

Khalid H. Gathwan[1], Ahmed Anwar Albir[1], Salim J. Attia[1*]

[1]Basic Science, College of Dentistry, University of Baghdad, 10071 Baghdad, IRAQ.

*Correspondent contact: salim.attia@codental.uobaghdad.edu.iq

Article Info

Received
01/03/2023

Revised
29/04/2023

Accepted
28/05/2023

Published
30/09/2023

ABSTRACT

The Ca^{2+}-Mg^{2+} ATPase are high attraction calcium pump, that contributes in maintaining plasma membrane of cytoplasm Ca^{2+}, Mg^{2+} homeostasis by source to the outside of cell. The aim of the study is to evaluate the effect of the ultraviolet radiation on the activity of the Ca^{2+}-Mg^{2+} ATPase in the membranous vesicles of ileum in rats. Thirty adult Sprague–Dawley rats (age, 3-4 months, weight range, 180 – 200 g) were used in this experiment, which divided in to 5 groups (n = 6 / group). The membrane vesicles isolated from smooth muscles of rats showed high activity Ca^{2+}-Mg^{2+} ATPase. All isolated membranous vesicles are irradiated with Ultraviolet radiation of 250 nanometers except control group. The irradiation period for each group was (5, 10, 30 and 45) minutes, respectively. The activity of Ca^{2+}-Mg^{2+} ATPase was decreased with increased time of irradiation. In conclusion, the increased time of irradiation inhibited Ca^{2+}-Mg^{2+} ATPase activity isolated from ileum smooth muscles of rats. The recommendations are to expose other organs like liver and kidney to UV radiation to explain its effect or using other range of UV radiation to reflect its effect.

KEYWORDS: Ca^{2+}-Mg^{2+} ATPase, Rats, Ultra Violet Radiation.

الخلاصة

Ca2+_Mg2+ ATPase هي مضخة الكالسيوم عالية الجاذبية، والتي تساهم في الحفاظ على توازن Ca2+_Mg2+ لغشاء بلازما السايتوبلازم من المصدر إلى خارج الخلية. الهدف من الدراسة هو تقييم تأثير الأشعة فوق البنفسجية على نشاط Ca2+_Mg2+ ATPase في الحويصلات الغشائية للفائفي وهو الجزء الاخير من الامعاء الدقيقة في الجرذان. تم استخدام ثلاثين جرذًا بالغًا من جرذان(Sprague-Dawley) لعمر من ٣-٤ أشهر، ونطاق الوزن(١٨٠-٢٠٠ غم) في هذه التجربة، والتي تم تقسيمها إلى ٥ مجموعات (ن = ٦ / مجموعة). أظهرت حويصلات الغشاء المعزولة من العضلات الملساء للفئران نشاطًا عاليًا Ca2+ _Mg2+ ATPase. يتم تشعيع جميع الحويصلات الغشائية المعزولة بأشعة فوق بنفسجية تبلغ ٢٥٠ نانومتر باستثناء المجموعة الضابطة. كانت فترة التشعيع لكل مجموعة (٥ ، ١٠ ، ٣٠ ، ٤٥) دقيقة على التوالي. انخفض نشاط Ca2+ _Mg2+ ATPase مع زيادة وقت التشعيع. في الختام أدى زيادة وقت التشعيع إلى تثبيط نشاط Ca2+ _Mg2+ ATPase المعزول من عضلات اللفائفي الملساء للجرذان. التوصيات هي تعريض الأعضاء الأخرى مثل الكبد والكلى للأشعة فوق البنفسجية لشرح تأثيرها أو استخدام نطاق آخر من الأشعة فوق البنفسجية ليعكس تأثيره.

INTRODUCTION

The knowledge of the enzyme Ca^{2+}-Mg^{2+} ATPase activity in the smooth muscles is important because it lacks of sarcoplasmic reticulum [1]. This led to search to the regulation of calcium ions during the contraction and relaxation [2]. The presences of membrane vesicles in addition of mitochondria regulate ionic activity during contraction and relaxation. In addition to fact these organelles have the ability to take calcium in the presence of ATP and magnesium, and this led to the belief that they may play the role of sarcoplasmic reticulum [3]. Therefore, the maintained of Ca^{2+} homeostasis integrated

process associated with cellular damage and loss of intracellular free Ca^{2+} [4-6].

The Ca^{2+}-ATPase is a protein pump in the plasma membrane that eliminates Ca^{2+} from the cytoplasm and releases it into the outside of the cell, lead to ATP hydrolysis and regulation of the cytoplasm Ca^{2+} concentration by plasma membrane. Ca^{2+}-ATPase is a critical for cell survival as disruptions in Ca^{2+} homeostasis can alter cellular physiology [7-8]. The plasma membrane Ca^{2+}-ATPase include a number of proteins of physiological and pathophysiological importance in vertebrates because the ions across the membranes [9-12].

Ultraviolet radiation is totally considered a carcinogen, mutagen, and non-specific damage [13]. These features can initiate and promote a tumor. In some conditions, ultraviolet radiation cause the skin cancer [14] and other effects like tanning. Nevertheless, Ultraviolet rays also benefit human health by controlling the natural amount of vitamin D and endorphins in the skin, So Ultraviolet radiation rays have other complications. Accordingly, exposure to ultraviolet radiation leads to health risks including changes pigments, degeneration, malignant cases and wrinkles and is absorbed by DNA causing mutations and cancer [15-19]. The objective of the study is to show the effect of UV radiation on the activity of Ca2+_Mg2+ ATPase in membranous vesicles isolated from smooth muscle of ileum in rats.

MATERIALS AND METHODS

Our investigational work was carried out using (30) male adult Sprague- Dawley rats (age 4- 5 months and weight range 190-200g), which divided into 5 groups (n=6/group). All the rats were housed under standard laboratory conditions of 12 hrs. light / 12 hrs. dark cycle, provided with standard laboratory food and water and at dominated temperature (22– 25^{o}C). The period of the study was 15 days for adaptation in animal house of Medicine college/University of Baghdad. The rats were sacrificed and their ilea were rapidly removed, and cut lengthwise into small pieces and then put into Krebs solution (NaCl, KCl, CaCl2, Mg Cl2, NaHco3 NaH2 Po4, Glucose), at temperature (37°C) and left for 10 minutes in order to make the smooth muscle preparations of the ileum stabilize. Then, they transferred to the separation solution Kcl - Imidazole (KCl, Imidazole). To obtain the membranous vesicles from the smooth muscle of the rat ileum, the preparations were crushed well using an electrolytic homogenizer, and the centrifuge was used to obtain the membranous vesicles as mentioned above [20-22].

The total protein activity for Ca^{2+}–Mg^{2+} ATPase was determined according to method lorry *et al.* [22]. The membranous vesicles were irradiated with UV radiation by using (UVGL-25. USA) of wavelength 252 nanometer except for control group (non - irradiated group) in order to compare with the other four irradiated groups. The irradiation period for each group was (5, 10, 30, and 45 minutes), respectively. After the end of the entire period of irradiation, the activity Ca^{2+}– Mg^{2+} ATPase in special protein of the membranous vesicles was measured using spectrophotometer of wavelength 636 nanometer [23].

RESULTS AND DISCUSSION

Smooth muscles of animals were found to be similar in functions including the ATPase activity of vesicles for pumping Ca^{2+}. The activity of Ca^{2+}–Mg^{2+} ATPase in membrane vesicles were isolated from ileum smooth muscles of rats, as shown in Table 1. In comparable, no change ($P > 0.05$) was observed with time of irradiation (5, 10 and 30) minutes, while, there was significant ($p < 0.05$) decreased in the activity of Ca^{2+}–Mg^{2+} ATPase with long time irradiation (45) minutes, (Table 1 and Figure 1).

ISSN: 1814-635X (print), ISSN:2521-3520 (online) Volume 34, Issue 3, 2023 DOI: http://doi.org/10.23851/mjs.v34i3.1326

Table 1: The activity of Ca^{2+}-Mg^{2+} ATPase on membrane vesicles isolated from ileum smooth muscles of rats irradiation with (5,10, 30 and 45) minutes, the amount of phosphate produced were estimated (µmole phosphate/mg protein), Mean± SE.

No.	Time (min)	µmole phosphate / mg protein				P-value
		Trial1	Trial 2	Trial3	Mean ± SE	
1	Control	9.1	9.0	9.2	9.10±0.4	-
2	5	9.0	9.1	8.9	9.00±0.5	P > 0.05
3	10	8.9	9.0	9.1	9.00±0.6	P > 0.05
4	30	8.8	8.7	8.8	8.76±0.4	P > 0.05
5	45	4.9	4.8	4.8	4.83±0.3	P< 0.05

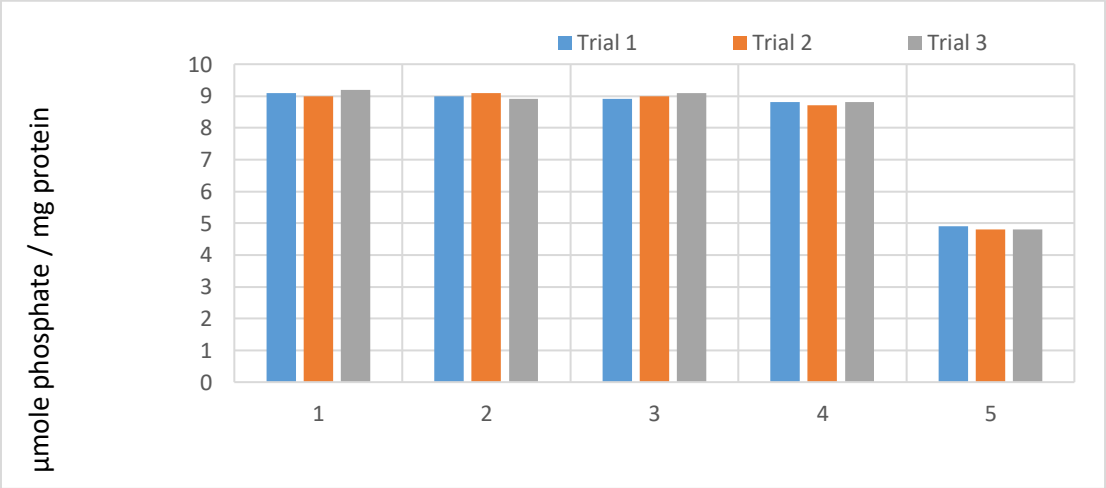

Figure 1: the activity of Ca^{2+}-Mg^{2+} ATPase on membrane vesicles: (control and irradiation for 5, 10, 30 and 45 minutes) marked with numbers 1, 2, 3, 4, and 5 respectively.

The Ca^{2+}– Mg^{2+} ATPase dependent sarcoplasmic reticulum plays an important role in promoting muscles relaxation by deposing Ca^{2+} from cytoplasm [24]. Guideline of the Ca^{2+} concentration in the plasma membrane is awkward for cell survival as impeding in Ca^{2+} homeostasis, which can change cellular physiology and lead to cell death [25]. The intracellular Ca^{2+} concentration is very low, Ultraviolet radiation may increase the level of Reactive Oxygen Species (ROS) and Ca^{2+} concentration as well as excessive Ca^{2+} concentration can be harmful to target cell [26]. Ultraviolet irradiation energy is subdivided into Ultraviolet radiation (A), (B) and (C) depend of electro physical properties. The Ultraviolet radiation-C has a wave length (100-280 nm), and Ultraviolet radiation -A photons have wave length (315-400 nm), while Ultraviolet radiation-B is falling in between [14]. The Ultraviolet radiation spectrum in our study was Ultraviolet Radiation-C.

A study suggests Ultraviolet radiation exposure can initiate the developments of cell, and has a strong irradiation energy to the tissue to cause cell damage. The Ultraviolet radiation dosing depends on solar radiation, and time spent outdoor professionally [26].

Ultraviolet radiation has many effects on skin physiology and the most acute effect on the skin is inflammation, and induces cascade of cytokines and neuron active [27]. The link between the effect of Ultraviolet radiation and Ca^{2+} homeostasis in cellular membrane is not clear [28], which has properties a calcium cell-signaling system, dependent on agonists known

to modulate growth, and proliferation of lens epithelial tissue.

In this study, the effect of radiation may be directly on the cell membrane, and in the particular on the binding site of Ca^{2+}, Mg^{2+} and thus a decrease in the cell activity and led to a decrease in enzyme activity and cell death. The activity of Ca^{2+}–Mg^{2+} ATPase in membrane vesicles isolated from membrane vesicles may play the role of sarcoplasmic reticulum since smooth muscles lack a well- distinguish sarcoplasmic reticulum that responsible of calcium regulation.

CONCLUSIONS

A general role of Ca^{2+}, Mg^{2+} is in controlling of Ca^{2+} homeostasis in the cell. The function of plasma membrane is to pump and maintain cellular calcium homeostasis. Exposing the tissue to ultraviolet radiation may have a direct effect on the cell membrane, calcium and magnesium ions, and thus a significant loss in enzyme activity. The recommendations are to expose other organs like liver and kidney to UV radiation to explain its effect or using other range of UV radiation to reflect its effect.

ACKNOWLEDGMENT

The authors would like to thank the Department of Basic Sciences at College of Dentistry, University of Baghdad for their support.

Ethical Statement: All procedures were conducted in accordance with the common animal welfare approved by most of ethical review committees in research institutes.

Disclosure and Conflict of Interest: The authors declare that they have no conflicts of interest.

REFERENCES

[1] Matthew A., Shmygol A. and Wray S. (2004). Ca2+ entry, efflux and release in smooth muscle, Biol Res 37: 617-624.

[2] Jack A., (2022). Discovery of the regulatory role of calcium ion in muscle contraction andrelaxation: Setsuro Ebashi and the international emergence of Japanese muscle research, Adv Physiol Educ, 46(3): 481–490.

[3] Bradley S. and George S., (2000). Effects of Mg2+ on Ca2+ release from sarcoplasmic reticulum of skeletal muscle fibres from yabby (crustacean) and rat, J Physiol., 526(2):299–312.

[4] Deshpande L., Delorenzo R., (2020). Novel therapeutics for treating organophosphate-induced status epilepticus co-morbidities, based on changes in calcium homeostasis, Neurobiol Dis., 133:104418.

[5] Choi H., and Lee C., (2016). Time-course change of hippocalcin expression in the mouse hippocampus following pilocarpine–induced status epilepticus, J. Vet. Sci.17:137-144.

[6] Umar S., Shahid N., Nazar L., Tanveer M., Devya G., Archoo S., (2021). Pharmacological Activation of Autophagy Restores Cellular Homeostasis in Ultraviolet-(B)-Induced Skin Photodamage, Front. Oncol., 11, 726066.

[7] Berridge, M., Lipp P., Bootman M., (2000). The Versatility and universality of calcium signalling. Nat. Rev. Mol. Cell Biol. 1, 11-21.

[8] Brini M., Cali T., Ottolini D., Carafoli E. (2013). The plasma membrane calcium pump in health and disease. FEBS J. 280, 5385-5387.

[9] Brini M., (2009). Plasm membrane Ca2+- ATPase: from a housekeeping function to a versatile signaling role. Pflugers Arch-Eur. J. Physiol. 457, 657-664.

[10] Joachim K., (2022). Structure, Function and Regulation of the Plasma Membrane Calcium Pump in Health and Disease, Int. J. Mol. Sci. 2022, 23(3), 1027; https://doi.org/10.3390/ijms23031027.

[11] Wenjuan D., Juefei Z., Wei L., Teng Z., Qianqian C., Fuyu Y., Taotao W., (2013), Plasma membrane calcium ATPase 4b inhibits nitric oxide generation through calcium-induced dynamic interaction with neuronal nitric oxide synthase, Protein & Cell, 4(4):286–298.

[12] Deshpande L., Delorenzo .J., Chrun S., and Parson J., (2020). Neural- specific inhibition of endoplasmic reticulum Mg -Ca ATPase uptake in a mixed primary hippocampal culture model, Brain. Scin. 10(7),438.

[13] Otilia G., Ioana M., Iulia P., Stefania D., Ramona P., Mioara C., Roxana B., Madalina C. and Sorin C., (2023), In Vitro Assessment of the Impact of Ultraviolet B Radiation on Oral Healthy and Tumor Cells, Photonics, 10(4), 464; https://doi.org/10.3390/photonics10040464.

[14] Zsófia S., Zsuzsanna N., József B., Györgyi K., Péter N.,Erika S., György T., Rosanna P. and Brahim S., (2023), Assessment of Inflammation in 3D Reconstructed Human Skin Exposed to Combined Exposure to Ultraviolet and Wi-Fi Radiation, Int. J. Mol. Sci., 24(3), 2853; https://doi.org/10.3390/ijms24032853.

[15] Orazio J., Jarret S., Amero A. and Scot T., (2013). UVradiationand skin. Int. J. Mol. Sci. 14, 12222-12248.
[16] Hoeijmakers, J., (2009). DNA damage, aging and cancer, J. Med. 361, 1475-1485.
[17] Naraman, D.L.; Saladi,R.N.and Fox, J. L. (2010). Ultraviolet radiation and skin cancer, Int. J. Dermatol. 49, 978-986.
[18] Ahmed U., Abdulla T., (2022). Ozone Layer Depletion and Emerging Public Health Concerns - An Update on Epidemiological Perspective of the Ambivalent Effects of Ultraviolet Radiation Exposure, Front. Oncol., 12, 866733.
[19] Kim J, Andrew T. (2020). Real-time and noninvasive detection of UV-Induced deep tissue damage using electrical tattoos. Biosens Bioelectron. 15, 150: 111909. Doi: 10.1016/j.bios.2019.111909. Epub 2019 Nov 23. PMID: 31786020.
[20] Zhongwei L. and Raouf A., (2018). Evolving Mechanisms of Vascular Smooth Muscle Contraction Highlight Key Targets in Vascular Disease, Biochem Pharmacol. 153: 91–122.
[21] Sanders K., (2008). Regulation of smooth muscle excitation and contraction, Neurogastroenterol Motil., 20 (Suppl 1): 39–53.
[22] Michael C., Neil H. and Cheryl A., (2021). Centrifugation Removes a Population of Large Vesicles, or "Macroparticles," Intermediate in Size to RBCs and Microvesicles, Int J Mol Sci., 22(3): 1243.
[23] Wu Q, Guo D, Bi H, Wang D, Du Y. (2013), UVB irradiation-induced dysregulation of plasma membrane calcium ATPase1 and intracellular calcium homeostasis in human lens epithelial cells. Mol Cell Biochem. 382(1-2):263-272. doi: 10.1007/s11010-013-1743-2. Epub 2013 Jul 2. PMID: 23817774.
[24] Kazi M., Kamrul H., Sufara A., Renu T., Narendra T., (2013), Global calcium transducer P-type Ca2+-ATPases open new avenues for agriculture by regulating stress signalling, Journal of Experimental Botany, 64(11):3099–3109.
[25] Tatalovich Z., Wilson J., Mack T., (2006). The objective assessment of lifetime cumulative ultraviolet exposure of determining melanoma risk, J. Photochem. photo. Biol. 85, 198-204.
[26] Balasubramanian D., (2000). Ultraviolet radiation and contaract, J. Ocul. Phamacol. Ther. 16:285-297.
[27] Lccas R., McMichael A., Armstrong B., (2008). Estimating the global disease burden due to Ultraviolet radiation exposure, Int. J. Epidemoil. 37,654-667.
[28] Brozyna A., Zbytek B., Granese J., Carlson J., Ross j., (2007), Mechanism of UV-related carcinogenesis and its contribution to nevi/melanoma, Expert Rev Dermatol. 2(4):451–469.

How to Cite

K. H. . Gathwan, A. A. . Albir, and S. J. Attia, "The Measurement of Activity of the Ca2+_Mg2+ ATPase in Membranous Vesicles Isolated from Smooth Muscle of Ileum in Rats Treated with Ultraviolet Radiation", Al-Mustansiriyah Journal of Science, vol. 34, no. 3, pp. 10–14, Sep. 2023.