Cumhuriyet Sci. J., 44(4) (2023) 645-649
DOI: https://doi.org/10.17776/csj.1340869

Cumhuriyet Science Journal

| csj.cumhuriyet.edu.tr | Founded: 2002 ISSN: 2587-2680 e-ISSN: 2587-246X Publisher: Sivas Cumhuriyet University

Investigation of the Effect of Indatraline on Oxidative Damage Induced by Hydrogen Peroxide in C6 Glioma Cell Line

Fatih Yulak [1,a,*], Bünyamin Üngür [2,b]

[1] *Department of Physiology, School of Medicine, Sivas Cumhuriyet University, Sivas, Türkiye.*
[2] *School of Medicine, Sivas Cumhuriyet University, Sivas, Türkiye.*
**Corresponding author*

Research Article

History
Received: 10/08/2023
Accepted: 12/12/2023

ABSTRACT

Oxidative stress is defined as an imbalance between the generation of reactive oxygen species (ROS) and their scavenging. Indatralin, which has serotonin reuptake inhibitory activity, has not yet been studied for its ability to prevent oxidative damage. Our research's objective was to find out how indatraline defends against oxidative damage. C6 cells were used in the study and four different cell groups were created. The control group received no therapy at all. For 24 hours, cells in the H_2O_2 group were exposed to 0.5 mM H_2O_2. The indatraline group received indatraline treatments for 24 hours at various doses (0.5, 1, 2.5, 5 and 10 µM). For one hour, indatraline was administered to the indatraline + H_2O_2 group at various concentrations (0.5, 1, 2.5, 5 and 10 µM) before the group was subjected to 0.5 mM H_2O_2 for 24 hours. Following the occurrence of oxidative damage, total antioxidant status (TAS) and total oxidant status (TOS) levels were determined. Cell viability was also evaluated using the XTT assay. As a result, after hydrogen peroxide-induced oxidative damage, indatraline at doses of 10, 5, and 2.5 µM showed a protective effect by significantly enhanced cell survival in C6 cells($p < 0.001$). Additionally, indatraline boosted the lowered TAS level while decreasing the elevated TOS levels following hydrogen peroxide-induced oxidative damage ($p<0.001$).

Keywords: Oxidative damage, Hydrogen Peroxide (H_2O_2), Indatraline, C6 Glioma.

[a] *fatihyulak@gmail.com* *https://orcid.org/0000-0003-3708-6752* [b] *ungurbunyamin@gmail.com* *https://orcid.org/0009-0003-7635-6820*

Introduction

Hydrogen peroxide (H_2O_2), superoxide anions (O2-), and hydroxyl radicals (OH-), which are generated in minute quantities during regular oxygen metabolism, are known as reactive oxygen species (ROS). In the dismutation reaction of superoxide, two superoxide molecules receive two protons to produce hydrogen peroxide and molecule oxygen. Because non-radical products are produced in this process, either naturally or as a result of the enzyme superoxide dismutase (SOD), it is known as the dismutation reaction. In addition, ROS play a crucial role for these cells[1] in crucial signal transduction processes including secondary messenger systems[1]. Free radicals are extremely harmful to cells and maintain homeostasis by interacting harmoniously with the antioxidant system[2]. Disruption of the oxidant/antioxidant balance causes the formation of free radicals, damaging important cell structures such DNA[3], lipids[4], proteins[5], and carbohydrates[6]. Cells lose their ability to function and finally perish as a result of genetic damage brought on by an excessive increase in H_2O_2 production [7,8]. Lipid peroxidation is the primary mechanism of ROS-induced oxidative damage[9]. Due to the brain's high lipid content and heightened sensitivity to ROS, many neurodegenerative disorders are caused by increased oxidative stress [10–12]. Long-term potentiation (LTP), a key process for learning, synaptic transmission, and neural plasticity, has been demonstrated to be dramatically reduced by elevated ROS concentrations[13,14]. Glial cells like microglia and astrocytes are the principal producers of ROS[15]. Therefore, lowering oxidative stress by regulating ROS generation in glial cells is crucial for the treatment of neurodegenerative disorders.

A strong inhibitor of serotonin (5-HT) reuptake is indatraline. By inhibiting 5-HT reuptake, serotonin reuptake medications boost serotonin transmission and have antidepressant effects by reducing the number and sensitivity of post synaptic receptors[16], [17]. Numerous psychiatric medications, including serotonin reuptake inhibitors, have been shown in studies to have neuroprotective benefits[18,19]. One study suggested that indatralin may slow the progression of various neurodegenerative disorders, including Alzheimer's disease, by inducing autophagy.[20]. Its effects on oxidative damage in C6 glioma cells, however, have not yet been studied and are still unknown. In this study, we looked at how indatraline affected the production of ROS and the viability of C6 glioma cells under an oxidative damage scenario generated by H_2O_2.

Material And Methods

Cell Culture

C6 Glioma (CRL107) cell lines were acquired from the American Type Culture Collection and cultured in DMEM (Thermo Fisher Scientific, Altrincham, UK) containing 10% Fetal Bovine Serum (FBS) (Sigma-Aldrich Co., St Louis, MO, USA), 1% L-glutamine (Sigma-Aldrich Co., St Louis, MO, USA), and 1% penicillin/streptomycin. The cells were kept at 37°C in a 5% CO_2 humidified environment. Before treatment, stock solutions were made by dissolving indatraline and H_2O_2 (Sigma-Aldrich Co., St. Louis, MO, USA) in DMEM.

Cell Viability Assay

The Roche Diagnostic, Massachusetts, USA, XTT assay was employed to measure cell viability. Prior to indatraline treatment, C6 glioma cells were seeded in 96-well plates at a density of $1x10^4$ cells per well in 100 mL of DMEM. Four cell groups were set up the following day to assess indatraline's protective effects. The control group did not receive any care. For 24 hours, cells in the H_2O_2 group were exposed to 0.5 mM H_2O_2. For 24 hours, cells in the indatraline group were exposed to indatraline at different concentrations (10, 5, 2.5, 1 ve 0.5 µM). For 24 hours, cells in the indatraline group were exposed to indatraline at different concentrations (10, 5, 2.5, 1 ve 0.5 µM). The indatraline + H_2O_2 group's cells had an hour of pretreatment with varying doses of indatraline (10, 5, 2.5, 1 or 0.5 µM) before being exposed to 0.5 mM H_2O_2 for 24 hours. After incubation, the medium was taken out, and phosphate buffered saline was used to wash the wells twice. The plates were then kept at 37°C for 4 hours after the last step of adding 100 µL of DMEM without phenol red and 50 µL of XTT labeling solution to each well. The plates were shaken, and an ELISA microplate reader (Thermo Fisher Scientific, Altrincham, UK) was used to measure the absorbance at 450 nm. The cell viability was determined by comparing the percentage of viable cells to the control, or untreated cells, in each experiment three times (Figure 1).

Preparation of Cells Homogenates

Sterilized tubes were used to collect the cells for each group. They underwent a 10-minute centrifugation at 2000 rpm. Supernatants were eliminated. PBS (pH: 7.4) is used to thin the cell suspension to a cell concentration of roughly 1 million cells per milliliter. This component of the cells that are in the under the tubes, suspended. Through numerous freeze-thaw cycles, the cells were destroyed in order to release the internal components. They underwent a 10-minute, 4°C, 4000 rpm centrifugation process. Then, using TAS and TOS commercial kits (Rel Assay Kit Diagnostics, Antep, Turkey), the supernatants were collected for biochemical examination of total antioxidant status (TAS) and total oxidant status (TOS). The total protein levels in samples were determined using a Bradford protein assay kit (Merck Millipore, located in Darmstadt, Germany)[21].

Measurement of TAS and TOS

An automated test technique that Erel had previously created was used to measure the TAS concentrations in the cell supernatants [22]. The technique is based on detecting the absorbance of colored diacidly radicals during free radical reactions, starting with the generation of hydroxyl radicals in the Fenton reaction, in order to track the reaction rate of free radicals. According to their quantities, antioxidants in the tissue samples should decrease coloring[22]. Micromolar Trolox equivalents per milligram of tissue protein (mol Trolox Eq/mg protein) were used to express the results. Using Erel's automated test approach, tissue TOS concentrations in cell supernatants were measured[23]. The method enables the measurement of tissue ferric ion levels using xylenol orange in order to quantify TOS levels because ferrous ion is converted to ferric ion when sufficient amounts of oxidants are present in the medium. The calibration of the assay was done using hydrogen peroxide[23]. In micromolar hydrogen peroxide equivalents per milligram tissue protein (µmol H_2O_2 Eq/mg protein), the assay's results were expressed.

Statistical Analysis

The results were expressed as a mean ± standard error of the mean (SEM). The data analyses were performed with SPSS Version 23.0 for Windows. The data were evaluated using a one-way analysis of variance (ANOVA) and a postdoc Tukey test was utilized to identify the differences between the experimental groups, and a value of $p < 0.05$ was accepted as statistically significant.

Results

Effects of Indatraline on Cell Viability in C6 Glioma Cells İnduced Oxidative Damage by H_2O_2

To assess the neuroprotective effects of indatraline on C6 glioma cell toxicity by, an XTT cell viability experiment was carried out. Our work employed the 0.5 mM H_2O_2 IC_{50} value that was discovered in earlier research on C6 glioma cells[24]. According to Figure 1, continuous H_2O_2 (0.5 mM) concentration significantly reduced cell viability when compared to control ($^{\gamma}P<0.001$, Figure 1). At concentrations of 10, 5, and 2.5 µM, indatraline treatment significantly boosted the viability of H_2O_2-induced C6 glioma cells ($\alpha P<0.001$, Figure 1). Additionally, there was no discernible difference between the control group and the C6 glioma cells treated with various amounts of indatraline in cells not treated with H_2O_2 ($P>0.05$).

Groups (XTT)	Cell Viability (% of control)	SEM
Control	100	0
H_2O_2	52.73	± 0.81
Ind 10 µM + H_2O_2	78.64	± 1.57
Ind 5 µM + H_2O_2	72.53	± 0.91
Ind 2.5 µM + H_2O_2	67.34	± 1.93
Ind 1 µM + H_2O_2	53.53	± 1.74
Ind 0.5 µM + H_2O_2	55.63	± 1.82
Ind 10 µM	105.52	± 1.42
Ind 5 µM	104.42	± 2.53
Ind 2.5 µM	103.56	± 2.41
Ind 1 µM	100.11	± 1.11
Ind 0.5 µM	99.22	± 2.12

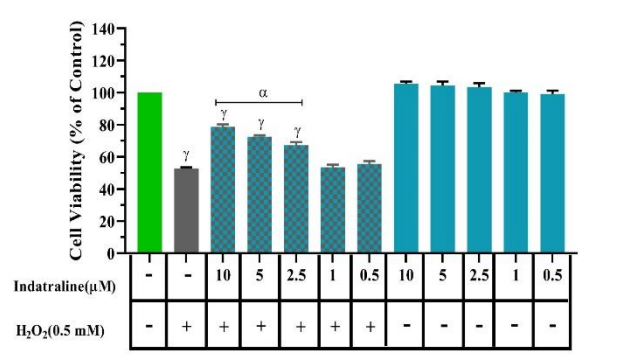

Figure 1. Effects of indatraline on C6 glioma cells' ability to survive after being subjected to H_2O_2-induced oxidative damage. Results are shown as the mean ± SEM ($^{\gamma}P<0.001$, $^{\alpha}P<0.001$).

Evaluation of the Effect of Indatraline on TAS and TOS in C6 Glioma Cells Induced Oxidative Damage by H_2O_2

Using commercial kits, TAS and TOS were evaluated in C6 glioma cells. H_2O_2-treated cells had considerably lower TAS levels than control cells in C6 glioma cells ($^{\gamma}P<0.001$, Figure 2). The TAS level in the H_2O_2 + indatraline group was considerably higher than that in the H_2O_2 group ($^{\alpha}P<0.001$, figure 2). In H_2O_2-treated cells compared to control, TOS level was considerably higher ($^{\gamma}P<0.001$, Figure 2). When compared to the H_2O_2 group, the TOS level was considerably lower in the H_2O_2 + indatraline group($^{\alpha}P<0.001$, Figure 2).

Groups (TAS)	mol Trolox Eq/mg protein	SEM
Control	0.76	± 0.041
H_2O_2 (0.5 mM)	0.49	± 0.022
Ind 10 µM + H_2O_2	0.72	± 0.025
Ind 10 µM	0.75	± 0.017

Groups (TOS)	µmol H_2O_2 Eq/mg protein	SEM
Control	1.54	± 0.085
H_2O_2 (0.5 mM)	4.01	± 0.094
Ind 10 µM + H_2O_2	2.54	± 0.076
Ind 10 µM	1.55	± 0.083

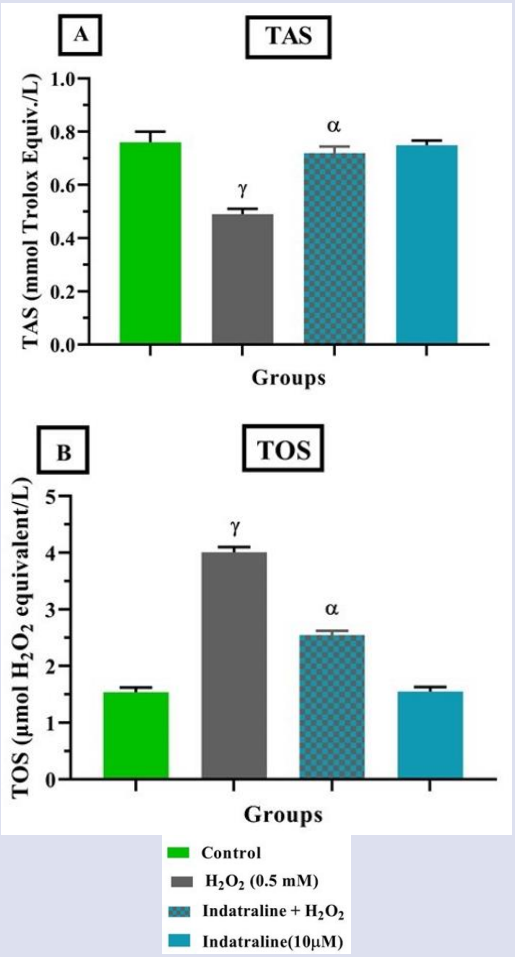

Figure 2. Effects of indatraline on TAS and TOS following H_2O_2 treatment in C6 glioma cells. Values are presented as mean ± SEM ($^{\gamma}P<0.001$, $^{\alpha}P<0.001$).

Discussion

Numerous mechanism can cause oxidative stress, which can cause cell death and brain dysfunction [25]. As a result, it may increase the risk of developing neurodegenerative illnesses like Alzheimer's, Parkinson's, Hungtinton, and others [26,27], with ROS and H_2O_2 in general, which are the main causes of oxidative stress, contributing to the underlying causes of these conditions [28]. Indatraline's ability to protect C6 glioma cells from oxidative damage brought on by hydrogen peroxide was assessed in this work for the first time. Here, indatraline significantly shielded C6 cells from the toxicity brought on by hydrogen peroxide. Additionally, indatraline pretreatment demonstrated a strong protective effect by raising TAS level and lowering TOS level against hydrogen peroxide-induced oxidative damage in C6 cells.

Numerous antidepressants, including indatraline, have been demonstrated in prior research to shield the brain from oxidative stress. In vitro research done concurrently with our findings revealed that indatraline greatly increased cell viability in HT-22 hippocampus cells subjected to glutamate-induced toxicity[29]. Indatraline

may mitigate various neurodegenerative illnesses marked by the buildup of fibrillary tangles, according to another in vivo study[20]. According to yet another study, serotonin reuptake inhibitors like indatraline exhibit brain-protective effects via boosting the brain's production of neurotrophic factor[30], [31]. Indatraline shown a neuroprotective effect against neuropathic pain in another trial of the condition[32]. Salam et al. demonstrated, in accordance with our findings, that serotonin reuptake medications boosted the antioxidant system and decreased oxidative stress by raising TAS levels in the brain and liver[33]. In a comparable manner it was demonstrated in a comparable in vivo investigation using the serotonin reuptake inhibitor fluvoxamine that elevated TAS levels lowered oxidative stress by activating the antioxidant system[34].

Our results support earlier research and demonstrate that indatraline protects against oxidative damage by enhancing the antioxidant system and suppressing the oxidant system in glial cells and makes a major contribution to their survival. In the pathophysiology of several neurodegenerative illnesses, glial cells are crucial. Consequently, in the treatment of such disorders, indatraline might function as a protective agent. However, further research is required in this area.

This article has some limitations. Instead of primary glial cells, glioma cells, a secondary cell line, were used for this study. The techniques used are insufficient to explain indatraline's protective effects. More methodologies and mechanisms need to be studied, such as measurement of oxidative stress parameters including ROS levels and lipid peroxidation.

Conclusion

The results of this investigation demonstrated that indatraline decreased the oxidative damage caused by hydrogen peroxide in C6 glial cells. These effects are most likely brought about by the antioxidant system being stimulated, which lowers oxidative stress. Indatraline may therefore have a calming impact on the central nervous system. To clarify this, more study is necessary.

Acknowledgment

This study was supported by TUBITAK as a 2209-A project (1919B012109251). The authors would like to thank TUBITAK and the management of Sivas Cumhuriyet University, Faculty of Medicine, CÜTFAM Research Center, Sivas, Turkey for providing the necessary facilities to conduct this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] H. J. Forman, Use and abuse of exogenous H2O2 in studies of signal transduction, *Free Radic. Biol. Med.*, vol. 42(7) (2007) 926–932.

[2] Karabulut H., Şükrü Gülay M., Serbest Radikaller., *MAKÜ Sag. Bil. Enst. Derg.*, 4(41) (2016) 50–59.

[3] Kuraoka B., Robins P., Masutani C., Hanaoka F., Gasparutto D., Harbiyeli J., Ahşap RD., Lindahl T., Oxygen free radical damage to DNA. Translesion synthesis by human DNA polymerase eta and resistance to exonuclease action at cyclopurine deoxynucleoside residues., *J. Biol. Chem.*, 276(52) (2001) 49283–49288.

[4] Devasagayam T. P. A., Boloor K. K., and Ramasarma T., Methods for estimating lipid peroxidation: an analysis of merits and demerits., *Indian J. Biochem. Biophys.*, 40(5) (2003) 300–308.

[5] Bengal W., Free Radicals and Their Role in Different Clinical Conditions : An Overview, 1(3) (2010) 185–192.

[6] Devasagayam T. P. A., Tilak J. C., Boloor K. K., Sane K. S., Ghaskadbi S. S., and Lele R. D., Free radicals and antioxidants in human health: current status and future prospects., *J. Assoc. Physicians India*, 52(10) (2004) 794–804.

[7] Gandhi S. and Abramov A. Y., Mechanism of Oxidative Stress in Neurodegeneration, *Oxid. Med. Cell. Longev.*, 2012 (2012) 428010. doi: 10.1155/2012/428010.

[8] Coyle J. T. and Puttfarcken P., Oxidative Stress, Glutamate, and Neurodegenerative Disorders, *Science.*, 262(5134) (1993)n689–695.

[9] Shirley R., Ord E. N. J., and Work L. M., Oxidative Stress and the Use of Antioxidants in Stroke., *Antioxidants*, 3(3) (2014) 472–501.

[10] Dias V., Junn E., and Mouradian M. M., The role of oxidative stress in Parkinson's disease., *J. Parkinsons. Dis.*, 3(4) (2013) 461–491.

[11] Gella A. and Durany N., Oxidative stress in Alzheimer disease., *Cell Adh. Migr.*, 3(1) (2009) 88–93.

[12] Jelinek M., Jurajda M., and Duris K., Oxidative Stress in the Brain: Basic Concepts and Treatment Strategies in Stroke., *Antioxidants*, vol. 10(12) (2021) 1886.

[13] Knapp L. T. and Klann E., Role of reactive oxygen species in hippocampal long-term potentiation: contributory or inhibitory?, *J. Neurosci. Res.*, 70(1) (2002) 1–7.

[14] Zhuo M., Small S. A., Kandel E. R., and Hawkins R. D., Nitric oxide and carbon monoxide produce activity-dependent long-term synaptic enhancement in hippocampus., *Science*, 260(5116) 1946–1950.

[15] Rodrigo R., Fernández-Gajardo R., Gutierrez R. , Matamala J.M. , Carrasco R., Miranda-Merchak A., Feuerhake W., Oxidative stress and pathophysiology of ischemic stroke: novel therapeutic opportunities., *CNS Neurol. Disord. Drug Targets*, 12(5) (2013) 698–714.

[16] Bøgesø K. P., Christensen A. V, Hyttel J., and Liljefors T., 3-Phenyl-1-indanamines. Potential antidepressant activity and potent inhibition of dopamine, norepinephrine, and serotonin uptake., *J. Med. Chem.*, 28(12) (1985) 1817–1828.

[17] Hyttel J. and Larsen J. J., Neurochemical profile of Lu 19-005, a potent inhibitor of uptake of dopamine, noradrenaline, and serotonin., *J. Neurochem.*, 44(5) (1985) 1615–1622.

[18] Hunsberger J., Austin D. R., Henter I. D., and Chen G., The neurotrophic and neuroprotective effects of psychotropic

agents., *Dialogues Clin. Neurosci.*, 11(3) (2009) 333–348.
[19] Young L. T., Neuroprotective effects of antidepressant and mood stabilizing drugs., *Journal of psychiatry & neuroscience : JPN*, 27(1) (2002) 8–9.
[20] Cho Y. S., Yen C., Shim J. S., Kang D. H., and Kang S. W., Antidepressant indatraline induces autophagy and inhibits restenosis via suppression of mTOR / S6 kinase signaling pathway, *Nat. Publ. Gr.*, 6(5) (2016) 1–9.
[21] Kruger N. J., The Bradford Method for Protein Quantitation BT - The Protein Protocols Handbook, Ed. Totowa, NJ: Humana Press, (2002)15–21.
[22] Erel O., A novel automated method to measure total antioxidant response against potent free radical reactions, *Clin. Biochem.*, 37(2) (2004) 112–119.
[23] Erel O., A new automated colorimetric method for measuring total oxidant status, *Clin. Biochem.*, 38(12) (2005) 1103–1111.
[24] Ergül M. and Taşkiran A. Ş., Thiamine protects glioblastoma cells against glutamate toxicity by suppressing oxidative/endoplasmic reticulum stress, *Chem. Pharm. Bull.*, 69(9) (2021) 832–839.
[25] Armstrong R. W. and Wu C. C., Lattice Misorientation and Displaced Volume for Microhardness Indentations in MgO Crystals, *J. Am. Ceram. Soc.*, 61(3–4) (1978) 102–106.
[26] Rao A. V and Balachandran B., Role of oxidative stress and antioxidants in neurodegenerative diseases., *Nutr. Neurosci.*, 5(5) (2002) 291–309.
[27] Niedzielska E., Smaga I., Gawlik M., Moniczewski A., Stankowicz P., Pera J., Filip M., Oxidative Stress in Neurodegenerative Diseases., *Mol. Neurobiol.*, 53(6) (2016) 4094–4125.
[28] Li J., O W., Li W., Jiang Z.-G., and Ghanbari H. A., Oxidative stress and neurodegenerative disorders., *Int. J. Mol. Sci.*, 14(12) (2013) 24438–24475.
[29] Maher P. and Davis J. B., The role of monoamine metabolism in oxidative glutamate toxicity., *J. Neurosci. Off. J. Soc. Neurosci.*, 16(20) (1996) 6394–6401.
[30] Nibuya M., Morinobu S., and Duman R. S., Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments., *J. Neurosci. Off. J. Soc. Neurosci.*, 15(11) (1995) 7539–7547.
[31] Lindén A. M., Väisänen J., Lakso M., Nawa H., Wong G., and Castrén E., Expression of neurotrophins BDNF and NT-3, and their receptors in rat brain after administration of antipsychotic and psychotrophic agents., *J. Mol. Neurosci.*, 14(1–2) (2000) 27–37.
[32] Hacısüleyman L., Saraç B., and Joha Z., Analgesic Effects of Vilazodone, Indatraline, and Talsupram in a Rat Model of Neuropathic Pain., *Turkish J. Pharm. Sci.*, 19(3) (2021) 336–342.
[33] Abdel-Salam O. M. E., Morsy S. M. Y., and Sleem A. A., The effect of different antidepressant drugs on oxidative stress after lipopolysaccharide administration in mice., *EXCLI J.*, 10 (2011) 290–302.
[34] Severcan S. M., Severcan C., Tazehkand M. N., and Oz Z. S., Evaluation of O xidant- A ntioxidant S tatus of F luvoxamine on H uman L ymphocyte C ell C ulture, 8(1) (2021) 79–83.