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Effects of the autophagy modulators d-limonene and chloroquine on vimentin levels in SH-SY5Y cells

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ABSTRACT

The molecular target and mechanism by which d-limonene induces LC3 lipidation and autophagosome formation remain elusive. Here, we report that this monoterpene rapidly enhances Ca^{2+} levels in SH-SY5Y cells; yet this effect does not lead to calpain- or caspase-mediated proteolysis of α-spectrin, nor calpain activity is required for the established enhancement of LC3-II levels by d-limonene. However, d-limonene rapidly reduced vimentin levels, an unexpected effect also induced by the autophagy inhibitor chloroquine (CQ). The magnitude of vimentin reduction parallels accumulation of LC3-II caused by a brief incubation with d-limonene or CQ. For longer exposure (48 h), d-limonene does not reduce vimentin, nor it increases LC3-II levels; conversely, a clear reduction of vimentin along with a massive accumulation of LC3-II is evident in cells treated with CQ. Vimentin participates in organelle positioning and in other cellular processes that have linked this intermediate filament protein to various diseases, including cancer, inflammatory and autoimmune disorders, and to virus replication and internalization. Our findings suggest an inverse relationship between vimentin reduction and LC3-II accumulation, whose causal link needs to be examined. Further experiments are needed to dissect the role of vimentin reduction in the mechanisms through which CQ impairs fusion of autophagosome with lysosomes as well as in other effects of this drug.

1. Introduction

d-Limonene is a natural monoterpene hydrocarbon that, in the past years, has attracted interest especially for its chemopreventive and antitumoral activities in preclinical animal models of chemically-induced carcinogenesis [1]. Along with this, d-limonene, which is abundant in *Citrus* essential oils, likely contributes to neuroprotection [2] and modulation of synaptic transmission [3] elicited by the essential oil of bergamot (*Citrus bergamia* Risso et Poiteau). Accordingly, some evidence has recently emerged that d-limonene improves the regeneration process and the sensory and motor function recovery after peripheral nerve injury in mice [4], and protects from Aβ42 toxicity in a Drosophila model of Alzheimer's disease [5]. However, the molecular target(s) responsible for the anticancer and neuroprotective effects of d-limonene remains still elusive. By using d-limonene at concentrations (250–750 μM) within the breast tissue levels measured in a clinical study, where limonene was administered orally at 2 g per day for 2–6 weeks before surgery in women with newly diagnosed operable breast cancer [6], we observed that d-limonene stimulates basal autophagy in human SH-SY5Y neuroblastoma cells [7,8] as well as in human HepG2 hepatocellular carcinoma and MCF7 breast cancer cells [8]. The ability of 500 μM d-limonene to stimulate autophagy was confirmed by Yu et al. [9] in A549 and H1299 lung cancer cells. Importantly, modulation of autophagy-related genes and proteins was reported in xenograft tumors of transplanted nude mice treated with d-limonene, along with a reduction of tumor size [9].

Macroautophagy (hereafter autophagy) is an intracellular process through which autophagosomes engulf cytoplasmic materials and deliver them to the lysosomes for degradation [10]. This

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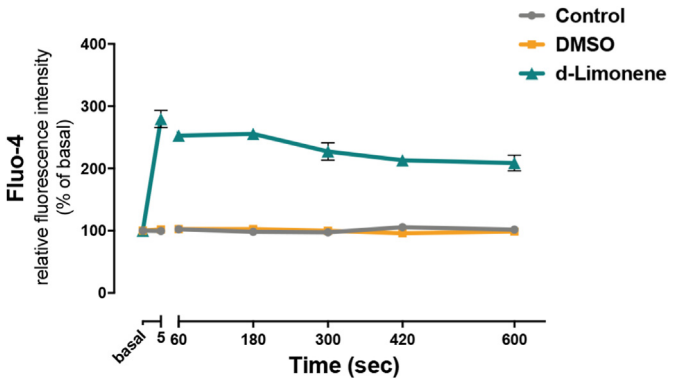

Fig. 1. d-Limonene increases intracellular Ca^{2+} levels. SH-SY5Y cells were loaded with Fluo-4 AM at 37 °C for 30 min and then washed twice with DPBS. The fluorescence intensity of the calcium sensor was measured by flow cytometry before (basal) and up to 10 min after treatment with DMSO (0.108%) or d-limonene (750 μM). Each point is the mean ± s.e.m. from three independent experiments. Results are expressed as percent of basal.

process enables the recycling of cellular components upon nutrient limitation; however, autophagy is more than just an adaptative metabolic response. It is critical for cellular homeostasis, allowing the removal of damaged organelles and the degradation of misfolded and aggregate-prone proteins. Accordingly, a deregulation of this quality control mechanism has been linked to several human pathologies, including neurodegeneration and cancer [11], paving the way for investigating the therapeutic potential of autophagy regulation. Thus, there is a need for additional autophagy modulators as valuable experimental tools for dissecting the complex biological processes of autophagy and validating the therapeutic efficacy of targeting autophagy.

d-Limonene appears a promising tool to modulate autophagy; in fact, it is regarded as a safe molecule [12] with minimal side effects [13]. However, the main limitation restraining its usefulness is that the target and the mechanisms through which it regulates autophagy are still unknown. One of the difficulties in identifying the exact mechanism involved stems from the various intracellular pathways affected by d-limonene, which may also have opposite effects in autophagy regulation [8]. Thus, not only LC3 lipidation by d-limonene is independent from inhibition of the mTOR pathway, which plays a key role in inhibiting autophagy initiation [14], but the rapid and transient increase in the phosphorylation levels of relevant mTOR substrates suggests that autophagy initiation and mTOR signalling are concurrently activated by d-limonene [8]. Furthermore, d-limonene rapidly activates ERK and stimulates ROS generation in SH-SY5Y cells, yet none of these events is implicated in its ability to stimulate autophagy [8].

Our previous data suggest the involvement of intracellular Ca^{2+} in the mechanism of LC3 lipidation initiated by d-limonene; in fact, the cell-permeant calcium chelator BAPTA-AM did not abrogate but significantly reduced the levels of LC3-II increased by d-limonene [8]. To get more insights into the mechanisms implicated in d-limonene action, here we investigated whether d-limonene affects intracellular Ca^{2+} levels and requires calpain activity to increase LC3-II levels in SH-SY5Y cells. We report that d-limonene rapidly enhances Ca^{2+} levels; this effect does not lead to calpain-mediated proteolysis of α-spectrin nor calpain activity is required for the enhancement of LC3-II levels by d-limonene. Moreover, by investigating the mechanisms through which d-limonene reduces vimentin levels, we describe here some effects of chloroquine (CQ) we have incidentally observed by using this drug to inhibit autophagy. In addition to causing cells to accumulate LC3-II, CQ provokes a rapid and long-lasting reduction of vimentin levels.

2. Materials and methods

2.1. Reagents

d-Limonene (Cas No 5989-27-5), dimethyl sulfoxide (DMSO) and chloroquine (CQ) were purchased from Sigma-Aldrich (St. Louise, MO, USA). 3-(4-Iodophenyl)-2-mercapto-(Z)-2-propenoic Acid (PD 150606) was obtained from Calbiochem (San Diego, CA, USA).

2.2. Cells, culture conditions and treatments

Adherent human SH-SY5Y neuroblastoma cells, from ICLC-IST (Genoa, Italy) were cultured as previously described [8] in RPMI 1640 medium (Gibco, Life Technologies, Paisley, UK) supplemented with heat-inactivated fetal bovine serum (10% v/v; Gibco). Cells were seeded in 6-well plates and 24 h after plating were treated for the indicated time. A stock solution (10%) of d-limonene in DMSO was further diluted in culture medium to obtain a final concentration of 750 μM; DMSO was added to the medium of parallel control cultures (vehicle-treated cells) to obtain a final percentage of 0.108% equal to that present in d-limonene-treated cells. Stock solutions of PD 150606 (20 mM in DMSO) and CQ (20 mM in water) were further diluted in culture medium and applied to SH-SY5Y cells together with (PD 150606) or 2 h before (CQ) the addition of d-limonene and DMSO for the indicated time.

2.3. Analysis of intracellular calcium

The relative levels of cytosolic free Ca^{2+} were measured as previously described [15] using the calcium indicator Fluo-4 AM (Molecular Probes), a cell permeable probe that is essentially non-fluorescent in the absence of Ca^{2} and exhibiting an increase in fluorescence upon Ca^{2+} binding. In brief, SH-SY5Y cells were seeded in 6-well plates and 24 h after seeding were resuspended in Dulbecco's phosphate-buffered saline (DPBS) with calcium chloride and magnesium chloride plus 20 mM HEPES and stained with 3 μM Fluo-4 AM for 30 min; after two washes with DPBS the cells were maintained at 37 °C and analyzed by BD FACSVerse flow cytometer. The fluorescence intensity was acquired before (basal) and up to 10 min after treatment with vehicle (DMSO, 0.108%) or d-limonene (750 μM).

2.4. Western blotting analysis

Cell lysis and immunoblotting were performed as previously described [7]. The following primary antibodies and dilutions were used: anti-LC3 1:2000 (code PD036; MBL, International Corporation, Nagoya, Japan); anti-spectrin 1:1000 (non-erythroid; clone AA6; Merk Millipore, MA, USA); anti-vimentin 1:1000 (Clone RV202; Abcam, Cambridge, UK); anti-actin 1:1000 (clone AC-40; Sigma-Aldrich, Milan, Italy); anti-β-tubulin 1:40000 (clone B-5-1-2; Sigma-Aldrich, Milan, Italy); anti-GAPDH 1:50000 (clone 6C5; Applied Biosystems, Carlsbad, CA, USA).

2.5. Statistical analysis

Data are expressed as the mean ± s.e.m. of the indicated number of independent experiments and evaluated statistically for difference by ANOVA followed by Tukey–Kramer test for multiple comparisons. A value of $P < 0.05$ was considered as significant.

3. Results and discussion

To investigate whether d-limonene affects intracellular Ca^{2+}

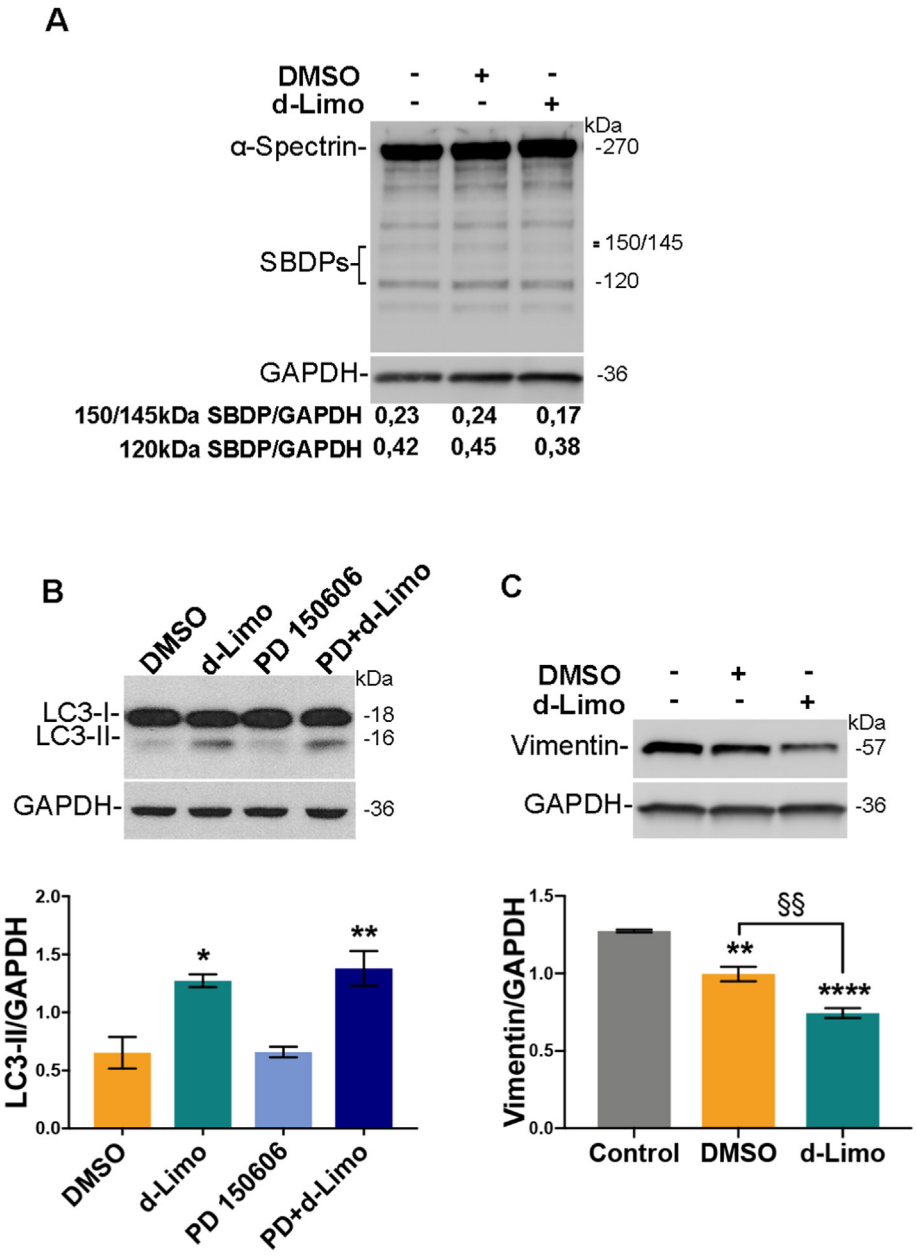

Fig. 2. Calpain is not involved in the enhancement of LC3-II levels induced by d-limonene. (**A**) Representative immunoblot of SH-SY5Y cells incubated for 30 min with DMSO (0.108%) or d-limonene (d-Limo, 750 μM) showing that d-limonene does not increase the levels of the calpain-specific 150–145 kDa α-spectrin breakdown products (SBDPs); also, note the lack of accumulation of 120 kDa SBDP derived from caspase-mediated proteolysis. GAPDH was used as loading control; 150/145 kDa and 120 kDa SBDPs/GAPDH optical density ratios for the reported blot are shown. (**B**) The calpain inhibitor PD 150606 does not reduce LC3-II levels increased by d-limonene. SH-SY5Y cells were treated with d-limonene (d-Limo, 750 μM) given alone or in combination with PD 150606 (PD, 20 μM) for 60 min and the total protein extracts were subjected to western blot analysis of LC3; GAPDH was used as internal control. Histograms show the results of densitometric analysis from three independent experiments (mean ± s.e.m.); *P < 0.05 and **P < 0.01 vs DMSO and PD 150606 given alone (ANOVA followed by Tukey-Kramer multiple comparison test). (**C**) d-Limonene (d-Limo; 750 μM) significantly reduces vimentin levels following a 30 min incubation. Representative immunoblot showing vimentin expression of SH-SY5Y cells treated as in (A). Histograms show the results of densitometric analysis from three independent experiments (mean ± s.e.m.). **P < 0.01 and ****P < 0.0001 vs Control; §§P < 0.01 (ANOVA followed by Tukey Kramer multiple comparison test).

levels, these were monitored by FACS analysis using the calcium indicator Fluo-4 AM. As shown in Fig. 1, d-limonene triggers a rapid increase of intracellular Ca^{2+} in SH-SY5Y cells. This finding is in line and corroborates our previous data showing that calcium participates in or, at least, it is partly necessary for autophagy initiation by d-limonene [8].

Enhancement of intracellular Ca^{2+} may activate calpain [16]. Calpain is required for autophagy [17] but activation of calpain, in turn, may negatively regulate autophagy [18]. To investigate whether d-limonene stimulates the Ca^{2+}-activated neutral protease calpain I, we examined the typical calpain-mediated generation of α-spectrin cleavage fragments (150–145 kDa), by western-blot analysis. As shown in Fig. 2A, no accumulation of the calpain-cleaved 150–145 kDa α-spectrin breakdown products (SBDPs) was detectable in cells treated for 30 min with d-limonene. These observations suggest that d-limonene does not activate calpain; however, to further assess whether calpain is required for d-limonene stimulated autophagy, the calpain inhibitor PD 150606 was also used. Pharmacological inhibition of calpain does not affect basal LC3-II levels nor it modifies LC3-II levels increased by d-

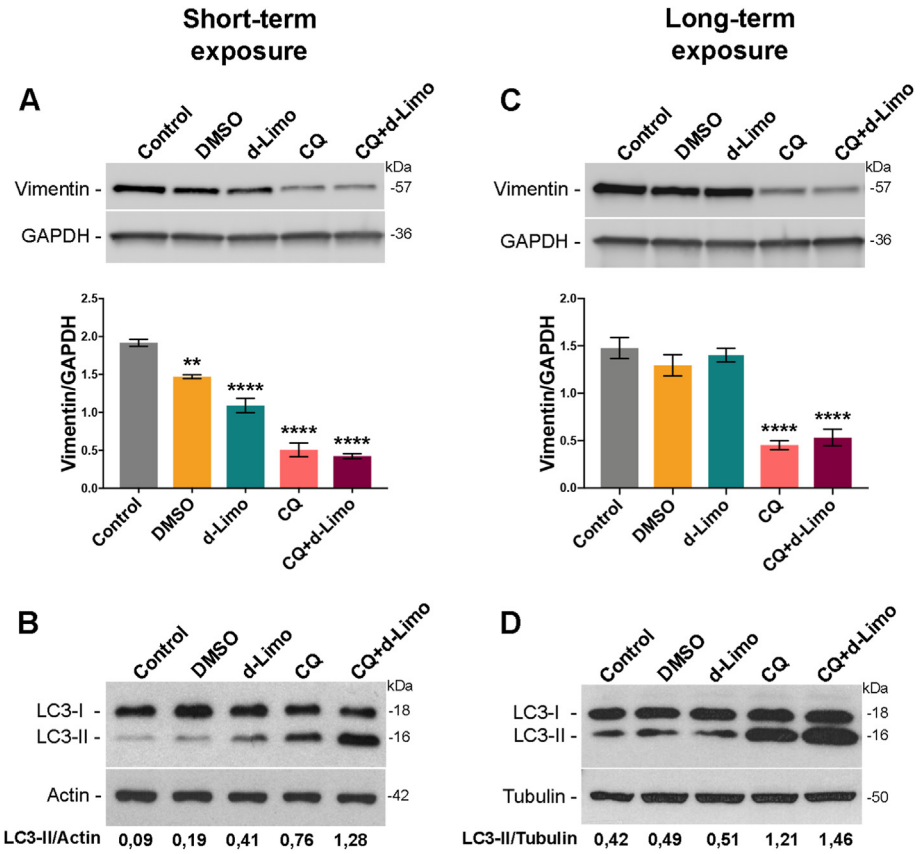

Fig. 3. Effects of short- and long-term exposure to d-limonene and chloroquine on vimentin and LC3-II levels. DMSO (0.108%) or d-limonene (d-Limo, 750 μM) were added for 30 min (**A, B**) or 48 h (**C, D**) to SH-SY5Y cells, with or without 2 h pretreatment with chloroquine (CQ, 50 μM). Total protein extracts were analyzed by western blotting for vimentin (A, C) and LC3 (B, D) levels; GAPDH, actin or tubulin were used as loading controls. Histograms in (A) and (C) show the results of densitometric analysis from three independent experiments (mean ± s.e.m.); **P < 0.01, ****P < 0.0001 vs Control (ANOVA followed by Tukey Kramer multiple comparison test). LC3-II/actin (B) and LC3-II/tubulin (D) optical density ratios for the reported blots are shown.

limonene (Fig. 2B). These findings indicate that enhanced autophagosome formation stimulated by d-limonene, though sensitive to Ca^{2+} buffering by BAPTA-AM [8], does not require calpain. An increase in intracellular Ca^{2+} can trigger autophagy by several mechanisms [19,20] and further experiments are needed to identify the Ca^{2+}-associated pathway that is recruited by d-limonene to stimulate autophagy.

Also, consistent with our previous observations that a brief exposure to d-limonene does not activate caspase-3 nor it induces a cleavage of PARP [21], here we observed that d-limonene does not enhance the levels of 120 kDa SBDPs indicative of caspase activation (Fig. 2A). However, when investigating the effects of d-limonene on vimentin, an intermediate filament protein, which is also a substrate of both calpain [22,23] and caspases [24], we observed that d-limonene caused a substantial reduction of this protein levels as compared to both control- and DMSO-treated cells, following a 30 min incubation (Fig. 2C). Because d-limonene does not appear to activate calpain or caspase-3 (Fig. 2A), we asked whether d-limonene could reduce vimentin levels through activation of autophagy and used CQ as acknowledged tool to inhibit the later stages of this pathway [10]. The results show that, following a short incubation time, CQ *per se* significantly reduced the levels of vimentin, and a reduction of a similar extent occurred in cells treated with both CQ and d-limonene (Fig. 3A). Moreover, the magnitude of vimentin reduction (Fig. 3A) parallels accumulation of LC3-II (Fig. 3B) caused by a brief incubation with d-limonene or CQ. However, upon treatment for 48 h, d-limonene does not reduce vimentin (Fig. 3C) nor it increases LC3-II levels (Fig. 3D) whereas a clear reduction of vimentin along with a massive accumulation of LC3-II is evident in cells treated with CQ, alone or in the presence of d-limonene (Fig. 3C and D). While these findings do not allow to establish a role for autophagic degradation in the reduction of vimentin caused by d-limonene, they uncover an inverse relationship between vimentin reduction and LC3-II accumulation, whose causal link needs to be examined.

CQ and d-limonene increase LC3-II via different mechanisms. While accumulation of LC3-II by CQ is due to inhibition of lysosomal degradation of LC3-II [10], we [7,8] and others [9] have demonstrated that d-limonene enhances the levels of LC3-II via increased LC3 lipidation and autophagosome formation. Also, LC3-II levels increase induced by d-limonene in SH-SY5Y cells is rapid but transient [7]. Accordingly, in CQ-pretreated cells, only a short (30 min; Fig. 3B [8]) co-incubation with d-limonene, but not a long one (48 h; Fig. 3D), markedly increases the LC3-II levels that are accumulating over time due to the blockage of autophagy induced by CQ.

The mechanism through which CQ inhibits autophagy has been ascribed to the lysosomotropic properties of CQ that accumulates into the lysosomes thereby raising intralysosomal pH and inhibiting lysosomal enzymes [10]. More recently, it has been suggested that CQ inhibits the later stages of autophagy by preventing the fusion of autophagosomes with lysosomes without substantially decreasing lysosomal acidity and the degradation capacity of lysosomes [25]. The present data demonstrate that in SH-SY5Y

neuroblastoma cells CQ accumulates LC3-II levels and induces a rapid and long-lasting reduction of vimentin levels.

Vimentin is involved in vesicular membrane traffic and vimentin filaments are required for endo-lysosomal vesicle transport and positioning of endosomes and lysosomes [26]. Recently, some evidence has emerged that vimentin is involved in autophagy progression [27]. In HEK293 cells, vimentin aggregation by whitaferin-A increases LC3-II levels and causes the juxtanuclear clustering of autophagosomes and lysosomes with only limited colocalization [27]. While the latter finding would suggest that vimentin aggregation interferes with the fusion of autophagosomes with lysosomes, vimentin depletion by gene silencing does not seem to affect basal autophagy or autophagosome distribution [27]. We investigated only the levels of the protein and no cell imaging study was performed that could inform us whether d-limonene and CQ affect the intracellular distribution of vimentin, which indeed deserves further investigation. Nonetheless, these findings represent an advance in our understanding of the mechanism of action of CQ considering that the intermediate filament protein vimentin participates in several critical functions including signal transduction and cell adhesion and migration [28]. Upregulation of vimentin is linked to epithelial-mesenchymal transition and vimentin overexpression has been associated with cancer progression and metastasis [29]. Furthermore, post-translational modifications of vimentin play a role in the pathogenesis of inflammatory and autoimmune disorders [30]. In addition, vimentin has been implicated in virus replication and in facilitating internalization of multiple viruses [31]. Thus, it has been suggested that vimentin directly binds to the severe acute respiratory syndrome coronavirus (SARS-CoV) spike protein and is involved in the entry of SARS-CoV, functioning as a putative co-receptor with ACE2 [32].

CQ is approved for the treatment of malaria and rheumatic diseases [33] and, due to its ability to inhibit autophagy, it is currently evaluated in clinical trials as adjuvant in anti-cancer chemotherapies [34]. Very recently, CQ and its analog, hydroxychloroquine, have received much attention because of their use to treat SARS-CoV-2 infection despite the paucity of preclinical evidence to support this indication and their dose-limiting toxicity [35]. To provide information that could be exploited in a better understanding of the pharmaco-toxicological profile of CQ, here we report some effects of CQ we have incidentally observed by using this drug as a tool to inhibit autophagy. In addition to causing cells to accumulate autophagosomes, CQ provokes a rapid and long-lasting reduction of vimentin expression.

We hope these findings may stimulate future studies to investigate the role of reduction of vimentin in the mechanisms through which CQ impairs fusion of autophagosome with lysosomes as well as in other effects of this drug.

Declaration of competing interests

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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References

[1] P.L. Crowell, Prevention and therapy of cancer by dietary monoterpenes, J. Nutr. 129 (1999) 775–778.
[2] M.T. Corasaniti, J. Maiuolo, S. Maida, V. Fratto, M. Navarra, R. Russo, D. Amantea, L.A. Morrone, G. Bagetta, Cell signaling pathways in the mechanisms of neuroprotection afforded by bergamot essential oil against NMDA-induced cell death in vitro, Br. J. Pharmacol. 151 (2007) 518–529, https://doi.org/10.1038/sj.bjp.0707237.
[3] L.A. Morrone, L. Rombolà, C. Pelle, M.T. Corasaniti, S. Zappettini, P. Paudice, G. Bonanno, G. Bagetta, The essential oil of bergamot enhances the levels of amino acid neurotransmitters in the hippocampus of rat: implication of monoterpene hydrocarbons, Pharmacol. Res. 55 (2007) 255–262, https://doi.org/10.1016/j.phrs.2006.11.010.
[4] H.G. Araújo-Filho, E.W.M. Pereira, L. Heimfarth, B. Souza Monteiro, F.R. Santos Passos, P. Siqueira-Lima, S.R. Gandhi, M.R. Viana dos Santos, J.R. Guedes da Silva Almeida, L. Picot, R. Grougnet, R.S. Almeida, H. Douglas Melo Coutinho, L.J. Quintans-Júnior, N. Martins, J.S.S. Quintans, Limonene, a food additive, and its active metabolite perillyl alcohol improve regeneration and attenuate neuropathic pain after peripheral nerve injury: evidence for IL-1β, TNF-α, GAP, NGF and ERK involvement, Int. Immunopharm. 86 (2020) 106766, https://doi.org/10.1016/j.intimp.2020.106766.
[5] M. Shin, Q.F. Liu, B. Choi, C. Shin, B. Lee, C. Yuan, Y.J. Song, H.S. Yun, I.S. Lee, B.S. Koo, K.S. Cho, Neuroprotective effects of limonene (+) against Aβ42-induced neurotoxicity in a Drosophila model of Alzheimer's disease, Biol. Pharm. Bull. 43 (2020) 409–417, https://doi.org/10.1248/bpb.b19-00495.
[6] J.A. Miller, J.E. Lang, M. Ley, R. Nagle, C.H. Hsu, P.A. Thompson, C. Cordova, A. Waer, H.H.S. Chow, Human breast tissue disposition and bioactivity of limonene in women with early-stage breast cancer, Canc. Prev. Res. 6 (2013) 577–584, https://doi.org/10.1158/1940-6207.CAPR-12-0452.
[7] R. Russo, M.G.V. Cassiano, A. Ciociaro, A. Adornetto, G.P. Varano, C. Chiappini, L. Berliocchi, C. Tassorelli, G. Bagetta, M.T. Corasaniti, Role of d-limonene in autophagy induced by bergamot essential oil in SH-SY5Y neuroblastoma cells, PLoS One 9 (2014) 1–19, https://doi.org/10.1371/journal.pone.0113682.
[8] L. Berliocchi, C. Chiappini, A. Adornetto, D. Gentile, S. Cerri, R. Russo, G. Bagetta, M.T. Corasaniti, Early LC3 lipidation induced by D-limonene does not rely on mTOR inhibition, ERK activation and ROS production and it is associated with reduced clonogenic capacity of SH-SY5Y neuroblastoma cells, Phytomedicine 40 (2018) 98–105, https://doi.org/10.1016/j.phymed.2018.01.005.
[9] X. Yu, H. Lin, Y. Wang, W. Lv, S. Zhang, Y. Qian, X. Deng, N. Feng, H. Yu, B. Qian, D-limonene exhibits antitumor activity by inducing autophagy and apoptosis in lung cancer, OncoTargets Ther. 11 (2018) 1833–1847, https://doi.org/10.2147/OTT.S155716.
[10] D.J. Klionsky, K. Abdelmohsen, A. Abe, M.J. Abedin, H. Abeliovich, A. A, et al., Guidelines for the use and interpretation of assays for monitoring autophagy, Autophagy 12 (2016) 1–222, https://doi.org/10.1080/15548627.2015.1100356, third ed.
[11] B. Levine, G. Kroemer, Autophagy in the pathogenesis of disease, Cell 132 (2008) 27–42, https://doi.org/10.1016/j.cell.2007.12.018.
[12] T.B. Adams, C.L. Gavin, M.M. McGowen, W.J. Waddell, S.M. Cohen, V.J. Feron, L.J. Marnett, I.C. Munro, P.S. Portoghese, I.M.C.M. Rietjens, R.L. Smith, The FEMA GRAS assessment of aliphatic and aromatic terpene hydrocarbons used as flavor ingredients, Food Chem. Toxicol. 49 (2011) 2471–2494, https://doi.org/10.1016/j.fct.2011.06.011.
[13] D.M. Vigushin, G.K. Poon, A. Boddy, J. English, G.W. Halbert, C. Pagonis, M. Jarman, R.C. Coombes, Phase I and pharmacokinetic study of D-limonene in patients with advanced cancer, Cancer Chemother, Pharmacology 42 (1998) 111–117, https://doi.org/10.1007/s002800050793.
[14] Y.C. Kim, K. Guan, Y.C. Kim, K. Guan, mTOR : a Pharmacologic Target for Autophagy Regulation Find the Latest Version : mTOR : a Pharmacologic Target for Autophagy Regulation, vol. 125, 2015, pp. 25–32, https://doi.org/10.1172/JCI73939.such.
[15] A. Anand, B. Liu, J.D. Giacobini, K. Maeda, M. Rohde, M. Jaattela, Cell death induced by cationic amphiphilic drugs depends on lysosomal Ca2+ release and cyclic AMP, Mol. Canc. Therapeut. 18 (2019) 1602–1614, https://doi.org/10.1158/1535-7163.MCT-18-1406.
[16] D.E. Goll, V.F. Thompson, H. Li, W. Wei, J. Cong, The calpain system, Physiol. Rev. 83 (2003) 731–801, https://doi.org/10.1152/physrev.00029.2002.
[17] F. Demarchi, C. Bertoli, T. Copetti, I. Tanida, C. Brancolini, E.L. Eskelinen, C. Schneider, Calpain is required for macroautophagy in mammalian cells, J. Cell Biol. 175 (2006) 595–605, https://doi.org/10.1083/jcb.200601024.
[18] R. Russo, L. Berliocchi, A. Adornetto, G.P. Varano, F. Cavaliere, C. Nucci, D. Rotiroti, L.A. Morrone, G. Bagetta, M.T. Corasaniti, Calpain-mediated cleavage of Beclin-1 and autophagy deregulation following retinal ischemic injury in vivo, Cell Death Dis. 2 (2011) e144–e149, https://doi.org/10.1038/cddis.2011.29.
[19] M. Høyer-Hansen, L. Bastholm, P. Szyniarowski, M. Campanella, G. Szabadkai, T. Farkas, K. Bianchi, N. Fehrenbacher, F. Elling, R. Rizzuto, I.S. Mathiasen, M. Jäättelä, Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-β, and Bcl-2, Mol. Cell. 25 (2007) 193–205, https://doi.org/10.1016/j.molcel.2006.12.009.
[20] A. Grotemeier, S. Alers, S.G. Pfisterer, F. Paasch, M. Daubrawa, A. Dieterle, B. Viollet, S. Wesselborg, T. Proikas-Cezanne, B. Stork, AMPK-independent induction of autophagy by cytosolic Ca2+ increase, Cell, Signals 22 (2010) 914–925, https://doi.org/10.1016/j.cellsig.2010.01.015.
[21] R. Russo, A. Ciociaro, L. Berliocchi, M.G. Valentina Cassiano, L. Rombolà, S. Ragusa, G. Bagetta, F. Blandini, M.T. Corasaniti, Implication of limonene and linalyl acetate in cytotoxicity induced by bergamot essential oil in human neuroblastoma cells, Fitoterapia 89 (2013) 48–57, https://doi.org/10.1016/

j.fitote.2013.05.014.
[22] H. Il Kwak, H. Kang, J.M. Dave, E.A. Mendoza, S.C. Su, S.A. Maxwell, K.J. Bayless, Calpain-mediated vimentin cleavage occurs upstream of MT1-MMP membrane translocation to facilitate endothelial sprout initiation, Angiogenesis 15 (2012) 287–303, https://doi.org/10.1007/s10456-012-9262-4.
[23] M.A. Davis, M.R. Fairgrieve, A. Den Hartigh, O. Yakovenko, B. Duvvuri, C. Lood, W.E. Thomas, S.L. Fink, M. Gale, Calpain drives pyroptotic vimentin cleavage, intermediate filament loss, and cell rupture that mediates immunostimulation, Proc. Natl. Acad. Sci. U.S.A. 116 (2019) 5061–5070, https://doi.org/10.1073/pnas.1818598116.
[24] N. Morishima, Changes in nuclear morphology during apoptosis correlate with vimentin cleavage by different caspases located either upstream or downstream of Bcl-2 action, Gene Cell. 4 (1999) 401–414, https://doi.org/10.1046/j.1365-2443.1999.00270.x.
[25] M. Mauthe, I. Orhon, C. Rocchi, X. Zhou, M. Luhr, K.J. Hijlkema, R.P. Coppes, N. Engedal, M. Mari, F. Reggiori, Chloroquine inhibits autophagic flux by decreasing autophagosome-lysosome fusion, Autophagy 14 (2018) 1435–1455, https://doi.org/10.1080/15548627.2018.1474314.
[26] M. Styers, G. Salazar, R. Love, A. Peden, A. Kowalczyk, V. Faundez, The endo-lysosomal sorting machinery interacts with the intermediate filament cytoskeletonle, Mol. Biol. Cell 15 (2005) 5369–5382.
[27] O. Biskou, V. Casanova, K.M. Hooper, S. Kemp, G.P. Wright, J. Satsangi, P.G. Barlow, C. Stevens, The type III intermediate filament vimentin regulates organelle distribution and modulates autophagy, PLoS One 14 (2019) 1–20, https://doi.org/10.1371/journal.pone.0209665.
[28] J. Ivaska, H.M. Pallari, J. Nevo, J.E. Eriksson, Novel functions of vimentin in cell adhesion, migration, and signaling, Exp. Cell Res. 313 (2007) 2050–2062, https://doi.org/10.1016/j.yexcr.2007.03.040.
[29] F. Danielsson, M. Peterson, H. Caldeira Araújo, F. Lautenschläger, A. Gad, Vimentin diversity in health and disease, Cells 7 (2018) 147, https://doi.org/10.3390/cells7100147.
[30] A. Musaelyan, S. Lapin, V. Nazarov, O. Tkachenko, B. Gilburd, A. Mazing, L. Mikhailova, Y. Shoenfeld, Vimentin as antigenic target in autoimmunity: a comprehensive review, Autoimmun. Rev. 17 (2018) 926–934, https://doi.org/10.1016/j.autrev.2018.04.004.
[31] I. Ramos, K. Stamatakis, C.L. Oeste, D. Pérez-Sala, Vimentin as a multifaceted player and potential therapeutic target in viral infections, Int. J. Mol. Sci. 21 (2020) 1–27, https://doi.org/10.3390/ijms21134675.
[32] Y.T.C. Yu, S.C. Chien, I.Y. Chen, C.T. Lai, Y.G. Tsay, S.C. Chang, M.F. Chang, Surface vimentin is critical for the cell entry of SARS-CoV, J. Biomed. Sci. 23 (2016) 1–10, https://doi.org/10.1186/s12929-016-0234-7.
[33] E. Schrezenmeier, T. Dörner, Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology, Nat. Rev. Rheumatol. 16 (2020) 155–166, https://doi.org/10.1038/s41584-020-0372-x.
[34] J.M.M. Levy, C.G. Towers, A. Thorburn, Targeting autophagy in cancer, Nat. Rev. Canc. 17 (2017) 528–542, https://doi.org/10.1038/nrc.2017.53.
[35] R. Kiplin Guy, R.S. DiPaola, F. Romanelli, R.E. Dutch, Rapid repurposing of drugs for COVID-19, Science (80- 368 (2020) 829–830, https://doi.org/10.1126/science.abb9332.