Neurochemistry International xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/neuint

Prolonged treatment with mevalonolactone induces oxidative stress response with reactive oxygen species production, mitochondrial depolarization and inflammation in human glioblastoma U-87 MG cells

Rossella Gratton[a,*], Paola Maura Tricarico[a,b], Fulvio Celsi[a], Sergio Crovella[a,b]

[a] Institute for Maternal and Child Health, IRCCS "Burlo Garofolo", Trieste, Italy
[b] University of Trieste, Trieste, Italy

ARTICLE INFO

Keywords:
Mevalonate kinase deficiency
Mevalonolactone
Human glioblastoma U-87 MG cells
Oxidative stress
Mitochondrial damage
Inflammation

ABSTRACT

Mevalonate pathway impairment has been observed in diverse diseases, including Mevalonate Kinase Deficiency (MKD). MKD is a hereditary auto-inflammatory disorder, due to mutations at mevalonate kinase gene (*MVK*), encoding mevalonate kinase (MK) enzyme. To date, the most accredited MKD pathogenic hypothesis suggests that the typical MKD phenotypes might be due to a decreased isoprenoid production rather than to the excess and accumulation of mevalonic acid, as initially supported. Nevertheless, recent studies provide clear evidences that accumulating metabolites might be involved in MKD pathophysiology by exerting a toxic effect. Our work aims at describing the effects of accumulating mevalonolactone, mostly produced by a dehydration reaction due to mevalonic acid accumulation, using an *in vitro* cellular model mimicking the glial component of the central nervous system (human glioblastoma U-87 MG cells). In order to mimic its progressive increase, occurring during the disease, U-87 MG cells have been treated repeatedly with growing doses of mevalonolactone, followed by the assessment of oxidative stress response (evaluated by measuring *SOD2 and HemeOX* expression levels), ROS production, mitochondrial damage and inflammatory response (evaluated by measuring *IL1B* expression levels). Our results suggest that protracted treatments with mevalonolactone induce oxidative stress with augmented ROS production and mitochondrial damage accompanied by membrane depolarization. Furthermore, an increment in *IL1B* expression has been observed, thus correlating the accumulation of the metabolite with the development of a neuroinflammatory response.

Our experimental work suggests to reconsider the presence of a possible synergy between the two major MKD pathogenic hypotheses in attempt of unravelling the different pathogenic pathways responsible for the disease.

1. Introduction

Mevalonate Kinase Deficiency (MKD; OMIM #610377) is a rare paediatric disease described as an autosomic recessive inborn error of metabolism with an autoinflammatory phenotype characterized by recurrent fevers and localised inflammatory episodes for which no infectious or autoimmune cause has been yet identified (van der Meer et al., 1984; McDermott and Frenkel, 2001). Attacks in MKD patients are commonly accompanied by cutaneous and skin rashes, arthralgia, aphtae, abdominal pain and diarrhoea; in addition the severe forms of the disease show neurological impairment with cerebellar atrophy, dysmorphic features, ataxia and psychomotor retardation (Hoffmann et al., 1993). The milder form of the disease does not seem to influence mortality and the severity and frequency of febrile attacks tends towards a reduced periodicity with increasing age even though symptoms continue to be present throughout life in most affected cases. On the contrary, in the severe manifestations the prognosis is poor and about half of affected patients die in infancy or early childhood (Haas and Hoffmann, 2006).

As with most rare disorders, the treatment of MKD is challenging being there a big variability in drug response, therefore no established therapeutic regimen results effective for all patients. For each case a specific therapeutic procedure has to be identified considering the individual balance between benefits perceived, risks and costs. Nevertheless, during febrile attacks the main treatments involve the use of: paracetamol, non-steroidal anti-inflammatory drugs, colchicine,

Abbreviations: ACTB, actin β gene; H2DCFDA, 2′,7′-dichlorofluorescein diacetate; *HemeOX*, heme oxygenase gene; HIDS, Hyper IgD Syndrome; *IL1B*, interleukin 1-β gene; JC-1, 5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzamidazolycarbocyanine iodide; MA, Mevalonic Aciduria; MEV, mevalonolactone; MK, Mevalonate Kinase enzyme; MKD, Mevalonate Kinase Deficiency; *MVK*, Mevalonate Kinase gene; ROS, reactive oxygen species; *SOD2*, superoxide dismutase 2 gene; ΔΨm, mitochondrial membrane potential

* Corresponding author. Institute for Maternal and Child Health, IRCCS "Burlo Garofolo", Via dell'Istria, 65/1, 34137, Trieste, Italy.
E-mail addresses: rossella.gratton@gmail.com (R. Gratton), tricaricopa@gmail.com (P.M. Tricarico), fulvio.celsi@gmail.com (F. Celsi), sergio.crovella@burlo.trieste.it (S. Crovella).

https://doi.org/10.1016/j.neuint.2018.05.003
Received 30 January 2018; Received in revised form 16 April 2018; Accepted 8 May 2018

cyclosporine, thalidomide and biological drugs able to oppose the action of pro-inflammatory cytokines. Amongst these biological drugs the most common are: tumor necrosis factor α antagonists (Infliximab, Abalimumab and Etanercept), IL-1 antagonists (Canakinumab and Rilonacept) and IL-1 receptor antagonist (Anakinra) (Favier and Schulert, 2016; Haas and Hoffmann, 2006). Recently, Canakinumab, has been approved for the treatment of MKD both by FDA (2016) and EMA (2017) and it constitutes the first registered treatment for the disease.

Unfortunately, all therapies given to patients are non-specific and act primarily on inflammatory symptoms.

MKD is associated with mutations at mevalonate kinase gene (*MVK*; 12q24.11 NG_007702.1) encoding for mevalonate kinase (MK; EC 2.7.1.36), a key enzyme in the mevalonate pathway. This metabolic route is involved in cholesterol metabolism and biosynthesis of branched unsaturated lipid chains called non-sterol isporenoids, essential molecules for the synthesis of diverse cellular compounds including dolichol, ubiquinone, heme A, geranylgeranyl-pyrophosphate (GGPP) and farnesylfarnesyl-pyrophosphate (FFPP); the latter are important for protein prenylation (Favier and Schulert, 2016). *MVK* mutations cause a decrement of MK catalytic activity which appears to be inversely related to the severity of the disease: MK residual activity between 1% and 8% is typically found in patients with the milder form of the disease, Hyper IgD Syndrome (HIDS; OMIM#260920); instead, MK residual activity below levels of detection (less than 1%) is characteristic of the more severe form, Mevalonic Aciduria (MA; OMIM #610377) (Drenth et al., 1994). The genotype/phenotype correlation within MKD is yet to be fully understood. Still, this aspect is confounded by the extremely variable spectrum of the disease and by the presumable role of additional genes that might act as modifiers of these phenotypes (Favier and Schulert, 2016; Mezzavilla et al., 2018; Moura et al., 2015).

A loss in MK activity has been linked to both an accumulation of mevalonic acid, which is mostly converted into mevalonolactone (MEV) in plasma and urine, accumulated in higher amounts by MA patients with respect to HIDS cases during febrile attacks, and a deficiency of downstream isoprenoid compounds (Prasad et al., 2012; Favier and Schulert, 2016; Hoffmann et al., 1993).

Moreover, during acute attacks, the increment of acute-phase reactants and pro-inflammatory cytokines has been observed in the serum of MKD patients. Specifically, interleukin 1β (IL-1β) is considered as a distinctive marker of the disease and its levels of secretion increase significantly during inflammatory and febrile attacks (van der Burgh et al., 2013; Houten et al., 2002).

The pathogenic mechanism of MKD has yet to be fully elucidated. Furthermore, the mechanisms concerning severe neurologic symptoms are neglected.

To date the most accredited MKD pathogenic hypothesis supports the assumption that MKD phenotypes, both HIDS and MA, could be due to a decrement of mevalonate pathway key products: GGPP and FFPP, important for protein prenylation, and 25-hydroxycholesterol, essential for its inhibitory action against expression and production of IL-1β. The shortage of these compounds has been associated to increased inflammation, mitochondrial dysfunction and defective autophagy (van der Burgh et al., 2014; Tricarico et al., 2017a, 2017b).

Due to the absence of genetic models, these mechanisms have been studied principally in MKD biochemical *in vitro* and *in vivo* models obtained by administration of biochemical blockers of mevalonate pathway such as lovastatin or alendronate with or without inflammatory stimulator LPS in various types of cellular lines including neuronal, microglial, glial and monocyte cell lines (van der Burgh et al., 2014; Tricarico et al., 2017a,b, 2015).

The impairment of mevalonate pathway has been seen to cause a reduction in protein prenylation levels which in turn is correlated to the release of pro-inflammatory cytokines, mitochondrial damage leading to subsequent oxidative stress response, defective autophagy and apoptosis both in systemic and in central nervous system cell models (van der Burgh et al., 2014; Tricarico et al., 2017a,b, 2015).

Recent studies propose to reconsider the previously discarded hypothesis by which MKD phenotypic manifestations might be also caused by an accumulation of mevalonic acid, substrate of the defective MK enzyme (Frenkel et al., 2002) (Ceccato et al., 2017). In fact, results presented by Cecatto C. et al. (2017), for the first time support the presence of a negative effect of accumulating metabolites in the context of MKD pathophysiology using an animal model (rat). The authors observed in rat brain mitochondria that MEV disrupts mitochondrial functions and induces permeability transition pore opening, concluding that disturbance in brain mitochondrial homeostasis could explain, at least partially, MKD neurologic symptoms.

Considering this interesting observation, our work aims at describing the effects of persistently accumulating metabolites, specifically MEV, in an *in vitro* cellular model mimicking the glial component of the central nervous system, in order to better characterise the severe neurological impairment and neuroinflammatory response found in the severe forms of MKD. Therefore, we treated human glioblastoma cells (U-87 MG) with increasing doses of MEV for 24 h, 48 h and 72 h, trying to mimic its progressive accumulation occurring during the disease, evaluating the influence of this treatment on oxidative stress response (*SOD2, HemeOX* expression levels), ROS production, mitochondrial damage and inflammation (*IL1B* expression levels).

2. Materials and methods

2.1. Cell cultures

Human glioblastoma U-87 MG cells, kindly provided by Prof. Del Sal (University of Trieste), were cultured in Minimum Essential Medium Eagle (MEME, Euroclone, Italy) supplemented with 10% foetal bovine serum (FBS, Euroclone, Italy), L-glutamine 4 mM and penicillin-streptomycin amphotericin B 1× solution 2 mM (Euroclone, Italy). Cells were cultured at 8×10^4 cells/well in 12 well plates for 24 h treatments, at 6×10^4 cells/well in 12 well plates for 48 h treatments and at a density of 4×10^4 cells/well in 12 well plates for 72 h treatments. 24 h after seeding cells were treated with 0.1 mM and 1 mM of mevalonolactone (MEV, Sigma-Aldricht, Saint Louis, MO) once every 24 h (repeated treatments at 24 h, 48 h and 72 h) or treated for 48 h without a second dose given at 24 h. The concentration of MEV at 1 mM was chosen based on literature data (Ceccato et al., 2017) (Frenkel et al., 2002), while the 0.1 mM dose was selected in order to dispose of a low dosage, which might be representative of a condition found in a pathological state (Houten et al., 2003).

2.2. RNA isolation and evaluation of SOD2, HemeOX and IL1B gene expression

Total RNA was extracted from U-87 MG cells using Direct-zol™ RNA MicroPrep Plus (Zymo Research, USA) and reverse transcribed in cDNA using High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems®, Monza, Italy) following manufactures' protocols. Semi-quantitative real-time PCR was performed using Taqman Gene Expression Assays for human *SOD2* (Hs00167309_m1), *HemeOX* (hs01110250_m1), *IL1B* (Hs01555410_m1) genes and human *ACTB* (Hs99999903_m1), used as endogenous control (Applied Biosystems ® Thermo Fisher, Monza, Italy). The PCR amplification cycle was executed with the ABI 7500 Fast Real-Time PCR platform (Applied Biosystems ® Thermo Fisher, Monza, Italy). All samples were analysed in triplicate employing the SDS 1.4 software (Applied Biosystems® Thermo Fisher, Monza, Italy).

2.3. ROS analysis

ROS production was evaluated by flow cytometry using 2′,7′-dichlorofluorescein diacetate (H2DCFDA) (Thermo Fisher Scientific)

molecular dye following manufactures' protocol. Cells were loaded with 100 μM of H2DCFDA for 30 min. Fluorescence was acquired using FACS calibur cytometer (Becton Dickinson) and Summit software (Beckman Coulter, Fort Collins, Colorado, USA). Acquired data were then analysed using FlowJo software (version7.6, TreeStar Inc., Ashland, OR, USA).

2.4. Mitochondrial membrane potential analysis

The characterisation of mitochondrial membrane potential ($\Delta\Psi_m$) was assessed with 5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzamidazolycarbocyanine iodide (JC-1) probe using the MitoProbe™ JC-1 Assay Kit for Flow Cytometry (M34152) (Life Technologies) following manufactures' instructions. The fluorescent signal of the dye was then collected using FACS calibur cytometer (Becton Dickinson) and Summit software (Beckman Coulter, Fort Collins, Colorado, USA). Acquired data were analysed using FlowJo software (version7.6, TreeStar Inc., Ashland, OR, USA).

2.5. Statistical analysis

Statistical significance was determined using one-way ANOVA with Bonferroni post-hoc test correction. Data analysis was performed using Graph Pad Prism software (version 5.0, GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1. Increment in SOD2 and HemeOX expression levels after prolonged treatment with mevalonolactone (MEV)

Following repeated treatments of U-87 MG cells with MEV, we observed a significant increase in *SOD2* and *HemeOX* expression levels in cells treated with MEV for 48 h, compared to the untreated condition (NT), for both tested concentrations (Fold increase *SOD2*: 0.1MEV 48 h: 2.50 ± 0.71, $p < 0.05$; 1MEV 48 h 2.44 ± 0.53, $p < 0.01$; NT 1) (Fold increase *HemeOX*: 0,1MEV 48 h: 5.90 ± 2.70, $p < 0.05$; 1MEV 48 h: 8.32 ± 1.52, $p < 0.001$; NT: 1) (Fig. 1a).

Instead, we did not observe variation in *SOD2* and *HemeOX* expression levels after 24 and 72 h of MEV treatment (Fig. 1a).

3.2. Prolonged treatment with mevalonolactone (MEV) leads to oxidative stress response with augmented production of ROS

We observed an augmented ROS production following treatment with 0.1 mM MEV for 24 h that became significant for 48 h and 72 h, compared to untreated condition (NT) (ROS production: 0.1MEV 48 h: 129.3 ± 9.17, $p < 0.01$; 0.1MEV 72 h 132.0 ± 21.21, $p < 0.01$; NT 100) (Fig. 1b). Significance was also registered after treatment with 1 mM MEV for 24 h and 72 h, compared to untreated condition (NT) (ROS production: 1MEV 24 h 144.7 ± 23.95, $p < 0.001$; 1MEV 72 h 157.3 ± 7.36, $p < 0.001$; NT 100) (Fig. 1b).

3.3. Prolonged treatment with mevalonolactone (MEV) decreases mitochondrial membrane potential (ΔΨm)

We also investigated the effects of MEV treatment on $\Delta\Psi_m$ and our findings suggest that the metabolite induces a significant decrease in the ratio between red/green JC-1, indicating mitochondrial depolarization, after 0.1 mM MEV treatment for 24 h and 72 h, that became more significant at 48 h, if compared to untreated condition (NT) (Red/green: 0.1MEV 24 h: 76.54 ± 17.60, $p < 0.05$; MEV0.1 48 h: 63.01 ± 14.50, $p < 0.001$; 0.1MEV 72 h: 75.60 ± 23.77, $p < 0.05$; NT: 100) (Fig. 1c). Also, 1 mM MEV treatment for 48 h caused mitochondrial depolarization, even if not reaching the statistical significance; instead, 1 mM MEV treatment for 24 and 72 h does not alter mitochondrial potential (Fig. 1c).

3.4. Increase in IL1B expression levels following protracted treatment with mevalonolactone (MEV)

After treatment with MEV, we observed a significant increase of *IL1B* expression following protracted administration of 0.1 and 1 mM MEV at 48 h, compared to untreated condition (NT) (Fold increase *IL1B*: 0.1MEV 48 h 4.60 ± 0.84, $p < 0.001$; 1MEV 48 h 5.90 ± 2.70, $p < 0.05$; NT 1) (Fig. 1d). Instead, we did not observe variation in *IL1B* expression levels for 24 and 72 h of MEV treatment (Fig. 1d).

3.5. Non-prolonged treatments with mevalonolactone (MEV) do not induce oxidative stress and inflammation

To confirm that the observed effects are due mainly to the prolonged MEV treatment, we examined *SOD2*, *HemeOX* and *IL1B* expression in U-87 MG cells treated with 0.1 mM and 1 mM MEV for 48 h without a second dose given at 24 h. For all tested concentrations, we did not register any significant change in *SOD2, HemeOX* and *IL1B* gene expression if compared to NT condition (Fig. 1e).

4. Discussion

The pathogenic mechanism of MKD is yet to be completely understood; indeed, the genetic defect (mutations at *MVK* gene) induces an accumulation of mevalonic acid, substrate of the defective MK enzyme, and a reduced synthesis of at least some of the branches of isoprenoid biosynthesis.

The results presented in a recent article by Cecatto et al. (2017), provide clear evidences that accumulating metabolites might exert a toxic effect being possibly implicated in MKD pathogenesis.

In our study we evaluated the oxidative stress, mitochondrial depolarization and inflammatory response following prolonged administration of MEV, thus mimicking what might be considered as a more physiological condition found in MKD patients, using a human glial cell line model (U-87 MG). We are aware that human glioblastoma U-87 MG cells, being an immortalised and not primary cell line, cannot fully recapitulate the behaviour of human glia in studying MKD pathogenesis, however it represents a widely accepted robust model (Zabłocka et al., 2015).

For this purpose, we initially assessed the gene expression levels of *SOD2* and *HemeOX,* being the induction of these genes in response to various stress conditions well related to an occurring oxidative stress response. Our findings confirm that enduring administration with MEV results in an altered redox state particularly significant following 48 h administration of the compound.

In addition, to confirm the oxidative stress caused by persistent administration of MEV in U-87 MG cells, we evaluated the levels of reactive oxygen species (ROS) production. In accordance with the results of *SOD2* and *HemeOX* expression levels, MEV treatment induced a significant increase in ROS production.

ROS are generated constantly under normal conditions as a consequence of aerobic metabolism, primarily at a mitochondrial level. ROS are particularly transient species and are highly reactive with DNA, carbohydrates, proteins and lipids in a detrimental manner. Clearly, cells are provided with diverse mechanisms able to combat ROS either directly or indirectly.

Specifically, the central nervous system is an aerobic compartment and therefore extremely receptive to oxidative stress. Due to the relatively low antioxidant defence capacity of the brain because of almost no catalase levels and low levels of glutathione, oxidative stress has been correlated to numerous neurodegenerative disorders (Cutin et al., 2002).

In accordance to the results of Cecatto et al. (2017), we observed that prolonged treatment with MEV markedly decreases mitochondrial

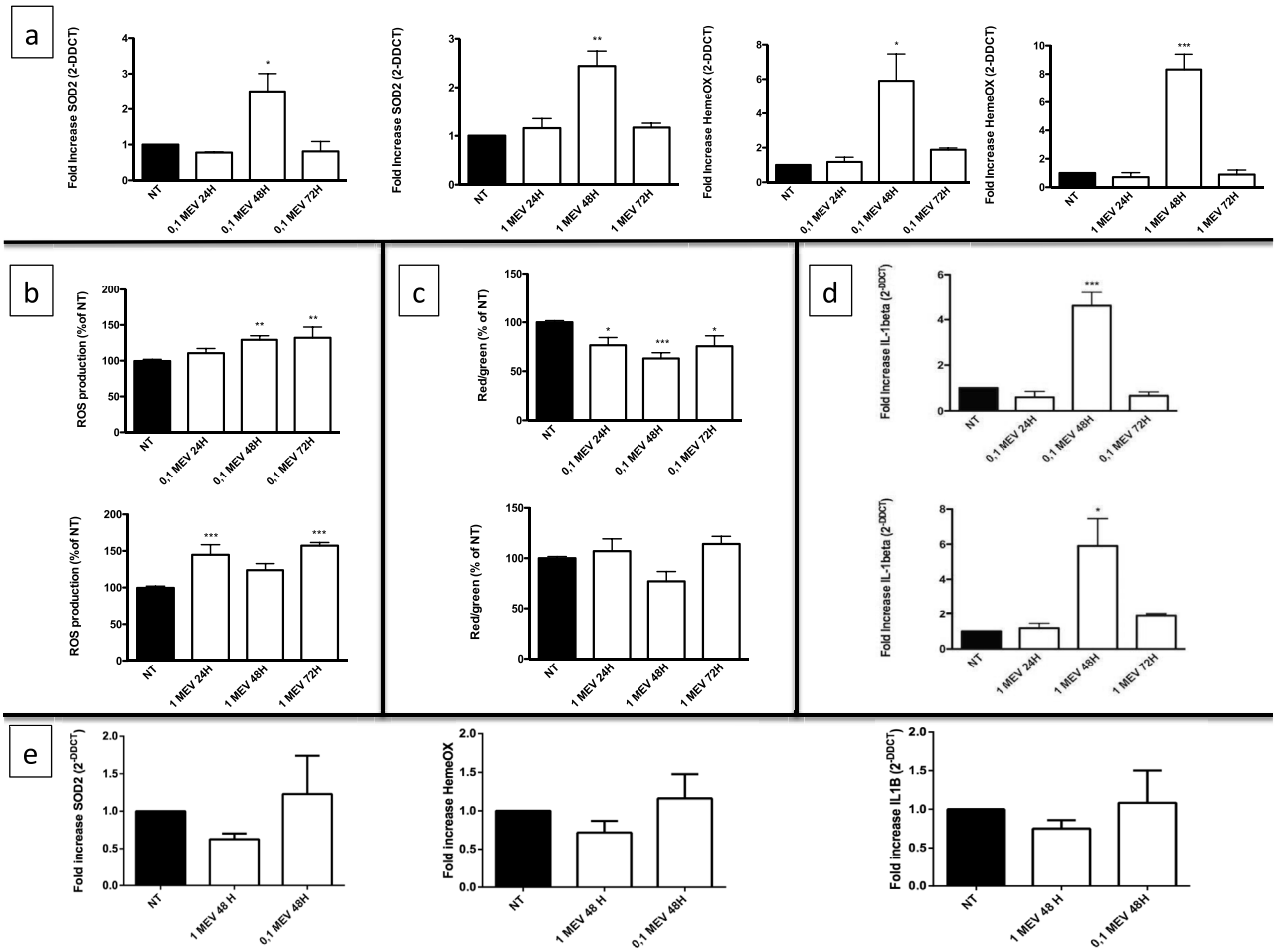

Fig. 1. Prolonged treatment of mevalonolactone (MEV) induces oxidative stress, ROS production, mitochondrial damage and inflammation in U87-MG cell line. (a) U-87 MG cells were treated with 0.1 and 1 mM of MEV every 24 h for all selected timings (24 h, 48 h and 72 h). Expression of *SOD2* and *HemeOX* were measured after MEV treatment. Analyses were performed using real time quantitative polymerase chain reaction (PCR), and results normalized to *ACTB* expression. Expression of untreated cells was normalized to 1. Expression data for the three experiments are reported as $2-\Delta\Delta Ct$ average ± SD, in which $\Delta\Delta Ct = \Delta Ct\_stimulated\ HC - \Delta Ct\_RHC$. (b) U-87 MG cells were treated with 0.1 and 1 mM of MEV every 24 h for all selected timings (24 h, 48 h and 72 h). ROS production was evaluated using 100 μM H2DCFDA and measured after MEV treatments. The results are represented as the mean ± standard deviation (SD) of three independent experiments. (c) U-87 MG cells were treated with 0.1 and 1 mM of MEV every 24 h for all selected timings (24 h, 48 h and 72 h). Mitochondrial damage was evaluated using the membrane-permeant dye JC-1. FlowJo software has been used for data analysis. Bars represent ratio of red to green fluorescence intensity, indicating the ratio of JC-1 between monomeric (green)/aggregated (red) JC-1 ± standard deviation (SD) of three independent experiments. (d) U-87 MG cells were treated with 0.1 and 1 mM of MEV every 24 h for all selected timings (24 h, 48 h and 72 h). Expression of *IL1B* was measured after MEV treatment. Analyses were performed using real time quantitative polymerase chain reaction (PCR), and results normalized to ACTB expression. Expression of untreated cells was normalized to 1. Expression data for the three experiments are reported as $2-\Delta\Delta Ct$ average ± SD, in which $\Delta\Delta Ct = \Delta Ct\_stimulated\ HC - \Delta Ct\_RHC$. (e) U-87 MG cells were treated with 0.1 and 1 mM of MEV for 48 h without a second dose given at 24 h. Expression of *SOD2, HemeOX* and *IL1B* was measured after MEV treatment. Analyses were performed using real time quantitative polymerase chain reaction (PCR), and results normalized to *ACTB* expression. Expression of untreated cells was normalized to 1. Expression data for the three experiments are reported as $2-\Delta\Delta Ct$ average ± SD, in which $\Delta\Delta Ct = \Delta Ct\_stimulated\ HC - \Delta Ct\_RHC$. All statistical analysis has been performed using GraphPad Prism software (version 5.0, GraphPad Software, Inc., La Jolla, CA, USA). Data analyses were performed with one-way ANOVA and Bonferroni correction comparing NT (not treated) cells with the other experimental conditions; *$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

membrane potential $\Delta\Psi_m$. In normal conditions mitochondrial membrane potential is maintained by cellular respiration; whereas, a dissipation of mitochondrial potential might reflect the occurrence of several processes (i.e. inhibition of respiration or failure of an uncoupling mechanism) that shunt the proton circuit therefore loosing the potential.

Being IL-1β the key regulator in the inflammatory response in MKD patients, we decided to assess whether mevalonolactone accumulation is associated to the induction of an autoinflammatory response.

An important study conducted by Frenkel et al. (2002) on peripheral blood mononuclear cells (PBMCs) isolated from paediatric HIDS or MA patients further confirmed that in MK-deficient PBMCs a decrement of mevalonate pathway isoprenoid end products might be associated to an increased IL-1β secretion observed in MKD patients, whereas the accumulation of mevalonate does not induce a detectable effect on IL-1β secretion in their *ex vivo* model (Frenkel et al., 2002). These data played a crucial role in considering MKD as a disorder whose inflammatory phenotype is most likely associated to a shortage of the pathways end products rather than to an adverse effect exerted by the presence of an accumulation of the substrate of the defective MK enzyme.

Contrary to what observed by Frenkel et al. (2002), the presence of exogenous mevalonolactone was responsible for the increased IL-1β secretion for both tested concentrations (0.1 mM and 1 mM) showing a significant peak for 48 h protracted treatments. This disagreement between our results and those previously obtained by Frenkel et al. could be due to the prolonged treatment that mimics MKD physiological condition, not evaluated in their work. So, for the first time we

observed the increase in *IL1B* expression in presence of MEV; this might imply that an excess of mevalonolactone is responsible for the increased cytokine secretion observed in MKD patients.

In addition, to confirm that the observed effects are due mainly to a prolonged MEV treatment, we evaluated *SOD2, HemeOX* and *IL1B* expression in U-87 MG cells treated with MEV for 48 h without a second dose given at 24 h. One dose of MEV does not induce changes in *SOD2, HemeOX* and *IL1B* gene expression. These results further corroborate our findings and provide additional evidences supporting the idea that the prolonged MEV treatment is able to induce oxidative stress response with reactive oxygen species production, mitochondrial depolarization and inflammation in U-87 MG cells.

Moreover, Bekkering S. et al. (2018) provided evidences that the accumulation of mevalonate, observed in MKD patients, induced a constitutive trained immunity phenotype at both immunological and epigenetic levels, which could explain the MKD inflammatory phenotype. Indeed the authors demonstrate that monocytes from MKD patients acquired a trained immunity phenotype, characterized by an increased expression of cytokines (Bekkering et al., 2018).

5. Conclusions

Our experimental work provides evidences that prolonged treatment with MEV induces in human glioblastoma U-87 MG cells the development of a significant oxidative stress, depolarization of mitochondrial membrane potential and the development of a neuroinflammatory response. Taken together, our results invite to reconsider and better investigate the presence of a possible synergy between the two major pathogenic mechanisms, shortage of isoprenoids and accumulation of mevalonic acid and its metabolites/MEV, in the attempt of unravelling different pathogenic pathways responsible for MKD and in particular MA phenotype.

Acknowledgements

RG, PMT and SC designed the experimental plan, performed the analysis of the results and wrote the manuscript; RG and PMT assessed the experiments on mRNA expression; RG, PMT and FC performed the flow cytometry analysis; RG, PMT and SC executed the statistical analysis and revised the style and grammar. All authors read and approved the final version of the manuscript.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Bekkering, S., Arts, R.J.W., Novakovic, B., Stunnenberg, H., Riksen, N.P., Netea, M.G., 2018. Metabolic induction of trained immunity through the Mevalonate pathway. Cell 172 (1–2), 135–146 e9.

Cecatto, C., Umpierrez Amaral, A., Camacho da Silva, J., Alessandro Wajner, dos Santos Godoy, K., Teixeira Ribeiro, R., de Mello Gonçalves, A., Regla Vargas, C., Wajner, M., 2017. Mevalonolactone disrupts mitochondrial functions and induces permeability transition pore opening in rat brain mitochondria: implications for the pathogenesis of mevalonic aciduria. Neurochem. Int. 108, 133–145.

Cutin, J.F., Donovan, M., Cotter, T.G., 2002. Regulation and measurement of oxidative stress in apoptosis. J. Immunol. Meth. 265 (1–2), 49–72.

Drenth, J.P., Haagsma, C.J., van der Meer, J.W., 1994. Hyperimmunoglobulinemia D and periodic fever syndrome. The clinical spectrum in a series of 50 patients. International Hyper-IgD Study Group. Medicine (Baltim.) 73 (3), 133–144.

Favier, L.A., Schulert, G.S., 2016. Mevalonate kinase deficiency: new perspectives. Appl Clin Gent 9, 101–110.

Frenkel, J., Rijkers, G.T., Mandey, S.H.L., Buurman, S.W.M., Houten, S.M., Wanders, R.J.A., Waterham, H.R., Kuis, W., 2002. Lack of isoprenoid products raises ex vivo interleukin-1beta secretion in hyperimmunoglobulinemia D and periodic fever syndrome. Arthritis Rheum. 46 (10), 2794–2803.

Haas, D., Hoffmann, G.F., 2006. Mevalonate kinase deficiencies: from mevalonic aciduria to hyperimmunoglobulinemia D syndrome. Orphanet J. Rare Dis. 1, 13.

Hoffmann, G.F., Charpentier, C., Mayatepek, E., Mancini, J., Leichsenring, M., Gibson, K.M., Divry, P., Hrebicek, M., Lehnert, W., Sartor, K., et al., 1993. Clinical and biochemical phenotype in 11 patients with mevalonic aciduria. Pediatrics 91 (5), 915–921.

Houten, S.M., Frenkel, J., Waterham, H.R., 2002. Isoprenoid biosynthesis in hereditary periodic fever syndromes and inflammation. Hum. Mol. Genet. 11 (25), 3115–3124.

Houten, S.M., Schneiders, M.S., Wanders, R.J., Waterham, H.R., 2003. Regulation of isoprenoid/cholesterol biosynthesis in cells from mevalonate kinase-deficient patients. J. Biol. Chem. 278 (8), 5736–5743.

McDermott, M.F., Frenkel, J., 2001. Hereditary periodic fever syndromes. Neth. J. Med. 59 (3), 118–125.

Moura, R., Tricarico, P.M., Campos Coelho, A.V., Crovella, S., 2015. GRID2 a novel gene possibly associated with mevalonate kinase deficiency. Rheumatol. Int. 35 (4), 657–659.

Mezzavilla, M., Moura, R.R., Celsi, F., Tricarico, P.M., Crovella, S., 2018. MMAB, a novel candidate gene to be screened in the molecular diagnosis of Mevalonate Kinase Deficiency. Rheumatol. Int. 38 (1), 121–127.

Prasad, C., Salvadori, M.I., Rupar, C.A., 2012. Severe phenotypic spectrum of mevalonate kinase deficiency with minimal mevalonic aciduria. Mol. Genet. Metabol. 107, 756–759.

Tricarico, P.M., Crovella, S., Celsi, F., 2015. Mevalonate pathway blockade, mitochondrial dysfunction and autophagy: a possible link. Int. J. Mol. Sci. 16 (7), 16067–16087.

Tricarico, P.M., Gratton, R., Braga, L., Celsi, F., Crovella, S., 2017a. 25-Hydroxycholesterol and inflammation in Lovastatin-deregulated mevalonate pathway. Int. J. Biochem. Cell Biol. 92, 26–33.

Tricarico, P.M., Romeo, A., Gratton, R., Crovella, S., Celsi, F., 2017b. Lack of prenylated proteins, autophagy impairment and apoptosis in SH-SY5Y neuronal cell model of mevalonate kinase deficiency. Cell. Physiol. Biochem. 41 (4), 1649–1660.

van der Burgh, R., Ter Haar, N.M., Boes, M.L., Frenkel, J., 2013. Mevalonate kinase deficiency, a metabolic autoinflammatory disease. Clin. Immunol. 147 (3), 197–206.

van der Burgh, R., Nijhuis, L., Pervolaraki, K., Compeer, E.B., Jongeneel, L.H., van Gijn, M., et al., 2014. Defects in mitochondrial clearance predispose human monocytes to interleukin-1β hypersecretion. J. Biol. Chem. 289, 5000–5012.

van der Meer, J.W., Vossen, J.M., Radl, J., van Nieuwkoop, J.A., Meyer, C.J., Lobatto, S., van Furth, R., 1984. Hyperimmunoglobulinaemia D and periodic fever: a new syndrome. Lancet 1087–1090.

Zabłocka, A., Mitkiewicz, M., Macała, J., Janusz, M., 2015. Neurotrophic activity of cultured cell line U87 is up-regulated by proline-rich polypeptide complex and its constituent nonapeptide. Cell. Mol. Neurobiol. 35, 977–986.