Mevalonate Kinase Deficiency and Squalene Synthase Inhibitor (TAK-475)

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Mevalonate Kinase Deficiency (MKD) is a rare inborn disease belonging to the family of periodic fever syndromes. The MKD phenotype is characterized by systemic inflammation involving multiple organs, including the nervous system. Current anti-inflammatory approaches to MKD are only partially effective and do not act specifically on neural inflammation. According to the new emerging pharmacology trends, the repositioning of drugs from the indication for which they were originally intended to another one can make mechanistic-based medications easily available to treat rare diseases. According to this perspective, the squalene synthase inhibitor Lapaquistat (TAK-475), originally developed as a cholesterol-lowering drug, might find a new indication in MKD, by modulating the mevalonate cholesterol pathway, increasing the availability of anti-inflammatory isoprenoid intermediates.

Keywords: mevalonate ; inflammation ; drug repositioning ; rare disease

1. Introduction

Mevalonate Kinase Deficiency (MKD) is a rare metabolic and autoinflammatory disorder caused by the mutation of the *MVK* gene (chromosome 12, q24). The mutation leads to a reduction in the mevalonate kinase enzyme activity, with a consequent decrease in a series of isoprenoid intermediate compounds and a mevalonate accumulation in plasma and urine in the acute phase [1] (**Figure 1**).

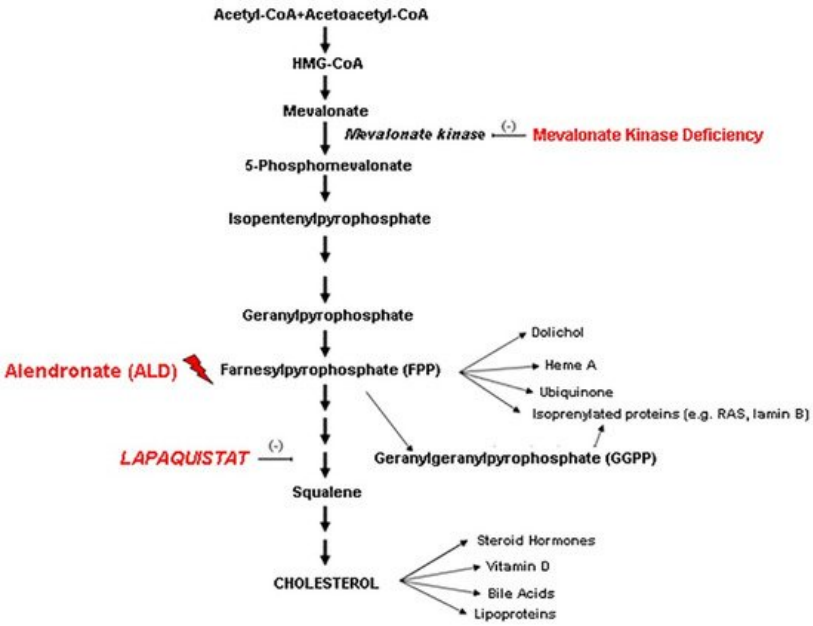

Figure 1. Schematic representation of the mevalonate pathway.

The clinical onset of the disease occurs in pediatric age but tends to last in life, presenting various degrees of severity, ranging from the less debilitating form (hyperimmunoglobulinemia D, HIDS, OMIM # 260920) to the most severe form (mevalonic aciduria, MA, OMIM # 610377). Both phenotypes are characterized by common symptoms that include periodic fever attacks associated with other inflammatory symptoms [2][3]. The common symptoms in HIDS are headache, splenomegaly, adenopathy, pharyngitis, abdominal and musculoskeletal pain. MA is also characterized, in addition to these symptoms, by significant psycho-motor and neurological involvement [4].

The most accredited hypothesis regarding MKD pathogenesis claims that the typical inflammation of the disease is caused by the deficiency of pre-squalenic isoprenoid intermediates, with a reduced prenylation of small GTPases, which, consequently, lose their membrane localization [5][6]. The lack of membrane-bound RhoA reflects on a reduced threshold of activation of the NLR family pyrin domain containing 3 (NLRP3) and pyrin inflammasomes and to the process of pyroptosis with a secretion of the inflammatory cytokines IL-1β, IL-6 and TNF-α [7][8][9]. An altered prenylation of KRAS (an isoform of RAS) is thought to play a role in the activation of PI3K-δ and in the lymphoproliferative phenotype characterizing MKD. Furthermore, the incorrect prenylation of the small GTPase Rab11 does not allow the formation of the autophagosome, leading to impaired mitophagy, which may contribute to the neurological damage observed in the most serious forms of MKD [10]. To date, MKD is no longer considered a treatment-orphan disease, because the IL-1 inhibitor treatments have allowed an acceptable control of the disease in most cases. However, not all patients fully respond to these therapies [11]. Furthermore, the anti-IL-1 biological treatments have a very high cost and require parenteral administration [12]. It remains, therefore, advisable to deepen the knowledge about the pathogenesis of MKD to identify more specific molecular targets for a mechanistic treatment.

Recent in vitro studies have shown a possible therapeutic role of a biochemical modulation of the mevalonate pathway [13]. For example, Lapaquistat (TAK-475), a compound belonging to the family of zaragozic acid, has been reported as a powerful competitive inhibitor of cholesterol synthesis in HepG2 cells [14]. Similarly, in a murine model, zaragozic acid was able to inhibit acute liver cholesterol synthesis accompanied by an accumulation of organic acids [15]. In summary, these experimental data highlighted the potential of squalene inhibitors in hypercholesterolemia [16]. The effect of zaragozic acid is related not only to the blockade of sterol synthesis, but also to a relative accumulation of geranylgeraniol due to both the blocking of its conversion to squalene and the induction of the mevalonate kinase [17]. Preliminary studies also suggest that this action could also help to protect neurons from a toxic effect that occurs under isoprenoid insufficiency conditions [18].

Of note, zaragozic acid could improve the MK residual enzymatic activity in skin fibroblasts derived from patients with MKD, increasing the transcription of the gene *Mvk* [13].

According to all evidence, TAK-475 could affect pathogenetic events relevant in MKD by increasing the levels of metabolites immediately upstream of the enzyme, including anti-inflammatory isoprenoids derived from the geraniol [19].

The study of the TAK-475 is also justified from the availability of safety data of the compound in phase three clinical trials at a lower dosage (50 mg/daily). However, the drug at a higher concentration (100 mg/daily) was halted because of a hepatic safety concern in addition to its comparable effectiveness as a cholesterol-lowering agent with respect to statins [20][21]. Nevertheless, even at a lower dosage, TAK-475 treatment led to reduction in C-reactive protein, highlighting its anti-inflammatory potential [22].

2. Discussion

MKD is an autosomal recessive rare disease, associated with mutations in the mevalonate kinase gene, coding for the homonymous enzyme that catalyzes the second step of the metabolic pathway that leads to the biosynthesis of cholesterol. The disease was discovered in 1980 and, to date, more than 250 mutations of this gene have been reported, all resulting in a significant decrease in residual enzyme activity (REA) [25]. The REA variability has allowed, over the years, to define a continuous spectrum of clinical phenotypes ranging from the less severe manifestation of the disease (HIDS) to the more severe one (MA). The pathogenic role of a decrement of mevalonate-derived intermediate compounds in MKD is well recognized: a GGPP-shortage is considered to have a key role in triggering the pathogenesis of the inflammatory MKD phenotype. Since 2016, MKD is no longer considered a treatment-orphan disease. Regulatory agencies have approved the extension of the therapeutic indications of the drug *Ilaris* (Canakinumab) previously prescribed for CAPS (periodic syndromes associated with cryopyrin), for active systemic juvenile idiopathic arthritis and for other diseases, including MKD, TRAPS (Tumor necrosis factor Receptor-Associated Periodic Syndrome) and FMF (Familial Mediterranean Fever) [11]. Previously, patients with MKD were treated with non-steroidal anti-inflammatory drugs, with poor effectiveness, with glucocorticoids, which were often burdened by serious adverse effects and by dependency, and with anti-cytokine biological drugs (anti TNF-α or anti IL-6) with partial benefit. The response to these pharmacological treatments in patients is variable and difficult to predict. This variability cannot be attributed to distinct MVK genotypes as patients with the same mutation may present significant differences in the clinical phenotype and response to medications. Among the hypotheses for these heterogeneous behaviors, epigenetic factors were suggested to have a discriminating role, but there is no doubt that further insights about MKD pathogenesis are necessary to give an answer to the many questions to establish a consolidated therapeutic line for MKD [26]. The study of TAK-475 can be inserted precisely in this context. The experimental design used in this study is a consolidated disease model that combines the

inhibitory effect of alendronate to block the cholesterol pathway and LPS to mimic the inflammatory stimulus. As shown by the impedancemetry analysis performed in our study, TAK-475 preserved the viability of the experimental cell population treated with alendronate and LPS. In addition, a morphological observation by electron microscopy showed that the changes induced by alendronate could be prevented at least in part by the addition of Lapaquistat. Notably, the mitochondrial damage induced by alendronate and LPS treatment, characterized by the presence of swollen mitochondria in the cytoplasm, was almost completely counteracted by Lapaquistat, especially in cells treated with a lower concentration of alendronate. Moreover, a TEM observation evidenced the ability of Lapaquistat to reduce autophagy induced by alendronate and LPS. Autophagy is an active mechanism in the process of cellular stress and in the removal of damaged organelles and intracellular pathogens [27][28]. In our model, alendronate and LPS induced an increase in the amount of autophagosomes, frequently containing mitochondria and autophagic compartments, and this induction was counteracted by Lapaquistat. The effect of Lapaquistat on autophagy could be associated with its restoring action on the inflammatory response induced by alendronate and LPS.

Regarding the cytokine profile, as expected, the production of inflammatory cytokines IL-6 and TNF-α was significantly increased following treatment with LPS in cells treated with alendronate in a dose-dependent manner. At both doses of alendronate (25 and 50 μM), TAK-475 was able to reduce the release of these cytokines, decreasing the inflammatory condition. Transposing these results to MKD, we could hypothesize TAK-475 efficacy limited to the cases with a partial enzyme defect (as in the syndrome with Hyper-IgD), while it seems more unlikely that the drug could be useful for the more serious forms of mevalonic aciduria. Indeed, it is possible that with this drug, as previously reported with other enzymatic modulators, paradoxical effects may be obtained linked to the involvement of different pathogenetic mechanisms in MKD, depending on the severity of the defect.

From all this evidence, the data project us to continue the Lapaquistat studies to evaluate the possibility of its future application in the treatment of MKD and, perhaps, other inflammatory diseases, in view of the repositioning of the drug. It is known that other medications acting on the cholesterol pathway have controversial results when used in patients affected by MKD. For example, statins could lead to a mild improvement in some subjects with MKD [29], but severely worsened the crises in subjects with MA [30]. Alendronate has also been proposed for therapy on the basis of possible benefit in a single patient, but it may also worsen the deficiency in mevalonate-derived isoprenoids, leading to worse inflammation. Indeed, the cholesterol pathway is finely regulated in vivo, and it may be difficult to predict how in vitro studies may reflect on the development of clinical studies. Unfortunately, murine models closely reproducing the human disease are lacking, hindering the translation of new treatments to the clinics. Moreover, considering the fine regulation of the cholesterol pathway, we should also consider the possibility that the blockade of squalene synthase could worsen the lack of post-squalene sterols deficiency in MKD, with possible detrimental clinical consequences. Indeed, while the safety profile of Lapaquistat in healthy subjects is good at low doses, this is not warranted for subjects with MKD, in whom the blockade of squalene synthase may lead to an excessive suppression of the synthesis of sterol derived molecules. The administration of coenzyme Q10, vitamin D and E is already part of the support therapy in MA-patients and could be even more necessary [31] to reduce the potential adverse effects of the inhibition of squalene synthase, such as myopathy. Finally, the antiviral 25-hydroxycholesterol (25-HC) has also been found reduced in membranes of subjects with MKD [32] and could be more strongly reduced in the presence of squalene inhibitors.

Thus, more data are still necessary to propose a possible trial of Lapaquistat in subjects with MKD.

3. Conclusions

Repositioned drugs, such as Lapaquistat, can have the advantage of reducing development costs and times since pharmacokinetics, toxicology and safety data have been previously collected [33][34]. Moreover, although IL-1 inhibitors are showing positive results in clinical trials to control MKD inflammatory phenotypes, TAK-475 may have the theoretical advantage of acting more directly on the biochemical mechanisms involved in the disease over a wider spectrum of clinical manifestations and controlling inflammatory disorders. In addition, TAK-475 is an oral available medicine with a short half-life, and this could be a valuable benefit for patients.

TAK-475 acts, as described, downstream of the statins and allows the production of early intermediates of the cholesterol pathway (geranylgeranylation) that would otherwise be limited by the statins. According to this hypothesis, TAK-475 has already been tested in in vivo preclinical studies [20]. To date, no data on the effects of TAK-475 in MKD-patients are available, but some results regarding the effect of TAK-475 treatment in an in vitro model of MKD are available. Suzuki et al., indeed, recently demonstrated that TAK-475 significantly increases levels of isoprenoids derived from mevalonate (FPP, GGPP and farnesol), in a dose-dependent manner, in human monocytic cells THP-1 [35].

The study of drugs that act on the mevalonate pathway, in general, has allowed us to control the mechanisms that go beyond the production of cholesterol. These results support the hypothesis about a potential pathogenic contiguity between different autoinflammatory diseases; therefore, it is possible that treatment with TAK-475 may also be useful for other autoinflammatory syndromes.

References

1. Buhaescu, I.; Izzedine, H. Mevalonate pathway: A review of clinical and therapeutical implications. Clin. Biochem. 2007, 40, 575–584.
2. Drenth, J.P.; Cuisset, L.; Grateau, G.; Vasseur, C.; van de Velde-Visser, S.D.; de Jong, J.G.; Beckmann, J.S.; van der Meer, J.W.; Delpech, M. Mutations in the gene encoding mevalonate kinase cause hyper-IgD and periodic fever syndro me. International Hyper-IgD Study Group. Nat. Genet. 1999, 22, 178–181.
3. Houten, S.M.; van Woerden, C.S.; Wijburg, F.A.; Wanders, R.J.; Waterham, H.R. Carrier frequency of the V377I (1129 G>A) MVK mutation, associated with Hyper-IgD and periodic fever syndrome, in the Netherlands. Eur. J. Hum. Genet. 2003, 11, 196–200.
4. Hoffmann, G.; Gibson, K.M.; Brandt, I.K.; Bader, P.I.; Wappner, R.S.; Sweetman, L. Mevalonic aciduria—An inborn erro r of cholesterol and nonsterol isoprene biosynthesis. N. Engl. J. Med. 1986, 314, 1610–1614.
5. Akula, M.K.; Shi, M.; Jiang, Z.; Foster, C.E.; Miao, D.; Li, A.S.; Zhang, X.; Gavin, R.M.; Forde, S.D.; Germain, G.; et al. Control of the innate immune response by the mevalonate pathway. Nat. Immunol. 2016, 17, 922–929.
6. Park, Y.H.; Wood, G.; Kastner, D.L.; Chae, J.J. Pyrin inflammasome activation and RhoA signaling in the autoinflammat ory diseases FMF and HIDS. Nat. Immunol. 2016, 17, 914–921.
7. Ibrahim, J.N.; Jéru, I.; Lecron, J.C.; Medlej-Hashim, M. Cytokine signatures in hereditary fever syndromes (HFS). Cytok ine Growth Factor Rev. 2017, 33, 19–34.
8. Marcuzzi, A.; Zanin, V.; Kleiner, G.; Monasta, L.; Crovella, S. Mouse model of mevalonate kinase deficiency: Comparis on of cytokine and chemokine profile with that of human patients. Pediatr. Res. 2013, 74, 266–271.
9. Marcuzzi, A.; Piscianz, E.; Vecchi Brumatti, L.; Tommasini, A. Mevalonate kinase deficiency: Therapeutic targets, treat ments, and outcomes. Expert Opin. Orphan Drugs 2017, 5, 515–524.
10. Marcuzzi, A.; Loganes, C.; Valencic, E.; Piscianz, E.; Monasta, L.; Bilel, S.; Bortul, R.; Celeghini, C.; Zweyer, M.; Tomm asini, A. Neuronal Dysfunction Associated with Cholesterol Deregulation. Int. J. Mol. Sci. 2018, 19, 1523.
11. De Benedetti, F.; Gattorno, M.; Anton, J.; Ben-Chetrit, E.; Frenkel, J.; Hoffman, H.M.; Koné-Paut, I.; Lachmann, H.J.; O zen, S.; Simon, A.; et al. Canakinumab for the Treatment of Autoinflammatory Recurrent Fever Syndromes. N. Engl. J. Med. 2018, 378, 1908–1919.
12. Malcova, H.; Strizova, Z.; Milota, T.; Striz, I.; Sediva, A.; Cebecauerova, D.; Horvath, R. IL-1 Inhibitors in the Treatment of Monogenic Periodic Fever Syndromes: From the Past to the Future Perspectives. Front. Immunol. 2021, 11, 619257.
13. Schneiders, M.S.; Houten, S.M.; Turkenburg, M.; Wanders, R.J.; Waterham, H.R. Manipulation of isoprenoid biosynthe sis as a possible therapeutic option in mevalonate kinase deficiency. Arthritis Rheum. 2006, 54, 2306–2313.
14. Nishimoto, T.; Amano, Y.; Tozawa, R.; Ishikawa, E.; Imura, Y.; Yukimasa, H.; Sugiyama, Y. Lipid-lowering properties of T AK-475, a squalene synthase inhibitor, in vivo and in vitro. Br. J. Pharmacol. 2003, 139, 911–918.
15. Amano, Y.; Nishimoto, T.; Tozawa, R.; Ishikawa, E.; Imura, Y.; Sugiyama, Y. Lipid-lowering effects of TAK-475, a squale ne synthase inhibitor, in animal models of familial hypercholesterolemia. Eur. J. Pharmacol. 2003, 466, 155–161.
16. Henneman, L.; van Cruchten, A.G.; Kulik, W.; Waterham, H.R. Inhibition of the isoprenoid biosynthesis pathway; detecti on of intermediates by UPLC-MS/MS. Biochim. Biophys. Acta 2011, 1811, 227–233.
17. Shidoji, Y.; Tabata, Y. Unequivocal evidence for endogenous geranylgeranoic acid biosynthesized from mevalonate in mammalian cells. J. Lipid Res. 2019, 60, 579–593.
18. Marcuzzi, A.; Piscianz, E.; Zweyer, M.; Bortul, R.; Loganes, C.; Girardelli, M.; Baj, G.; Monasta, L.; Celeghini, C. Geran ylgeraniol and Neurological Impairment: Involvement of Apoptosis and Mitochondrial Morphology. Int. J. Mol. Sci. 2016, 17, 365.
19. Marcuzzi, A.; Loganes, C.; Celeghini, C.; Kleiner, G. Repositioning Of Tak-475 in Mevalonate Kinase Disease: Translati ng Theory into Practice. Curr. Med. Chem. 2018, 25, 2783–2796.
20. Ebihara, T.; Teshima, K.; Kondo, T.; Tagawa, Y.; Moriwaki, T.; Asahi, S. Pharmacokinetics of TAK-475, a Squalene Synt hase Inhibitor, in Rats and Dogs. Drug Res. 2016, 66, 287–292.

21. Seiki, S.; Frishman, W.H. Pharmacologic inhibition of squalene synthase and other downstream enzymes of the cholesterol synthesis pathway: A new therapeutic approach to treatment of hypercholesterolemia. Cardiol. Rev. 2009, 17, 70–76.
22. Liao, J.K. Squalene synthase inhibitor lapaquistat acetate: Could anything be better than statins? Circulation 2011, 123, 1925–1928.
23. Martorana, D.; Bonatti, F.; Mozzoni, P.; Vaglio, A.; Percesepe, A. Monogenic Autoinflammatory Diseases with Mendelian Inheritance: Genes, Mutations, and Genotype/Phenotype Correlations. Front. Immunol. 2017, 8, 344.
24. Jeyaratnam, J.; Frenkel, J. Management of Mevalonate Kinase Deficiency: A Pediatric Perspective. Front Immunol. 2020, 11, 1150.
25. Filomeni, G.; De Zio, D.; Cecconi, F. Oxidative stress and autophagy: The clash between damage and metabolic needs. Cell Death Differ. 2015, 22, 377–388.
26. Bao, M.; Yi, Z.; Fu, Y. Activation of TLR7 Inhibition of Mycobacterium Tuberculosis Survival by Autophagy in RAW 264.7 Macrophages. J. Cell. Biochem. 2017, 118, 4222–4229.
27. Simon, A.; Drewe, E.; van der Meer, J.W.; Powell, R.J.; Kelley, R.I.; Stalenhoef, A.F.; Drenth, J.P. Simvastatin treatment for inflammatory attacks of the hyperimmunoglobulinemia D and periodic fever syndrome. Clin. Pharmacol. Ther. 2004, 75, 476–483.
28. Hoffmann, G.F.; Charpentier, C.; Mayatepek, E.; Mancini, J.; Leichsenring, M.; Gibson, K.M.; Divry, P.; Hrebicek, M.; Lehnert, W.; Sartor, K.; et al. Clinical and biochemical phenotype in 11 patients with mevalonic aciduria. Pediatrics 1993, 91, 915–921.
29. Hübner, C.; Hoffmann, G.F.; Charpentier, C.; Gibson, K.M.; Finckh, B.; Puhl, H.; Lehr, H.A.; Kohlschütter, A. Decreased plasma ubiquinone-10 concentration in patients with mevalonate kinase deficiency. Pediatr. Res. 1993, 34, 129–133.
30. Olsen, B.N.; Schlesinger, P.H.; Ory, D.S.; Baker, N.A. 25-Hydroxycholesterol increases the availability of cholesterol in phospholipid membranes. Biophys. J. 2011, 100, 948–956.
31. Finsterer, J.; Frank, M. Repurposed drugs in metabolic disorders. Curr. Top. Med. Chem. 2013, 13, 2386–2394.
32. Lotfi Shahreza, M.; Ghadiri, N.; Mousavi, S.R.; Varshosaz, J.; Green, J.R. A review of network-based approaches to drug repositioning. Brief. Bioinform. 2018, 19, 878–892.
33. Suzuki, N.; Ito, T.; Matsui, H.; Takizawa, M. Anti-inflammatory and cytoprotective effects of a squalene synthase inhibitor, TAK-475 active metabolite-I, in immune cells simulating mevalonate kinase deficiency (MKD)-like condition. Springer plus 2016, 5, 1429.
34. Maryam Lotfi Shahreza; Nasser Ghadiri; Sayed Rasoul Mousavi; Jaleh Varshosaz; James Green; A review of network-based approaches to drug repositioning. *Briefings in Bioinformatics* **2017**, *19*, 878-892, 10.1093/bib/bbx017.
35. Nobutaka Suzuki; Tatsuo Ito; Hisanori Matsui; Masayuki Takizawa; Anti-inflammatory and cytoprotective effects of a squalene synthase inhibitor, TAK-475 active metabolite-I, in immune cells simulating mevalonate kinase deficiency (MKD)-like condition. *SpringerPlus* **2016**, *5*, 1429-1429, 10.1186/s40064-016-3125-1.