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Targeting farnesyl-transferase as a novel therapeutic strategy for mevalonate kinase deficiency: *In vitro* and *in vivo* approaches

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ABSTRACT

Mevalonate kinase deficiency (MKD) is a rare inborn auto-inflammatory disease due to the impairment of the pathway for the biosynthesis of cholesterol and non-sterol isoprenoids. The shortage of isoprenoids compounds and in particular of geranylgeranylpyrophosphate (GGPP) was recently associated to the MKD characteristic inflammatory attacks.

The aim of this study is to demonstrate that the normalization of the mevalonate pathway intermediates levels and in particular of GGPP, through the specific inhibition of farnesyl-transferase (FT) with Manumycin A could ameliorate the inflammatory phenotype of MKD patients.

The effect of Manumycin A was first evaluated in MKD mouse and cellular models, chemically obtained using the aminobisphosphonate alendronate (ALD), and then in monocytes isolated from 2 MKD patients. Our findings were compared to those obtained by using natural exogenous isoprenoids (NEIs).

Manumycin A was able to significantly reduce the inflammatory marker serum amyloid A in ALD-treated Balb/c mice, as well as IL-1β secretion in ALD-monocytes and in MKD patients. These results clearly showed that, through the inhibition of FT, an increased number of mevalonate pathway intermediates could be redirected towards the synthesis of GGPP diminishing the inflammatory response. The importance in limiting the shortage of GGPP was emphasized by the anti-inflammatory effect of NEIs that, due to their biochemical structure, can enter the MKD pathway.

In conclusion, manumycin A, as well as NEIs, showed anti-inflammatory effect in MKD models and especially in MKD-monocytes, suggesting novel approaches in the treatment of MKD, an orphan disease without any efficacious treatment currently available.

1. Introduction

Mevalonate kinase deficiency (MKD) is a rare autosomal recessive disease (OMIM #610377) due to mutations in the *MVK* gene (12q24) coding for the enzyme mevalonate kinase (MK) (Fig. 1). Males and females are equally affected and most patients are of Dutch or French origin (www.hids.net). Worldwide, nearly 200 patients are affected with the mild form of the disease (Hyper IgD Syndrome, HIDS). Mevalonic aciduria (MA) the most severe form of MKD is even more sporadic (till now about 15 subjects have been described worldwide). It was supposed that the disease could be under-estimated especially in the milder forms and in developing countries (www.hids.net).

The disease usually starts within the first year of life and shows a variable spectrum of clinical manifestation, ranging from recurrent acute inflammatory episodes, characterized by fever, lymphoadenopathy, joint abdominal and skin involvement (HIDS), to psychomotor delay (MA) [1]. The quality of life of MKD patients is poor for the frequent inflammatory episodes, and, in the most severe cases, for neurological involvement and/or amyloidosis. Various treatments have been considered to treat patients affected by this disease, with variable and only partial success, and, in MA, the overall prognosis remains extremely poor.

MK is an essential enzyme in the biosynthesis of isoprenoids (Fig. 1); this pathway produces cholesterol as well as non-sterol intermediate compounds (farnesyl pyrophosphate, FPP and geranylgeranyl pyrophosphate, GGPP) involved in the control of several cell functions through protein prenylation (farnesylation and geranylgeranylation, respectively) [2]. The lack of GGPP and the consequent shortage of protein geranylgeranylation, have been

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associated with the activation of caspase-1 (Fig. 1) and the high IL-1β release, both representing important pathogenetic events in the MKD [3–6].

MKD still remains an orphan disease without an established treatment; hence the manipulation of isoprenoid biosynthesis pathway and the increase of GGPP levels could represent a novel approach for a specific treatment.

We recently reported an anti-inflammatory effect of natural exogenous isoprenoids (NEIs) in a mouse model of MKD [3–6]. These compounds, due to their isoprenoid structure, enter the mevalonate pathway (Fig. 1) and are supposed to limit the shortage of GGPP. Moreover Mandey et al. tested *in vitro* an inhibitor of squalene synthase (zaragozic acid, ZAA) and of geranylgeranil-transferase (GGTI-298) (Fig. 1) with opposite results [5].

Considering all these findings we hypothesized that manipulation of the mevalonate pathway, aimed to increase the GGPP levels, could represent a novel approach for a specific treatment; so we evaluated the effect of the inhibition of farnesyl-transferase (FT), one of the final enzyme of the pathway (Fig. 1), using farnesyl-PP for protein farnesylation *in vitro* and *in vivo* models [7]. Since FT inhibition could lead to an increased availability of FPP for GGPP synthesis. We evaluated the effect of the FT inhibitor Manumycin A [8] in animal and cellular MKD models, chemically obtained treating Balb/c mice or human monocytes with the aminobisphosphonate alendronate (ALD) [9]. ALD inhibits farnesyl pyrophosphate synthase (FPPS), an enzyme of the mevalonate pathway downstream MK (Fig. 1), reproducing, at least in part, the genetic defect of MKD. As recently reported, the blockage of the pathway increases the susceptibility to pro-inflammatory compounds such as bacterial muramyldipeptide [9] or lipopolysaccaride [5].

To emphasize the effect of FT inhibition on the ALD-induced inflammation, healthy monocytes were treated with NEIs, zaragozic acid and GGTI-298 and results were compared.

Manumycin A and NEIs was finally tested on monocytes isolated from two MKD patients.

2. Materials and methods

2.1. Reagents

Lipopolysaccharide (LPS) (*E. coli*-serotype 055:B5), muramyldipeptide (MDP), zaragozic acid, geraniol (GOH) and farnesol (FOH) (Euphar group s.r.l., Piacenza, Italy) were dissolved in a saline solution. GGTI-298 and geranylgeraniol (GGOH) were dissolved in ethanol before dilution in tissue culture medium, so that the final concentration of ethanol would not exceed 0.1% (v/v). Manumycin A was dissolved in dimethylsulfoxide (DMSO) before dilution in tissue culture medium so that the final concentration of DMSO would not exceed 0.1% *in vitro* and 1% *in vivo*. All the reagents were from Sigma–Aldrich (Milano, Italy), except where differently specified.

2.2. Animals

BALB/c male mice (Harlan, Udine, Italy) aged 6–8 weeks and weighting between 25 and 30 g were randomly divided in groups of 5 animals each: group 1, saline containing 1% DMSO; group 2, ALD 13 mg/kg on day 0; group 3, ManA 7.5 mg/kg on day 0; group 4, MDP 100 μg/kg on day 3; group 5, ALD 13 mg/kg on day 0 and MDP 100 μg/kg on day 3; group 6, ManA 7.5 mg/kg on day 0 and MDP 100 μg/kg on day 3; group 7, ALD 13 mg/kg and ManA 7.5 mg/kg on day 0; group 8, ALD 13 mg/kg and ManA 7.5 mg/kg on day 0 and MDP 100 μg/kg on day 3. All the solutions were administered *via* the intraperitoneal route (injection volume: 10 μl/g). Animals were sacrificed on day 3, 2 h after MDP or saline solution administration, whole blood was collected and the serum stored for serum amyloid A (SAA) measurement. Animal experimentation was carried out in accordance with Italians laws and was approved by the Ethical Committee of the University of Trieste (Ministry of Health registration n 62/2000-B, October 6, 2000).

2.3. SAA concentration

SAA concentration was measured in duplicate, using an enzyme-linked immunosorbent assay (ELISA) kit (Biosource, Camarillo, CA, USA).

2.4. Human peripheral blood monocytes

Monocytes isolated from healthy subjects ($n = 8$) were cultured at 2×10^5 cells/well in RPMI 1640-10% FBS (Euroclone, Milano, Italy) with 100 μM ALD for 20 h and then with 1 μg/ml LPS for additional 24 h. 10 μM ManA or 10 μM GOH or 10 μM FOH or 10 μM GGOH or 50 μM ZAA or 10 μM GGTI-298 were added together with ALD. Written informed consent was obtained from healthy controls according to the protocol of Children Health "Burlo Garofolo" (Trieste, Italy) (n.185/08, 19/08/2008).

2.5. MKD patients

Monocytes isolated from 2 MKD subjects (Patient 1, P1: 3 years old, male, *MVK*:S135L/V377I; Patient 2, P2: 20 years old, female, *MVK*:c.16-24del/V377I) were cultured at 2×10^5 cells/well in RPMI 1640-10% FBS (Euroclone, Milano, Italy) with 10 μM GOH or 10 μM FOH or 10 μM GGOH or 10 μM ManA for 20 h and then with 1 μg/ml LPS for additional 24 h. Written informed consent was obtained from patients' parents, according to the protocol of the Institute of Children Health "Burlo Garofolo" (Trieste, Italy) ethical board (n.185/08, 19/08/2008).

2.6. IL-1β concentration

IL-1β concentration was measured in duplicate, using an enzyme-linked immunosorbent assay (ELISA) kit (Endogen Human IL-1β ELISA Kit, Pierce Biotechnology Inc., USA).

2.7. Data analysis

All results are expressed as the mean ± SEM. Statistical significance was calculated using two-way analysis of variance (ANOVA), and Bonferroni post-test. Analysis was performed through GraphPad Prism software (version 5.0).

3. Results

ALD and ALD-MDP induced a marked increase of SAA mean level in Balb/c mice Manumycin A was able to significantly reduce ALD and ALD-MDP-induced SAA production. ManA did not affect the low SAA concentration induced by MDP alone, even if it showed a little but significant increase in SAA levels in untreated animals.

All the above mentioned results are shown in Fig. 2.

A similar, anti-inflammatory effect of Manumycin A was evident on LPS-induced IL-1β secretion from human monocytes treated with ALD (Fig. 3). In this cellular model of MKD, ManA significantly reduced cytokine secretion (241.3 ± 64.63 pg/ml versus 635.0 ± 115.1 pg/ml; $p < 0.05$).

In Fig. 3 we reported the effect of ManA and of two other mevalonate pathway inhibitors, squalene synthase (SS) inhibitor, ZAA, and geranylgeranyl-transferase (GGT) inhibitor, GGTI-298 as percentage of ALD-LPS-induced IL-1β release. ZAA significantly lowered ALD-LPS-induced IL-1β secretion (166.5 ± 55.1 pg/ml versus 338.7 ± 73.59 pg/ml; $p < 0.05$). On the contrary GGTI-298 induced a