Biochemical and genetic aspects of mevalonate kinase and its deficiency
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Summary

Mevalonate kinase (MK) deficiency is a metabolic disorder with a diverse and broad clinical spectrum, and includes two separately described inherited diseases, called mevalonic aciduria (MA) and hyperimmunoglobulinaemia D and periodic fever syndrome (HIDS). All MA patients show congenital or developmental features. In addition, they also suffer from recurrent episodes of fever associated with lymphadenopathy, arthralgia, subcutaneous edema, gastrointestinal problems and skin rash. HIDS patients only suffer from fever episodes that are strikingly similar to the ones observed in MA. The fever episodes occur every 2 to 6 weeks, last 3 to 7 days and are associated with malaise, chills, headache, arthralgias, arthritis, nausea, abdominal pain, diarrhea, skin rash, hepatosplenomegaly and lymphadenopathy. Several laboratory observations are suggestive of a systemic inflammatory response. The fever episodes can be triggered by infections, minor trauma, childhood immunizations, menses or physical and emotional stress, but usually occur without any clear precipitating event.

MK is an enzyme functioning in the isoprenoid biosynthesis pathway. Isoprenoids make up a large group of essential compounds involved in diverse cellular processes. Among these are: (1) ubiquinone-10, (2) heme A, (3) dolichol, (4) isopentenyl tRNAs, (5) isoprenylated proteins, and (6) cholesterol. In contrast to other disorders of isoprenoid biosynthesis, MK deficiency can affect the biosynthesis of all isoprenoids since it occurs early in the pathway. MK catalyzes the first reaction after the rate-limiting enzyme step of the pathway, which is performed by the highly regulated HMG-CoA reductase (HMGR).

Chapter 1 is a concise summary of the current knowledge on isoprenoid biosynthesis and defects therein. Chapter 2 is an updated, extensive review on the diverse aspects of MK, MA and HIDS.

At the beginning of this PhD project, the biochemical and genetic basis of HIDS was still elusive. Chapter 3 describes how the detection of an abnormal concentration of mevalonic acid in the urine of a HIDS patient led to the unraveling of the underlying defect in this disease. The accumulation of mevalonic acid suggested a defect in the metabolism of this compound. Indeed, HIDS patients have depressed activity of MK due to functionally significant mutations in the encoding gene (MVK). Immunoblot analysis with a MK-specific antibody, showed low protein levels indicating that the mutations predominantly affect the stability of the protein.

MA is a rare disease. Most of the reported patients were characterized only at the biochemical level and not at the genetic level. Chapter 4 reports the biochemical and genetic basis in three MA patients. Three novel missense mutations have been identified and characterized. As in chapter 3, this was done by heterologous expression of these mutant proteins in *Escherichia coli* and immunoblot analysis of lysates of patients' cells with a MK-specific antibody.

The discovery of the molecular basis of HIDS led to an improved diagnosis of this syndrome. As a result, many HIDS patients have been analyzed at the biochemical (MK activity measurements) and genetic level (mutation analysis of *MVK* cDNA/gene). Chapter 5 reports the results of the mutation analysis at the cDNA level, whereas in chapter 6 these studies are extended with mutation analysis at the genomic level. This was possible after the elucidation of the structure of the *MVK* gene. It appears that the most common *MVK* mutation, 1129G>A (V377I), is specific for HIDS. Most HIDS patients are compound heterozygotes for this mutation and a second missense mutation that could be identified in
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both MA and HIDS patients (803T>C and 59A>C). The 1129G>A has not been described in MA patients, all together strongly suggesting that the 1129G>A mutation is responsible for the HIDS phenotype.

Chapter 7 describes an attempt to estimate the incidence of MK deficiency in the Netherlands. To this end, the carrier frequency of the 1129G>A mutation in the Dutch population was determined by a PCR-RFLP method. Together with an estimation of the proportion of this allele in patients with MK deficiency, the carrier frequency of all MVK mutations can be calculated. This predicts a high disease-incidence varying between 1 in 5608 to 1 in 58785, which is far more than observed. Although it is reasonable to suggest that not all patients with MK deficiency are diagnosed, this large difference is probably due to an incomplete penetrance of 1129G>A homozygosity. As a consequence, the true disease-incidence will be lower than this estimation.

Chapter 8 and 9 report the results of biochemical studies toward the effect of MK deficiency on cellular isoprenoid metabolism and provide a clue for the possible pathogenetic mechanism for the periodic fever episodes. Earlier studies already suggested that cells with MK deficiency are able to synthesize near normal levels of isoprenoids. This is possible by the elevation of the activity of HMG-CoA reductase (HMGR), the rate-limiting enzyme of the pathway. In chapter 9 is described that this elevated HMGR activity can be downregulated by isoprenoid precursors, including mevalonate. Furthermore, MK deficient cells are capable of isoprenylating proteins to the same degree as normal cells, however, this process is more sensitive to depletion of mevalonate by statins. Statins are competitive inhibitors of HMGR and consequently lower the endogenous synthesis of mevalonate. These results suggest that MK deficient cells are able to compensate for this deficiency by elevating intracellular mevalonate levels. As a consequence there is an increased leakage of mevalonate from the cell, which is compensated for by the elevated HMGR activity.

When cells with MK deficiency are able to compensate for the deficiency, the question remains why HIDS and MA patients have periodic fever episodes. Several lines of evidence points toward a shortage of end-products as the cause of the disease. The most important one being a therapeutic trial with lovastatin in two MA patients. This caused severe clinical crises in these patients. Another piece of evidence is the pro-inflammatory effect of statins on monocyte function. The observation that MK activity in HIDS cell lines was extremely sensitive to temperature and decreased substantially during fever episodes in HIDS patients, led to the proposal of a model for the pathogenesis in chapter 8. In this model, minor elevations in temperature as can be caused by several trivial events, may set off a chain of events, with MK becoming progressively rate-limiting, leading to a temporary deficiency of isoprenoid end-products, followed by inflammation and fever.

The research described in this thesis led to the discovery of the genetic and biochemical basis of HIDS and has extended the knowledge of MK deficiency disorders. The direct cause of fever and inflammation still remains elusive, however, and is an important research field for the next years. The proposed pathogenetic mechanism suggests that supplementation of isoprenoid precursors may be beneficial in these syndromes. Therefore, current research should be focused also on the possible beneficial and side effects of such a treatment.