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REVIEW ARTICLE

Hyperhomocysteinemia, MMPs and Cochlear Function: A Short Review

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Abstract Hyperhomocysteinemia (HHCY) has been demonstrated to affect cochlear microvasculature as well as cochlear epithelial cells directly, with a resultant alteration of the expression of matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs). Hence, ascertaining the optimum concentration of MMPs and TIMPs in the cochlea could help to inhibit hearing loss due to HHCY by the administration of appropriate MMP inhibitors, Since infections/inflammations as well as ototoxic antibiotics have a similar mechanism of otic pathology, the cochlear damage they cause could also be similarly prevented.

Keywords Hyperhomocysteinemia · MMPs · TIMPs · Cochlea · Hearing loss

Hyperhomocysteinemia

Homocysteine is a non-essential sulfur-containing amino acid which has a metabolically strategic involvement at the crossroad of three pathways—the folate cycle, the remethylation pathway and the transsulfuration pathway [1]. In the remethylation cycle, methylation of homocysteine to methionine occurs and this is the mainstay for every cell in the body as the very survival and individuality of every cell or cell system is dependent on the one-carbon reactions and epigenetic alteration of protein expression which are supported by adequate methionine (Fig. 1). Disruption of the fine equilibrium between the remethylation cycle, the transsulfuration pathway and the folate cycle, leads to an accumulation of homocysteine, termed hyperhomocysteinemia (HHCY). It is caused by deficiency of the vitamins folate, B_{12} and B_6, or by polymorphisms of the genes for the rate-limiting enzymes MTHFR and CBS of these pathways [2]. HHCY is not only associated with decreased methionine production leading to a marked decrease in the methylation reactions in every cell, but also it promotes vascular atherothrombosis via multiple mechanisms, thus leading to a reduced blood flow to any of the various organ systems (depending on the blood vessel which is first occluded) [3]. Furthermore, HHCY is neurotoxic, as well, through several mechanisms. One of the mechanisms of injury to tissue is mediated through increased expression of matrix metalloproteinases (MMP's) and a decrease in their naturally occurring inhibitors, the tissue inhibitor of metalloproteinases (TIMP) [4].

Matrix Metalloproteinases and Their Tissue Inhibitors

MMP's were first described by Cross and Lapiere in 1962 when they observed collagen triple helix degradation during tadpole tail metamorphosis [5]. MMPs, as their name suggests, are proteolytic enzymes which are involved in the major cellular processes—proliferation, migration, adhesion, angiogenesis, wound healing and bone development [6, 7]. They are essential for the remodeling of tissues that occurs during development. They are numbered 1–28; as

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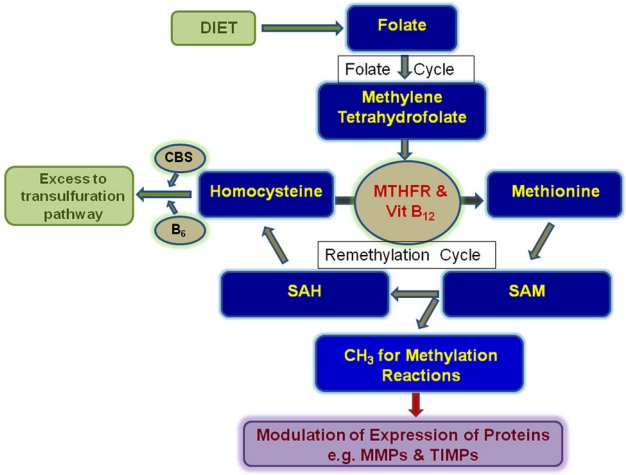

Fig. 1 Metabolism of homocysteine via the remethylation cycle results in the formation of SAM, the one-carbon-moiety donor, and consequent methylation reactions leading to altered expression of proteins like the MMPs and TIMPs. *SAM* S-adenosyl methionine; *SAH* S-adenosyl homocysteine

per their structural biology and primary protein target, they have been grouped into collagenases, stromelysins, gelatinases, matrilysins, secreted MMPs, membrane-type MMPs and Type II transmembrane MMPs [8]. These are a group of functionally diverse enzymes some of which are protective and some destructive. They function synergistically through dynamically interconnected reaction networks, along with their inducing and suppressing pathways, and form a constantly shifting 'protease web' [9]. The MMP's are regulated by several mechanisms—(a) transcriptional regulation of their genes; (b) activation of secreted proenzymes; and (c) via specific inhibitors—the tissue inhibitors of metalloproteinases [10]. In fact, MMP's are produced only when necessary in response to exogenous stimuli by growth factors and cytokines; MMP-2 is the exception which is constitutively expressed in various tissues [11]. Genetically, the MMPs have originated in life forms before the emergence of vertebrates. The MMP genes have undergone evolutionary processes and have emerged as a diverse group of interdependent genes with multiple ramifications in their individual expression [12]. Epigenetic mechanisms have been shown to alter the expression of these genes; for e.g. hypomethylation of the MMP-2 and MMP-9 genes increases its expression [13, 14].

TIMP's are a group of four inhibitors (TIMP-1 to TIMP-4) of MMP's whose proteolytic activities they modulate. TIMP-1 and TIMP-2 are secreted complexed with MMP-2 and MMP-9 in their latent form. TIMP's thus cause inhibition of the following MMP-mediated processes:

- active MMP's
- proMMP activation
- cell growth promotion
- matrix binding
- inhibition of angiogenesis
- induction of apoptosis [15]

Regulation of the TIMP genes goes hand-in-hand with that of the MMP genes, resulting in a constant modulation of MMP activity towards a healthy extracellular milieu.

Hyperhomocysteinemia and MMPs

HHCY is accompanied by decreased SAM synthesis and consequent hypomethylation of several genes and their promoter regions, e.g. MMPs 2 and 9, leading to a increased expression of these MMPs. Another mechanism by which HHCY alters the expression of these MMP's and TIMP's is the endogenous endothelial folate deficiency—EEFD. In vascular endothelial cells which are inherently deficient in the enzyme cystathionin-beta-synthase (CBS) of the transsulfuration pathway, more folate is being diverted towards the remethylation cycle in an attempt to correct the HHCY; hence there is a relative deficiency of folate in the folate shunt where it is the cofactor for the enzyme nitric oxide synthase. Consequently, there is a decreased synthesis of nitric oxide and hence more reactive oxygen species in the microenvironment of the endothelial cell—a hypothesis developed by Hayden and Tyagi [16].

Homocysteine, the Cochlea and Hearing

The exact mechanisms of sensorineural hearing loss have been elusive. Amongst other possible contributing factors, homocysteine has been implicated in noise-induced hearing loss (NIHL). Gok et al. demonstrated that patients with NIHL had higher serum levels of Hcy and lower serum levels of folate and vitamin B_{12} as compared to normal controls [17].

The inner ear or cochlea of mammals is a snail-shell like structure which is divided into three fluid-filled canals. Two of these transmit the pressure changes ensuing from sound waves. The third houses the very sensitive organ of corti which actually perceives these sound wave-pressure changes as mechanical impulses and converts them into electrical impulses which then travel along the auditory nerve to the brain. The organ of corti have highly specialized cells, the outer and inner hair cells, which are embedded in a highly organized extracellular matrix (ECM). Any alterations in this ECM, in terms of its turnover or composition, would therefore interfere with the normal functioning of the hair cells which are the actual impulse detectors of our ears.

In 2009, Kundu et al. demonstrated that HHCY in the CBS± mice was associated with increase in the level of MMP-2, -9 and -14 in the brain and in the cochlea. They also demonstrated a decrease in the TIMPs-1, -2 and -3 in the cochlea of these mice as compared to their wild type counterpart. It is known that acute hearing loss as well as age-related hearing loss can be a sequel of altered blood flow to the inner ear, specifically the cochlea. They postulated that HHCY may affect the cochlear microcirculation in the same way as it does the BBB [18].

Other causes of cochlear trauma are also known to act through the MMPs. Setz et al. demonstrated that exposure of the organ of corti to gentamycin increased the expression of MMP-2 and MMP-9 and exposure to MMP inhibitors resulted in hair cell death in wild type mice. This indicated that optimum concentrations of MMP-2 and MMP-9 are required for the normal functioning of the organ of corti hair cells [19].

The cochlear epithelium at its base and its apex respond differentially to acoustic trauma [20]. This could probably be due to differing patterns of expression of MMPs in these two regions. Hu et al. demonstrated that in addition to MMP-2 and -9,and the TIMP-1, -2 and -3, several other MMPs (MMP-7, -11, -13, and -14) were expressed in the normal mammalian cochlea (wild type mice). They also elucidated that the expression of MMPs was significantly upregulated in the apical region of the cochlea as compared to the basal region. After acoustic trauma, several MMPs and TIMPs were upregulated in the early phase (up to 1 day); in the recovery phase (at day 28) the upregulation of only TIMP-1 was still demonstrable, indicating that modulation of the MMP and TIMP genes is time-dependent [21].

In experimentally induced pneumococcal meningitis, inhibition of MMPs, by MMP inhibitor ilomastat, results in reduced concentration of TNF-α and MMP-9 in the CSF and attenuates the extent of cortical brain damage. As in the blood brain barrier (BBB), MMP production occurs in the blood-labyrinth barrier (BLB) not only by the microvascular endothelial cells, but also by the inflammatory response cells—the macrophages. Middle ear toxins and inflammatory cytokines enter the inner ear via the round window, causing spread of the inflammatory and infective processes, and damage to the organ of Corti. This damage is permanent as the cochlea does not have regenerative capabilities. Intratympanic instillation of lipopolysaccharide (LPS) causes cochlear lateral wall damage mimicking labyrinthitis [22]. Choi et al. used these facts to demonstrate the protective effect of MMP inhibitors (oxytetracycline and ilomastat) against inflammatory or infective cochlear damage [23]. Nam et al. observed that the inflammatory cytokine IL-1β induced the expression of MMP 9 in the organ of corti, and that dexamethasone caused transcriptional down-regulation of this MMP 9 [24].

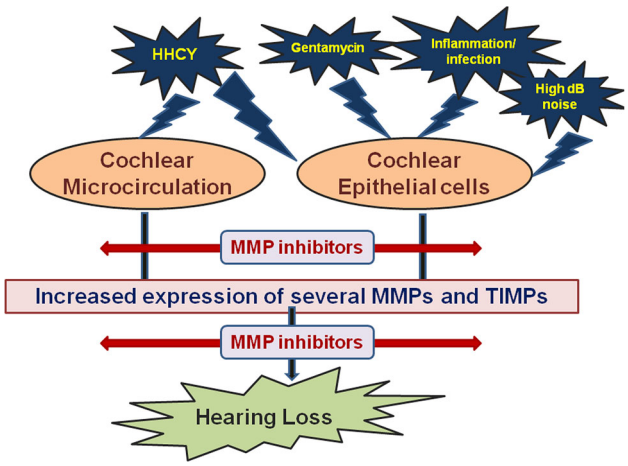

Fig. 2 Hyperhomocysteinemia affects the microcirculation as well as the epithelial cells of the cochlea leading to an increased expression of several MMPs and their corresponding TIMPs which interfere with its normal functioning. These effects could be prevented by specific MMP inhibitors. Other types of insult to the cochlea—by noise of high decibel (dB), some antibiotics (gentamycin), inflammation/infection—also act through increased expression of the MMPs and TIMPs in the epithelial cells and could be similarly prevented

Conclusion

Thus it is evident that hearing loss due to several causes—HHCY or noise, antibiotics or infection/inflammation—is mediated through altered expression of the MMPs specifically 2 and 9 (Fig. 2). Hence, it could be modified by administration of MMP inhibitors (in case of noise in the first month after exposure). At the same time, we must keep in mind that administering these inhibitors in the normal state (without HHCY) can also cause hair cell death in the cochlea. This would require further studies on the optimum concentration of MMPs required for normal functioning in the cochlear endolymph and the specific MMP inhibitors that may be used to attenuate hearing loss.

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