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Review

Pyridoxamine as a multifunctional pharmaceutical: targeting pathogenic glycation and oxidative damage

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Abstract. The discovery that pyridoxamine (PM) can inhibit glycation reactions and the formation of advanced glycation end products (AGEs) stimulated new interest in this B_6 vitamer as a prospective pharmacological agent for treatment of complications of diabetes. The mechanism of action of PM includes: (i) inhibition of AGE formation by blocking oxidative degradation of the Amadori intermediate of the Maillard reaction; (ii) scavenging of toxic carbonyl products of glucose and lipid degradation; and (iii) trapping of reactive oxygen species. The combination of these multiple activities along with PM safety posture it as a promising drug candidate for treatment of diabetic complications as well as other multifactorial chronic conditions in which oxidative reactions and carbonyl compounds confer pathogenicity.

Key words. Pyridoxamine; AGE; reactive oxygen species; reactive carbonyl compounds; diabetes; kidney stones; atherosclerosis; ageing.

Introduction

Pyridoxamine (PM) was discovered in animal tissues, and its structure was determined as part of the study of pyridoxine, the only known form of vitamin B_6 at the time [1, 2]. Subsequent research demonstrated the ability of PM and another B_6 vitamer, pyridoxal, to catalyze in vitro non-enzymatic transamination reactions between amino and α-keto acids [3]. The analogy between these in vitro reactions and the transamination reactions discovered earlier in animal tissues [4, 5] led to the proposal of a general mechanism of vitamin B_6 enzymes [6, 7]. This mechanism established PM as a transient intermediate in enzymatic transamination reactions. In the absence of enzyme, the catalytic reaction requires binding and coordination of metal ion by PM [7]. In enzymatic catalysis, metal ion is not required because of the stabilization of the intermediate complex by the amino acid side chains of the enzyme [8]. The binding of metal by PM at physiological pH is possible due to the unusually acidic ionization constant of the phenolic hydroxyl, pK ~ 3.4 [9, 10]. PM can form complexes with a number of transition metal ions but has a preference for Cu^{2+} and Fe^{3+} [11, 12]. PM metal binding is relatively weak, with a stability constant for Cu^{2+} several orders of magnitude lower than that of strong chelators such as EDTA or DTPA [13].

Dietary PM is consumed mostly in the form of PM-5′-phosphate, which is hydrolyzed to PM by intestinal phosphatases [14]. PM is converted back to PM-5′-phosphate and further to pyridoxal-5′-phosphate by the action of PM-5′-phosphate(pyridoxine-5′-phosphate) oxidase in liver and, to a lesser degree, in intestinal tissues [15, 16]. The reported tissue concentrations of PM range from 0.01–0.1 µM to 1.9–15 µM in rat brain and kidney, respectively [17, 18]. PM concentration in rat plasma is lower, 0.001–0.02 µM [17, 18]. The reported PM levels in human plasma range from 0.003 to 0.03 µM [18, 19]. Upon pharmacological supplementation in rats, plasma

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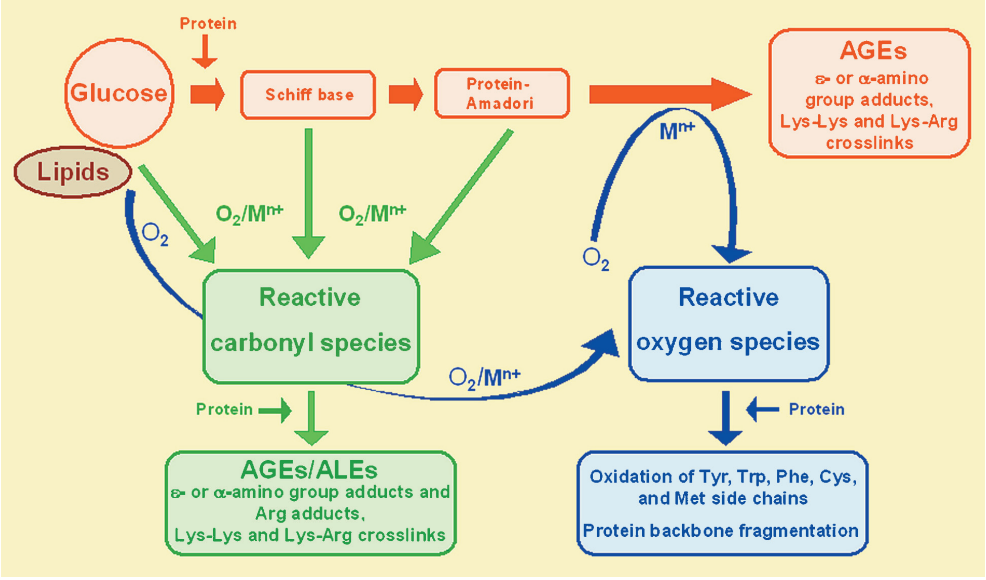


Figure 1. The pathways of protein damage by glycation reactions: via the Amadori pathway (shown in red); via the reactive carbonyl species (shown in green); and via the reactive oxygen species (shown in blue). Lipids as a source of both carbonyl compounds and free radicals are shown in brown. AGEs, advanced glycation end products; ALEs, advanced lipoxidation end products.

PM levels rise significantly, in some cases reaching values ~100 μM [20].

More recently, PM has also emerged as a promising pharmacological agent for protecting against the progressive tissue damage that occurs in diabetes and other diseases. This development is based on the initial discovery that PM is a potent inhibitor of the formation of advanced glycation end products (AGEs), protein modifications that have been implicated in the pathogenesis of vascular complications of diabetes and ageing [21, 22]. The inhibitory activity of PM has provided the impetus for a number of studies on mechanisms, toxicity and efficacy in animals and humans for protecting against diabetic nephropathy, neuropathy, retinopathy, and in kidney stone disease. In this article, we will review the pharmacological effects of PM with a focus on the potential mechanisms of PM action. We will also compare PM mechanisms with those of aminoguanidine (AG). AG was the first AGE inhibitor to demonstrate pharmacological effects in clinical trials and, thus, provided the initial support for the concept that the inhibition of glycation reactions and AGE formation is beneficial in treatment of diabetic complications [23, 24]. Even though a number of different inhibitors of AGE formation have been developed for prospective therapeutic use [25], AG and PM remain the only AGE inhibitors tested in clinical trials. Therefore, the comparison of therapeutic effects and mechanisms of action of PM and AG may provide new insights into the development of safer and more effective therapies targeting glycoxidative reactions and AGE formation.

There is a plethora of evidence that accumulation of AGEs, along with lipid-derived advanced lipoxidation end products (ALEs), plays a major role in the development of different diabetic complications [26–28]. The fact that glycoxidative and lipoxidative reactions cause protein dysfunction strongly suggests a role for such reactions as pathogenic factors in diabetes. There are three major avenues through which these reactions can cause pathogenic damage to protein structure: the Amadori pathway of the Maillard reaction, via low molecular weight carbonyl compounds and via reactive oxygen species (fig. 1).

Inhibition of post-Amadori pathways of AGE formation

Glucose, a reducing sugar elevated in diabetes, can modify proteins via reversible condensation of its aldehyde group with protein amino group, forming a Schiff base followed by essentially irreversible rearrangement to an Amadori intermediate (fig. 1, pathway shown in red). This intermediate can form on the ε-amino group of lysine residue as well as on an N-terminal α-amino group. The Amadori intermediate undergoes further condensation, dehydration and oxidative fragmentation reactions to yield heterogeneous chemical compounds, AGEs (fig. 1, shown in red). The structures of many AGEs, derived from the Amadori pathway, have been identified in vitro and found in a number of pathological conditions in vivo (reviewed in [29]). AGE-modified proteins may also participate in pathogenic signaling such as that mediated by the receptor for advanced glycation end products (RAGE) [30]. Modification of protein amino groups by glucose leading to formation of AGEs is considered one of the pathogenic

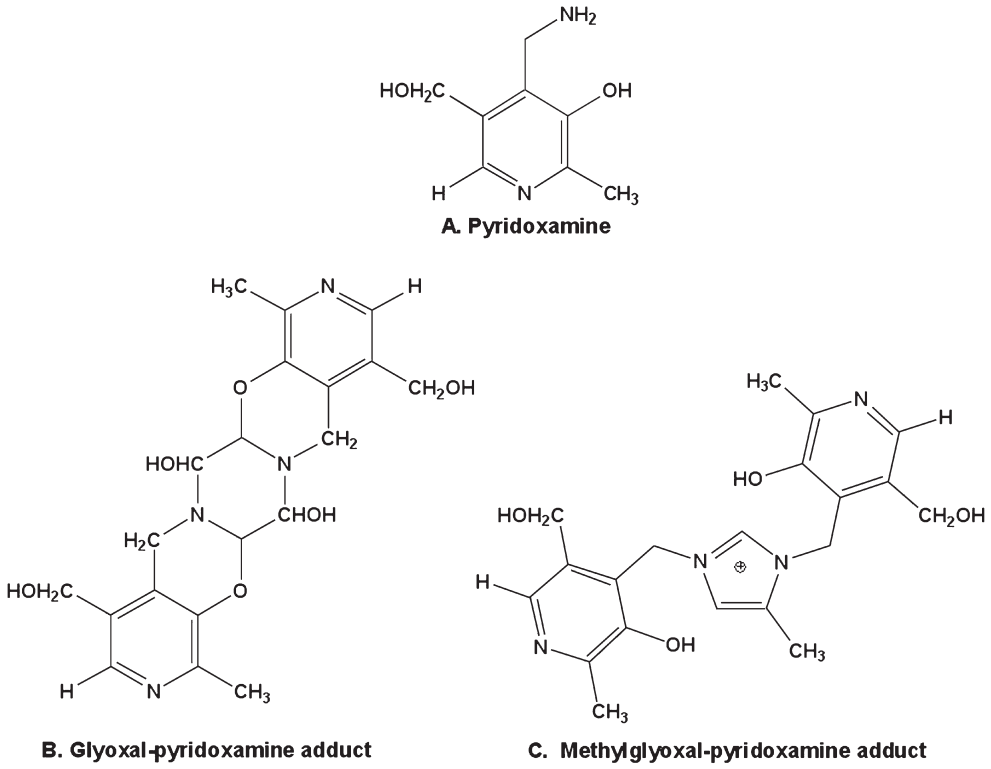


Figure 2. Structures of pyridoxamine (*A*), glyoxal-pyridoxamine adduct (*B*, [56]) and methylglyoxal-pyridoxamine adduct (*C*, [57]).

mechanisms of diabetic complications, atherosclerosis and neurodegenerative diseases; it may also be important in ageing [31–33].

The idea of using PM to inhibit AGE formation was based on its known reactivity with carbonyl groups in transamination reactions, which led to the hypothesis that PM could form an adduct with the Amadori intermediate and thereby block the formation of AGEs [21, 22]. Indeed, PM inhibited this reaction and was more effective compared with other B_6 vitamers, pyridoxal, pyridoxal-5′-phosphate or pyridoxine, in preventing antigenic AGE formation on model proteins incubated with glucose [22]. The mechanism of PM inhibition was difficult to study because the initial reaction between protein and sugar was followed by multiple pathways leading to AGEs. To circumvent this challenge, a method was developed for isolating the protein-Amadori intermediate, the first committed intermediate of the Maillard reaction [34]. This method is based on use of a high concentration of ribose to inhibit the conversion of ribose-Amadori to AGEs, which allowed for accumulation of the protein-Amadori intermediate. After removal of the sugar, the isolated intermediate underwent oxidative degradation to AGEs, enabling specific investigation of post-Amadori pathways [34]. PM strongly inhibited the formation of AGE N^{ε}-(carboxymethyl)lysine (CML) from the isolated protein-Amadori intermediate, suggesting a novel mechanism of interference with one of the post-Amadori steps of the Maillard reaction [21, 35]. More recently, this mechanism was investigated using the protein-Amadori intermediate prepared with physiological concentrations of glucose [36]. Utilizing ^{13}C nuclear magnetic resonance (NMR) and the structural analogs of PM, these studies demonstrated that PM does not directly interact with the carbonyl moiety of the Amadori intermediate. Instead, it interferes with post-Amadori oxidative reactions by binding catalytic redox metal ions [36]. Experiments with PM structural analogs have demonstrated the absolute requirement of both the phenolic hydroxyl group and the aminomethyl group at positions 3 and 4 of the pyridinium ring (see fig. 2A) for inhibition by PM [36]. Interestingly, AG can inhibit the metal-catalyzed oxidation of ascorbic acid [37], but is completely inactive as an inhibitor of post-Amadori oxidative reactions leading to AGEs [35]. These findings suggest that, in addition to metal binding, the PM inhibitory mechanism may involve specific interactions with either protein or the Amadori moiety. The precise nature of such possible interactions is yet to be defined.

The results of animal studies suggest a link between PM inhibition of AGE formation and its therapeutic effects in diabetic complications. PM inhibited the progression of early renal disease, decreased hyperlipidemia and protected against a range of pathological changes in the retina of STZ-diabetic rats [20, 38, 39]. Importantly, PM treatment also inhibited AGE formation in skin collagen and in the retina of diabetic animals [20, 38, 39]. These

studies suggest a relationship between therapeutic effects of pharmacological doses of PM and its AGE inhibitory activity.

When the effects of PM and AG in an STZ-diabetic animal model were compared directly, PM was significantly more effective at retarding the development of early renal disease as measured by the increase in urinary albumin and plasma creatinine [20]. This lower efficacy of AG may, in part, be due to its inability to inhibit post-Amadori AGE formation.

Scavenging of toxic carbonyl compounds

Another mode of AGE formation on proteins is mediated by low molecular weight reactive carbonyl compounds (fig. 1, shown in green). Carbonyl species glyoxal (GO), methylglyoxal (MGO) and glycolaldehyde (GLA) can derive from autoxidation of glucose or Schiff base intermediate formed during the reaction of glucose with protein amino groups [40–42]. A protein-Amadori adduct, another glycation intermediate, is a major source of 3-deoxyglycosone (3-DG) [43]. MGO can also originate from either spontaneous or enzymatic degradation of triose phosphates derived from glucose [44]. Moreover, reactive carbonyl species can derive from other sources such as ascorbate, polyunsaturated lipids and amino acids [45–47]; among these, lipids are probably the more physiologically important source (fig. 1, shown in brown). Unlike glucose, which reacts predominantly with amino groups, these much more reactive species can modify both α- and ε-amino groups as well as the guanidinium group of arginine (fig. 1, shown in green). These small compounds diffuse easily from the sites of formation, thus further propagating glycation damage. Their accumulation has been documented in a number of pathologic conditions. In particular, concentrations of the carbonyl compounds GO, MGO and 3-DG are increased severalfold above normal levels in serum and tissues of diabetic patients and in experimental animal models of diabetes [48–54].

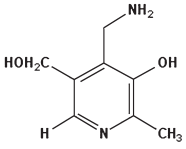
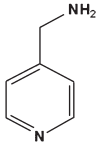
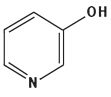
Experimental conditions	Chemical structure of the additive	Fraction of integrin binding remaining
MGO alone		0.14±0.02
MGO+Pyridoxamine		0.70±0.05
MGO+4-aminomethylpyridine		0.69±0.02
MGO+3-hydroxypyridine		0.13±0.02

Figure 3. The aminomethyl group of PM is required for protection from the methylglyoxal (MGO)-induced inhibition of integrin binding to collagen IV. Microtiter plates were coated with α3(NC1)-RGD domain of collagen IV (20 µg/ml) overnight at 4 °C followed by several washes with incubation buffer [100]. Coated plates were incubated either in 200 mM sodium phosphate buffer, pH 7.5, supplemented with MGO alone (0.5 mM) or with MGO and different additives (5 mM). The control incubation contained only the buffer alone. Incubations were carried out at 37 °C for 16 h. Binding of $\alpha_v\beta_3$ integrin to α3(NC1)-RGD protein was determined in solid-phase binding assay [100]. Data are expressed as fractions of integrin binding measured in control incubations.

PM can directly scavenge the reactive carbonyl compounds. This PM mechanism was initially demonstrated in vitro for reactive intermediates forming during peroxidation of arachidonic and linoleic acids [55]. Later, studies from several laboratories showed that PM can scavenge a range of toxic carbonyl species derived from both sugars and lipids such as glyoxal, methylglyoxal, glycolaldehyde and 1,4-dicarbonyls [56–58]. Scavenging of GO and GLA by PM requires both aminomethyl and phenolic hydroxyl substituents of the PM pyridinium ring to form relatively stable GOPM and GLAPM adducts (fig. 2B, [56]). The same structural requirement may apply to scavenging of dicarbonyl products of fatty acid oxidation, as suggested by the proposed mechanism of reaction between PM and 2-ketoheptanal [59]. However, the reported structure of MGOPM adduct suggested the requirement of only the aminomethyl ring substituent [57]. To corroborate these structural data in the functional studies, we measured endothelial cell adhesion to the MGO-modified NC1 domain of collagen IV (fig. 3). Modification of collagen IV by MGO strongly inhibited cell adhesion; however, adhesion was only slightly affected when modification was performed in the presence of PM, suggesting MGO trapping by PM (fig. 3). A PM structural analog, 4-aminomethylpyridine, was as active as PM at trapping MGO, while 3-hydroxypyridine had no effect. These results confirmed the aminomethyl group requirement, consistent with the reported structure of the MGOPM adduct (fig. 2, [57]).

Animal studies have suggested the relevance of the carbonyl scavenging mechanism to the therapeutic effects of PM. In the Zucker rat model of diabetes and hyperlipidemia, characterized by elevated levels of plasma α-dicarbonyl compounds and protein AGEs/ALEs, PM treatment had a significant renoprotective effect [60, 61]. This was accompanied by a substantial decrease in plasma levels of GO and MGO, by decreased AGE/ALE formation

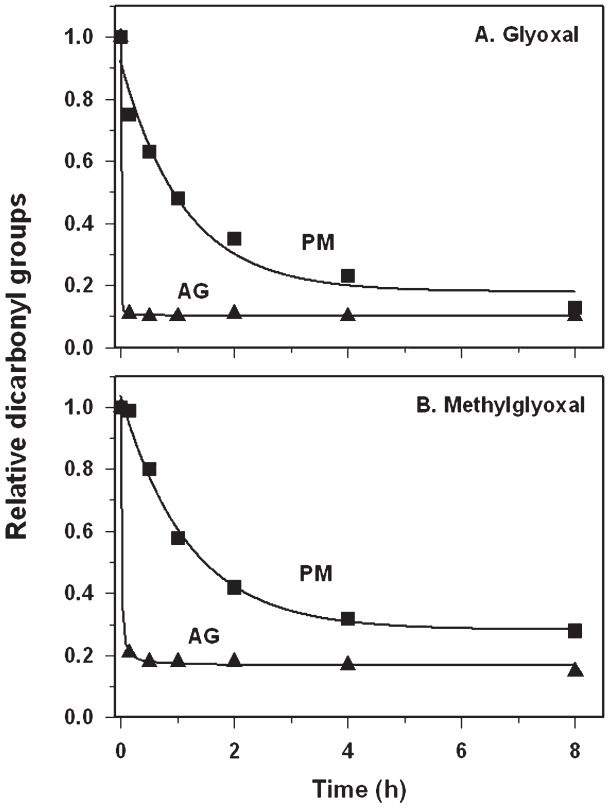


Figure 4. PM has a more moderate dicarbonyl scavenging activity when compared with aminoguanidine. Samples of 10 mM glyoxal (*A*) or 10 mM methylglyoxal (*B*) were incubated with 15 mM pyridoxamine (PM) squares or 15 mM aminoguanidine (AG) triangles. The incubations were carried out at 37 °C in 200 mM Na-phosphate buffer, pH 7.5, containing 0.02% sodium azide. The loss of carbonyl moieties in the course of reaction was measured spectrophotometrically using Girard's reagent T [56]. Parallel experiments with carbonyl compounds incubated under the same conditions but without PM or AG were used as references for calculating the relative amount of the reactive carbonyl groups.

in collagen and by decreased levels of MGO lysine dimer, a specific MGO-derived lysine-lysine crosslink, in plasma proteins [57, 60, 61]. Moreover, adducts of PM and carbonyl products of degradation of arachidonic and linoleic acids were detected in urine of PM-treated animals, indicating that the carbonyl trapping mechanism is operative in vivo [59]. Under cell culture conditions, PM protected DNA of human endothelial cells from hyperglycemia-induced modifications by a mechanism consistent with trapping of GO [62].

The ability of PM to scavenge reactive carbonyl compounds may also underlie its therapeutic effect in elevated urinary oxalate, hyperoxaluria, one of the major risk factors of kidney stones [63, 64]. Oxalate is synthesized enzymatically, mainly in liver, in the glyoxylate pathway via the carbonyl intermediates glycolate and glycolaldehyde [65]. In the rat model of hyperoxaluria, PM treatment significantly reduced urinary excretion of oxalate and inhibited the formation of kidney calcium oxalate crystals, consistent with trapping of carbonyl precursors in oxalate biosynthesis [66]. These preclinical trials suggested a new therapeutic use of PM in the treatment of hyperoxaluria and kidney stone disease.

The experiments using animal models demonstrated that PM might be effective in the treatment of diabetic nephropathy, retinopathy and neuropathy, as well as in hyperoxaluria and in complications of hyperlipidemia [20, 38, 61, 66–68]. The therapeutic effects of PM were accompanied by a decrease in circulating reactive carbonyl species and/or protein AGE/ALE modifications [20, 38, 60, 61] (most of these results are more fully discussed in a recent review [69]). These in vivo findings together with mechanistic insights from in vitro studies strongly suggest that the reported pharmacological effects of PM may result from the combination of (i) inhibition of post-Amadori AGE formation and (ii) trapping of carbonyl precursors of either AGE/ALE or oxalate.

Aminoguanidine can also efficiently scavenge α-oxoaldehyde compounds such as GO, MGO and 3-DG by forming amino-triazine derivatives [70]. In fact, when compared with PM, reaction rates between AG and either GO or MGO were over 20-fold faster (fig. 4). However, as discussed above, AG did not inhibit post-Amadori formation of AGEs [35], and was inferior to PM at retarding the development of early renal disease in diabetic animals [20]. This suggests that, unlike PM, AG exerts its therapeutic effects via only one mechanism, i.e. efficient scavenging of reactive carbonyl compounds.

Scavenging of ROS

The third major mechanism of protein damage by glycation reactions is based on generation of reactive oxygen species (ROS) either from autoxidation of glucose or in the process of oxidative degradation of Amadori intermediate [71–73]. Major ROS generated from these reactions are superoxide anion, hydrogen peroxide, and hydroxyl radical (fig. 1, shown in blue). Other ROS, such as MGO radical, have also been shown to form during the reaction of carbonyl species with proteins [74]. Along with glycoxidative reactions, lipid peroxidation is another major source of ROS such as peroxyl and alkoxyl radicals [75]. ROS can cause oxidative damage to several protein amino acid side chains, including oxidation of cysteine to sulfonate, conversion of methionine to its sulphoxide, hydroxylation of aromatic amino acids as well as fragmentation of protein backbone [75–77]. ROS themselves can also trigger pathogenic signaling, which is suggested to play an important role in the development of diabetic complications [78, 79]. Besides their suggested role in the mechanism of diabetic complications, ROS have also been implicated the in pathogenesis of other diseases, such as atherosclerosis and cancer [78, 80, 81]. Therefore,

scavenging of ROS or inhibition of ROS production may have a significant therapeutic effect.

PM can potentially trap oxygen radicals via the hydrogen atom-donating ability of its phenolic group, although this PM activity may be relatively weak [55]. Since post-Amadori oxidative reactions generate ROS [71, 72], (fig. 1), inhibition of these reactions by PM could also decrease ROS production. Indeed, PM significantly inhibited accumulation of hydroxyl radical, •OH, generated in vitro by the Fenton reaction ([82] and fig. 5). The PM structural analog 3-hydroxypyridine was as efficient as PM at inhibiting •OH accumulation, indicating that the phenolic hydroxyl ring substituent of PM is sufficient for this activity (fig. 5). It was reported that PM can also react with hypochlorous acid, HOCl, a highly reactive ROS produced by activated neutrophils and monocytes at the site of inflammation [83]. In cell culture, PM inhibited production of superoxide radical, $O_2^{\bullet-}$, in red blood cells treated with glucose, apoptotic signaling triggered by nitric oxide, NO• in insulin-secreting RINm5F cells, and H_2O_2-induced lipid peroxidation in U937 monocytes [84–86]. In STZ-diabetic hamsters, PM reduced oxidative stress as measured by accumulation of malondialdehyde, a marker for free radical lipid peroxidation [87]. Taken together, these data indicate that PM may have a protective effect in ROS-related toxicity.

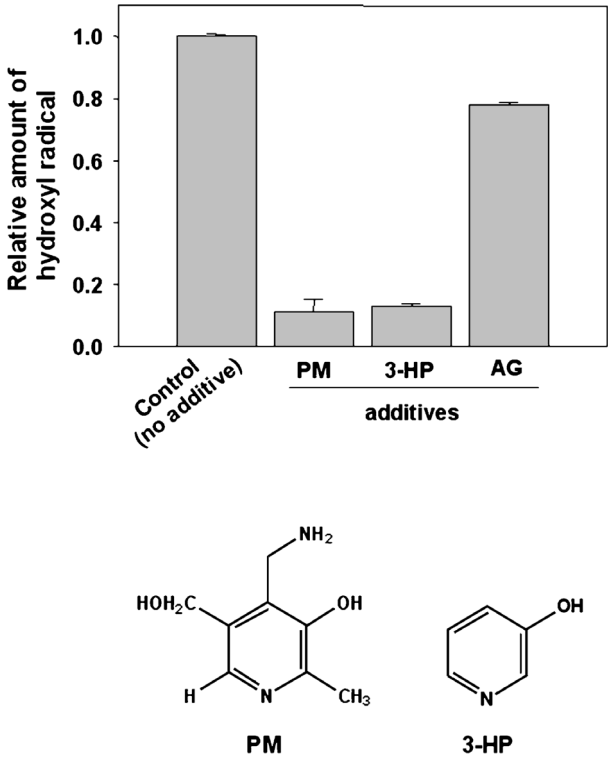


Figure 5. The 3-hydroxyl group of PM is required for efficient hydroxyl radical scavenging. Hydroxyl radicals were generated using a Fe(II)/hydrogen peroxide system. The final concentration of all additives was 20 mM. Hydroxyl radicals produced in a 90-min reaction at 37 °C were determined using the salicylate hydroxylation method [101].

Although it has been reported that AG can inhibit generation of hydroxyl radical [88], this inhibitory activity is very weak when compared directly with that of PM (fig. 5). Thus, inhibition of major pathogenic glycation pathways by PM and AG occurred via somewhat different overall mechanisms. PM was a stronger inhibitor of post-Amadori AGE formation and ROS accumulation but showed relatively weak carbonyl scavenging activity compared with that of AG. Aminoguanidine, on the other hand, demonstrated virtually no inhibition of the post-Amadori AGE pathway, very weak ROS inhibition but strong scavenging of reactive carbonyl species. It is likely that the carbonyl scavenging activity of AG was solely responsible for its AGE inhibition and therapeutic effects in preclinical and clinical trials. However, stronger carbonyl scavenging by AG may be a significant drawback when the safety of the treatment is considered [82].

Treatment safety

Treatment of multifactorial chronic diseases such as diabetes is characterized by the absence of a single molecular target for which a high-specificity pharmacological agent could be developed. Targeting of multiple pathways and the necessity of long-term treatment put very heavy demands on drug tolerability and safety. In toxicity studies, PM was well tolerated and showed a favorable safety profile with no reported adverse effects [89, 90]. PM safety may be explained, in part, by proper balance of different therapeutic mechanisms, each possessing relatively moderate activity. Arguably, this makes PM less likely to interfere with processes which are not the intended targets of treatment. For AG, the benefits in treatment of diabetic complications [23, 24] were compromised by the toxic effects possibly related to its high reactivity towards the biologically important carbonyl species [82, 91]. As shown above, AG is a much stronger scavenger of α-dicarbonyl compounds compared with PM (fig. 4), and it binds strongly to pyridoxal-5′-phosphate [82]. On the other hand, because PM is a more moderate carbonyl scavenger, it does not react with pyridoxal-5′-phosphate [82]. Similarly, agents with stronger metal cation binding activity than that of PM may interfere with the functioning of metalloproteins. Yet, some of these strong metal ion scavengers still cannot inhibit post-Amadori AGE formation [92]. Recently, a PM analog possessing stronger free-radical trapping activity was synthesized [93, 94]. However, its safety is unknown and may be compromised.

Another important safety factor is that PM is a natural compound. Although PM pharmacological concentrations are significantly higher than its physiological levels, the adaptation of the organism to low levels of the drug mini-

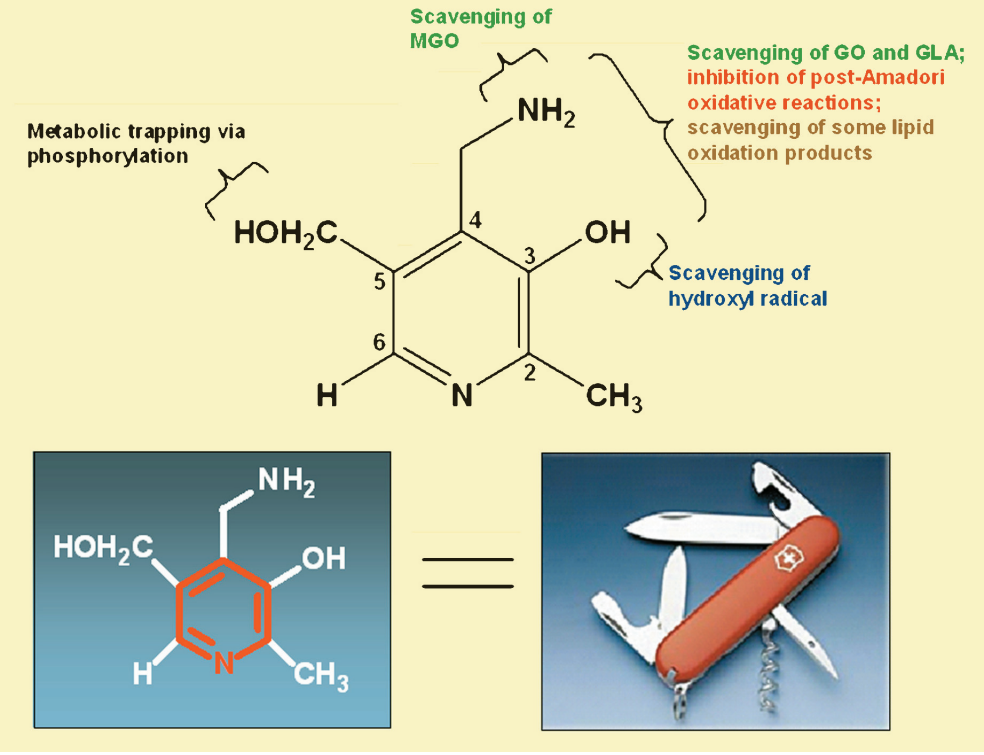


Figure 6. The illustration to the concept of PM polyfunctionality: the functional groups of pyridoxamine responsible for inhibition of different pathogenic factors of glycoxidation and lipoxidation reactions and for its intracellular accumulation. The 4-aminomethyl ring substituent is required for scavenging the reactive carbonyl compound methylglyoxal (MGO); the 3-hydroxyl ring substituent is required for scavenging hydroxyl radicals; both of these functional groups are required either for scavenging glyoxal (GO), glycolaldehyde (GLA) and some lipid-derived carbonyl species or for inhibition of oxidative degradation of the Amadori intermediate to AGEs. The 5-hydroxymethyl ring substituent is required for PM phosphorylation and intracellular accumulation via the 'metabolic trapping' mechanism [102].

mizes toxicity, especially the immune response. As a part of such adaptation, high-affinity PM binding sites with the potential to confer toxicity are likely to be saturated at physiological PM levels, making them insensitive to pharmacological doses.

Conclusion

Diabetes is quickly becoming one of the major human diseases exhibiting features of a worldwide epidemic [95, 96]. The development of multiple diabetic complications is a multifactorial systemic disorder similar to accelerated ageing and as such cannot be 'cured'. The realistic goal is to slow the development of these complications, thus delaying their life-threatening or even comfort-threatening consequences beyond the human lifespan. One approach could be either inhibition of production or scavenging of toxic AGEs, reactive carbonyl species and ROS derived from the accelerated glycoxidative and lipoxidative reactions in diabetes. A small excess of these toxic species over the capacity of natural defense systems can, over time, lead to significant cumulative damage through modification of long-lived proteins, inhibition of enzyme activities and induction of pathogenic signaling. On the other hand, the therapies that produce even a small decrease in these toxic species may go a long way in delaying the development of diabetic complications. Such therapies are based on targeting of a broad spectrum of toxic compounds with relatively diverse structures and therefore cannot be tuned for specific molecular targets without losing effectiveness. This puts a very strong demand on the safety of the treatment.

Aminoguanidine therapy has experimentally confirmed the concept that the inhibition of AGE formation may delay the onset of diabetic complications. Unfortunately, the toxicity of the treatment prevented this therapy from clinical use. Pyridoxamine is emerging as the next promising therapeutic agent, now on the FDA 'fast track' to phase III clinical trials for treatment of diabetic nephropathy. The advantages of PM appear to be its properly balanced inhibitory activities towards the major toxic factors of glycoxidation and lipoxidation reactions (figs 6, 7) and safety. In fact, PM safety may result, at least in part, from this combination of different mechanisms confined within one simple molecular structure, each producing a relatively moderate effect. In this context, it appears that compounds possessing strong carbonyl-scavenging activ-

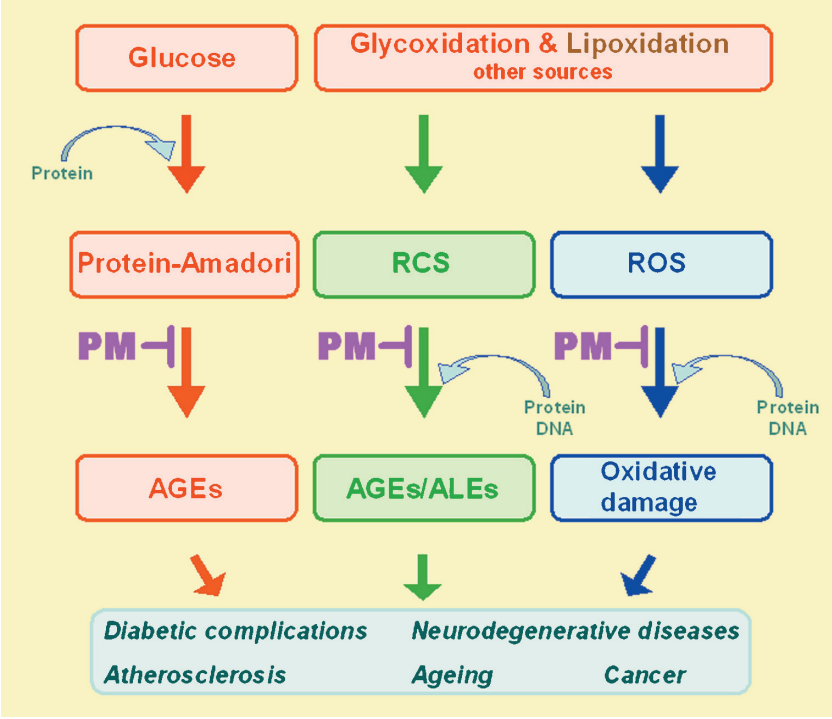


Figure 7. PM inhibition of the pathways leading to formation of AGEs (red), reactive carbonyl species (green) or reactive oxygen species (blue) and its potential in retarding the development of diseases for which these pathways confer pathogenicity. RCS, reactive carbonyl species; ROS, reactive oxygen species; AGEs, advanced glycation end products; ALEs, advanced lipoxidation end products.

ity, such as AG and its analogs, have compromised safety. In either preclinical or clinical trials PM has demonstrated a pharmacological potential for treatment of diabetic nephropathy, diabetic retinopathy, hyperlipidemia, and for use in kidney stone prevention therapies. Although the precise mode of action of PM in vivo is not yet clear, the available body of scientific data is consistent with its usefulness for long-term treatment of chronic conditions in which oxidative reactions and carbonyl compounds confer pathogenicity (fig. 7). Therefore, the pharmacological applications of PM may be expanded to include such conditions as atherosclerosis, cancer, neurodegenerative diseases and ageing [62, 97–99].

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