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Minireview

Pyridoxamine, an inhibitor of advanced glycation and lipoxidation reactions: a novel therapy for treatment of diabetic complications☆

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Abstract

Pyridoxamine (PM), originally described as a post-Amadori inhibitor of formation of advanced glycation end-products (AGEs), also inhibits the formation of advanced lipoxidation end-products (ALEs) on protein during lipid peroxidation reactions. In addition to inhibition of AGE/ALE formation, PM has a strong lipid-lowering effect in streptozotocin (STZ)-induced diabetic and Zucker obese rats, and protects against the development of nephropathy in both animal models. PM also inhibits the development of retinopathy and neuropathy in the STZ-diabetic rat. Several products of reaction of PM with intermediates in lipid autoxidation have been identified in model reactions in vitro and in the urine of diabetic and obese rats, confirming the action of PM as an AGE/ALE inhibitor. PM appears to act by a mechanism analogous to that of AGE-breakers, by reaction with dicarbonyl intermediates in AGE/ALE formation. This review summarizes current knowledge on the mechanism of formation of AGE/ALEs, proposes a mechanism of action of PM, and summarizes the results of animal model studies on the use of PM for inhibiting AGE/ALE formation and development of complications of diabetes and hyperlipidemia.

Keywords: Advanced glycation end-product; Advanced lipoxidation end-product; Hyperlipidemia; Maillard reaction; Diabetic nephropathy

Sources of advanced glycation and lipoxidation end-products

The Maillard hypothesis on diabetic complications proposes that these complications develop as a result of chronic, increased chemical modification of tissue proteins by glucose during hyperglycemia. According to the classical (Hodge) pathway, reversible Schiff base and Amadori adducts of glucose to protein gradually rearrange to form permanent chemical modifications of proteins, including both amino acid adducts and cross-links [1,2]. These reactions, described as Maillard or browning reactions, proceed normally in the body at homeostatic concentrations of glucose, and AGEs[1] accumulate with age in long-lived tissue proteins such as collagen in the extracellular matrix. AGEs are also formed more rapidly on intracellular proteins, probably from glycolytic intermediates, such as glyceraldehyde 3-phosphate and dihydroxyacetone phosphate [3], but may not accumulate because of the more rapid rate of turnover of intracellular proteins. A wide range of AGEs have been described in tissue proteins, and increased, age-adjusted levels of two of these AGEs, N^{ε}-(carboxymethyl)lysine (CML) and pentosidine, in skin collagen correlate with the severity of diabetic

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[1] *Abbreviations used:* AG, aminoguanidine; AGE, advanced glycation end-product; ALE, advanced lipoxidation end-product; CEL, N^{ε}-(carboxyethyl)lysine; CML, N^{ε}-carboxymethyl(lysine); 1DG, 1-deoxyglucosone; 3DG, 3-deoxyglucosone; FAPM, *N*-formyl-PM; GA, glycolaldehyde; GLO, glucosone; GO, glyoxal; HAPM, *N*-hexanoyl-PM; HNE, hydroxynonenal; MDA, malondialdehyde; MGO, methylglyoxal; PM, pyridoxamine; STZ, streptozotocin.

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complications, including nephropathy, retinopathy, and vascular disease [1,2].

There are multiple pathways to the formation of AGEs from glucose, including formation of AGEs from products of autoxidation of glucose, Schiff bases, and Amadori adducts (Fig. 1). Specific AGEs, such as CML, may also be formed from multiple carbohydrate precursors, including glucose, fructose, ascorbate, and metabolic intermediates. Polyunsaturated fatty acids in lipoproteins also contribute to the chemical modification of proteins, forming ALEs [4,5]. Some compounds, such as CML and the homologous compound N^{ε}-(carboxyethyl)lysine (CEL), may be formed from both carbohydrates and lipids (Fig. 1) and are therefore described as AGE/ALEs. Products of autoxidation or metabolism of amino acids are also sources of AGE/ALEs, such as CML [6], so that it is difficult by chemical analysis alone to determine the actual source of AGE/ALEs in tissues, even during hyperglycemia in diabetes.

The formation of AGEs and ALEs may be accelerated not only by an increase in concentration of an oxidizable substrate, such as blood glucose in diabetes and blood lipids in atherosclerosis, but also by increases in oxidative stress or overload on detoxification pathways for dicarbonyl and reactive carbonyl compounds. Exposure of proteins to glucose under air leads to generation of reactive oxygen species and formation of glycoxidation products, such as CML and pentosidine, and also to formation of *o*- and *m*-tyrosine and methionine sulfoxide [7]. Compared to normal tissue, atherosclerotic plaque also contains increased levels of CML and tyrosine oxidation products [8,9]. AGE, ALEs, and amino acid oxidation products, including chloro- and nitro-tyrosine, also appear together at other sites of tissue injury and inflammation and in protein deposits in neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases [10,11]. AGE/ALEs also

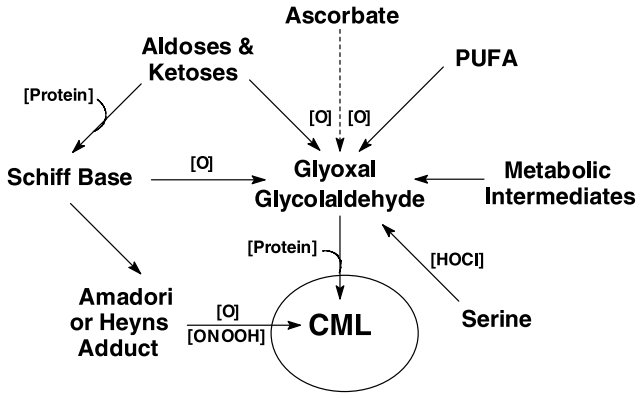

Fig. 1. Alternative pathways to CML, illustrating the range of reactions that may lead to the same product from a variety of precursors. PUFA, polyunsaturated fatty acids; HOCl, hypochlorous acid. ONOOH, peroxynitrite; [O], autoxidation by other reactive oxygen species.

increase in tissue proteins in uremia [12] and severe hepatic disease [13], in these cases probably because of deficiencies in renal or hepatic detoxification of intermediates in AGE/ALE formation.

In summary, the accumulation of AGE/ALEs is recognized as a common feature of the aging of tissue proteins, and levels of these compounds are increased either systemically or locally in a broad range of diseases, including diabetes, atherosclerosis, renal, hepatic, and neurodegenerative diseases. Formation of AGE/ALEs is not an isolated process, but part of a range of oxidative chemical modifications of tissue proteins that increase in aging and disease. Because AGE/ALEs may also catalyze oxidative damage [14–16], AGE/ALE inhibitors should limit the formation of not only AGE/ALEs, but also a range of other oxidative chemical damage to proteins and provide broad protection against tissue damage in disease. While they may not cure the underlying disease process, AGE/ALE inhibitors should retard the development of complications resulting from the fundamental disorder.

Treatment of diabetic complications with AGE/ALE inhibitors

The design of drugs to inhibit AGE/ALE formation and thereby to protect against diabetic complications is a novel challenge for the pharmaceutical industry. Drugs are commonly targeted at specific proteins or other biomacromolecules—they are designed to bind with high affinity, in many cases irreversibly, to some protein, enzyme or receptor in a specific tissue, and frequently have half-maximal activity at nanomolar to micromolar concentrations. In contrast, AGE/ALE inhibitors must trap low molecular mass, soluble, reactive intermediates in AGE/ALE formation and they must react stoichiometrically with a wide range of structures. They must also intercept these intermediates in the presence of much higher concentrations of reactive functional groups on proteins. Lysine, for example, which is a major site of chemical modification of proteins by AGE/ALEs, is present in plasma (on plasma proteins) at nearly 50 mM concentration. Thus, an AGE/ALE inhibitor, which is unlikely to achieve even 100 μM concentration in plasma during therapy, must be significantly more reactive than lysine residues on proteins with intermediates in AGE/ALE formation. Alternatively, the inhibitor may intercept the formation of AGE/ALEs at a stage either preceding the formation of the reactive carbonyl intermediates or after formation of a reactive adduct with protein. At the same time, it must display these activities without interfering with the intermediary metabolism of aldehydes or ketones, or trapping coenzymes or their precursors that contain reactive aldehydes, such as pyridoxal phosphate and retinal.

Reactive carbonyl or dicarbonyl intermediates, such as glyoxal (GO), glycolaldehyde (GA) or methylglyoxal (MGO), are recognized as common, late-stage intermediates in the formation of AGE/ALEs from a variety of carbohydrate and lipid precursors. Aminoguanidine (AG), the prototype AGE inhibitor [17], is a dinucleophile that forms stable hydrazone or triazine adducts on reaction with these compounds [18,19]. In numerous studies in animal models of diabetes [17], AG has been effective in retarding the full range of diabetic complications—nephropathy, neuropathy, retinopathy, and vasculopathy. Further, the protective action of AG against diabetic complications in animal models is associated with a decrease in the formation of fluorescence and crosslinks in collagen. Several studies have also shown a decrease in CML in tissue proteins, measured by immunohistochemistry, and in recent studies AG was shown to inhibit the formation of CML, CEL, Maillard-type fluorescence, and crosslinking of skin collagen in diabetic rats, concurrent with inhibition of development of renal disease [20]. Notably, all of these positive effects were observed in animal models without any effect on blood glucose concentration or glycation of hemoglobin or collagen, consistent with the proposed mechanism of the action of AG as an inhibitor of advanced glycation reactions. Clinical trials of AG yielded both promising and disappointing results. AG significantly reduced proteinuria and inhibited progression of nephropathy in diabetic patients, but failed to reduce the risk for doubling of serum creatinine, the primary endpoint of the study [21]. The usefulness of AG for treatment of diabetic complications was also compromised by toxicity in the high dose arm of the study, and AG reacts with pyridoxal phosphate and may adversely affect vitamin B_6 metabolism [19]. Despite its limitations, however, AG has provided "proof of concept" that inhibition of AGE formation is a reasonable therapeutic modality for treatment of diabetic complications.

Pharmaceutical scientists have a maxim that: "all drugs have at least two mechanisms of action." AG is typical in this respect. It has a number of alternate activities, in addition to its AGE inhibitory activity, including both anti- and pro-oxidant effects on lipid peroxidation [22], chelation of redox-active metal ions [23], inhibition of amine oxidases and inducible nitric oxide synthase [24], and lipid-lowering effects [20]. These activities of AG may affect the progression of complications, independent of the effects on AGE formation. Other AGE inhibitors with some of the structural features of AG have been introduced, including ALT-946 diaminophenazine and OPB-9195. Although these compounds have shown promise in animal model studies, their mechanism of action is less well understood and none have progressed, as yet, to the stage of clinical trials.

Effects of pyridoxamine in STZ-diabetic rats

In a search for novel, less toxic AGE inhibitors, Hudson and colleagues [25,26] evaluated the AGE inhibitory activity of analogs of vitamins involved in the metabolism of carbonyl compounds, focusing on vitamin B_1 (thiamine) and B_6 (pyridoxine) derivatives. Because Amadori adducts were considered important intermediates in AGE formation in vivo, an experimental system, described as "interrupted glycation" [27], was developed in which Amadori proteins were prepared by short-term pre-incubation of the protein with ribose or glucose under antioxidative conditions. The antioxidative conditions promoted the formation of Amadori adducts, but limited formation of AGEs, yielding a glycated (Amadori-modified) protein. AGE inhibitors were then studied for their effects on AGE formation from glycated proteins using an AGE-ELISA assay. Both AG and pyridoxamine (PM; Fig. 2) inhibited AGE formation from the free sugars; however, in contrast to AG, PM proved to be a potent inhibitor of AGE formation from glycated proteins. Because it inhibited the formation of AGEs from Amadori-modified protein, PM was described as a post-Amadori inhibitor of AGE formation, an *Amadorin* [28].

In model studies, Degenhardt et al. [20] showed that, at comparable doses (1 g/L in drinking water), PM was superior to AG in retarding the development of renal disease, measured by increases in urinary albumin and total protein and plasma creatinine, in the streptozotocin (STZ)-induced diabetic rat. This might result, in

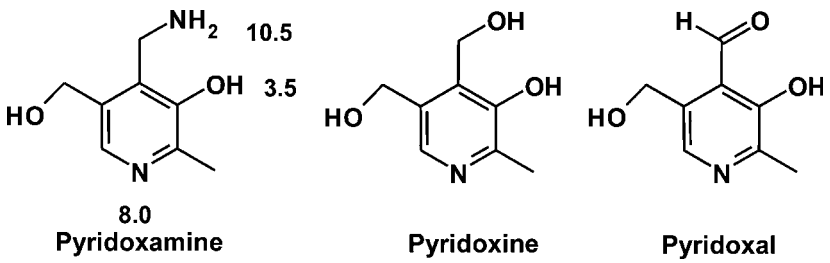

Fig. 2. Structure of Vitamers B_6. Pyridoxamine is shown at the left; numbers indicate the pK_a values of the functional groups. Pyridoxine is the vitamin form of B_6. Pyridoxal, in its phosphorylated form, is the coenzyme.

part, from the longer half-time for plasma clearance of PM, compared to AG, 1.5 vs. 0.5 h, respectively, following gavage administration, (T.P. Degenhardt, unpublished). PM was also more effective in reducing hypercholesterolemia and hypertriglyceridemia (Fig. 3) and in correcting redox imbalances (increased lactate/pyruvate ratio) in this diabetic rat model [20]. The increase in lactate/pyruvate in the diabetic rat was interpreted as evidence of peripheral hypoxia, possibly the result of decreased tissue oxygenation because of microvascular disease, leading to increased anaerobic metabolism of glucose. Despite differences in their effects on indicators of renal disease, dyslipidemia, and redox imbalances, AG and PM were comparable in inhibiting the ~2-fold increase in CML, CEL, Maillard-type fluorescence, and crosslinking of skin collagen of diabetic rats, compared to non-diabetic controls, after 7 months of diabetes. Unexpectedly, neither AG nor PM had an effect on the increase in pentosidine. Effects of PM (and AG) on renal dysmorphology were mixed [20]. After 7 months of diabetes, there were small, but significant, increases in glomerular and mesangial volume and glomerular basement membrane thickening. PM caused a partial correction of the increase in glomerular volume, but effects on GBM thickness and mesangial volume were not statistically significant. Failure to correct all pathology may be attributed to the severity of diabetes in these animals or to other conditions in the STZ-diabetic rats, e.g., hypertension. In this study [20], PM was recovered nearly quantitatively in urine, indicating limited metabolism of the drug.

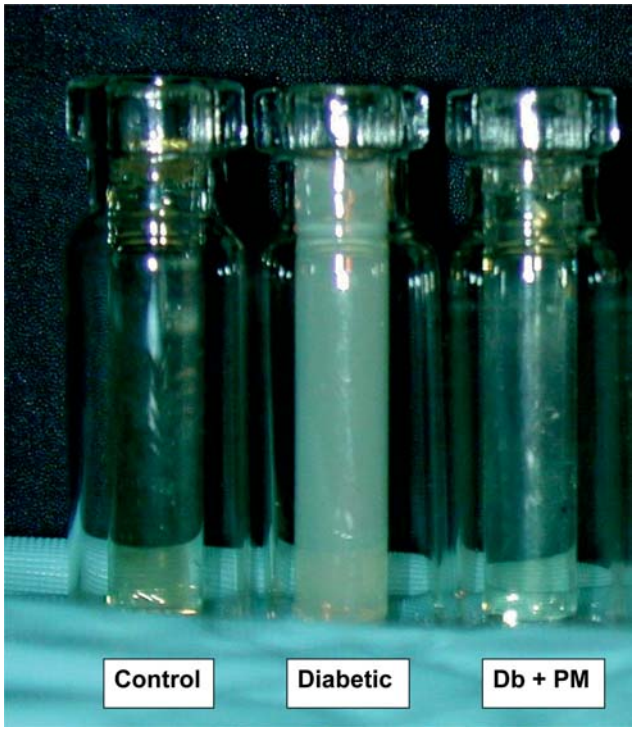

Fig. 3. Plasma from control, diabetic, and PM-treated diabetic rats. The lipemia observed in the STZ-diabetic rat is reversed by treatment with PM.

In other studies in STZ-diabetic rats, Nagaraj et al. [29] reported that PM, at 0.4 g/L in drinking water, inhibited modest increases (20–25%) in MOLD (methylglyoxal–lysine dimer; imidazolium salt) and pentosidine concentration in plasma proteins, and also inhibited a nearly 3-fold increase in plasma and erythrocyte MGO concentrations. Plasma thiobarbituric acid reactive substances (TBARS) also increased by nearly 2-fold and protein carbonyls by ~30% in diabetic rats, and these changes were also corrected by PM treatment. The measurements of TBARS and protein carbonyls were normalized to plasma protein, but not corrected for plasma lipid concentration, so the decrease in these indicators might be secondary to the effects of PM on lipemia. These authors also noted a significant decrease in erythrocyte glutathione concentration in diabetic rats, which was not affected by PM, and observed a 2-fold increase in glyoxalase 1 activity in PM-treated diabetic rats, compared to controls. They concluded, based on the effects on protein carbonyls, TBARS, and glyoxalase 1 activity that PM provided some protection against oxidative stress in the diabetic rat, although the decrease in erythrocyte GSH concentration suggests otherwise. The effects of PM on diabetic complications were not reported in this study.

In recent studies, Stitt et al. [30] demonstrated that PM retarded the development of retinopathy in the STZ-diabetic rat, as measured by protection against pericyte loss and formation of acellular capillaries. PM also inhibited the increase in synthesis of mRNA for collagen, laminin, and fibronectin in the retina, measured by real time PCR, and inhibited the increase in laminin and CML in retinal basement membrane protein, measured by immunohistochemistry. In recent studies, PM has been shown to inhibit the development of peripheral neuropathy in the STZ-diabetic rat (personal communication, N.E. Cameron and M.A. Cotter, University of Aberdeen). In these experiments, the effects of PM were observed within 2 weeks of treatment of animals with 6-weeks' duration of diabetes. Because of the short-term of the diabetes and intervention with PM, there were no measurable differences in the AGE/ALE (CML) content of total nerve tissue in control vs. diabetic or PM-treated diabetic rats. The rapid development of neuropathy and the acute response to PM suggest that changes in AGE/ALEs in long-lived extracellular proteins are not involved in the development of peripheral neuropathy. Localized changes in AGE/ALEs, e.g., in neuronal endothelial cells, have not been evaluated by higher resolution immunohistochemical techniques, so that the role of AGE/ALEs in the pathogenesis of diabetic neuropathy is still uncertain [31].

Effects of pyridoxamine in Zucker obese rats

One of the unexpected observations in the studies on the STZ-diabetic rats was that these animals were severely hyperlipidemic. Further, both AG and PM had profound lipid-lowering effects in these animals, and effects of PM on levels of triglycerides and cholesterol correlated with the loss in renal function and the increase in CML content of skin collagen [20]. Because of the correlation between plasma triglycerides and CML and the lack of an effect of AG or PM on blood glucose, glycation of collagen or levels of the carbohydrate-derived AGE pentosidine, these results suggested that lipids might be the primary source of chemical modification of collagen in the diabetic rat. Fu et al. [4] had demonstrated previously that CML and CEL were formed during metal-catalyzed oxidation of low density lipoproteins and that these adducts were formed from the lipids rather than Amadori adducts on the protein.

To address the role of lipids in chemical modification of proteins and development of renal disease in a non-diabetic model, Alderson et al. [32] studied the chemical modification of proteins in the Zucker obese (*fa*/*fa*) rat. This animal is characterized by obesity, hyperlipidemia, hypertension, and insulin resistance, and may be considered a model of the metabolic syndrome (Syndrome X) in humans. Despite the fact that they are normoglycemic, the increases in CML, CEL, pentosidine, and fluorescence in the skin collagen of obese, hyperlipidemic rats, compared to lean littermates, were similar to those observed in STZ-diabetic vs. control rats, suggesting that lipids may be the primary source of CML and CEL in both the diabetic and obese rats. As in the diabetic rat, PM inhibited the increases in fluorescence, CML, and CEL in skin collagen, as well as the increase in malondialdehyde and hydroxynonenal adducts to lysine (MDA-Lys and HNE-Lys), which are derived exclusively from lipids. These changes were accompanied by a profound lipid-lowering effect of PM in the Zucker rat, without any effect on food intake, insulin resistance or obesity. As in the STZ-diabetic rat, PM protected against renal disease, inhibiting the development of both albuminuria and creatinemia in the Zucker rat. PM also prevented the increase in systolic blood pressure and mean arterial pressure in the Zucker rat, and inhibited thickening of the aortic wall and the walls of small arteries in the heart and kidney. Overall, with the exception that PM did not affect blood pressure in the STZ-diabetic rat, the effects of PM in the Zucker rat were similar to those observed in the diabetic model (Table 1). One important indication from the studies in both STZ-diabetic and Zucker obese rats is that lipids may be as important as, if not more important than, carbohydrates in the chemical modification of proteins and development of complications in diabetes. Indeed, lipids are more readily oxidized than carbohydrates and induce a wide range of chemical modifications of proteins, and dyslipidemia and hyperlipidemia have been recognized as major risk factors for development of renal and vascular diseases in diabetes [33,34] and obesity [35,36].

Table 1
Effects of pyridoxamine in STZ-diabetic rats

- Retards early nephropathy
  - Albuminuria
  - Proteinuria
  - Plasma creatinine
- Inhibits dyslipidemia
  - Plasma triglycerides
  - Plasma cholesterol
- Decreases chemical modification of skin collagen
  - CML and CEL
  - Fluorescence
  - Pepsin digestibility
- Reverses redox imbalances
  - Lactate/pyruvate ratio
- Has no effect on:
  - Plasma glucose concentration
  - Glycated hemoglobin
  - Glycation of collagen
  - Hypertension
  - Pentosidine content of skin collagen

Studies on the mechanism of AGE/ALE inhibition by pyridoxamine

A major advantage of PM for mechanistic studies is the fact that it is a highly fluorescent, aromatic compound and that its derivatives are detectable by absorbance and/or fluorescence following reaction with intermediates in glycoxidation and lipoxidation reactions. PM adducts are also readily ionized for electrospray analysis, so that they can be detected and quantified in complex mixtures, including urine, by multiple reaction monitoring LC/MS/MS. Products formed during autoxidation of linoleate in the presence of PM in vitro were fractionated by reversed phase HPLC and readily detected by their characteristic fluorescence [5]. Both *N*-hexanoyl-PM (HAPM) and *N*-nonanedioyl-PM monoamide (NDAPM) were identified in reactions between PM and linoleate or arachidonate and were subsequently detected in the urine of PM-treated diabetic and obese rats [37]. Other urinary products that have now been identified include the pentanedioic acid amide derivative of PM (PDAPM), derived from the α-terminus of arachidonate, and *N*-formyl-PM (FAPM) (Fig. 4). Not all compounds formed in model lipid peroxidation systems were detected in urine, but the substantial overlap between products formed in vitro and in vivo establishes that PM acts, at least in part, as an ALE inhibitor in vivo.

There were two unexpected observations from our mechanistic studies on PM: first, we did not detect the

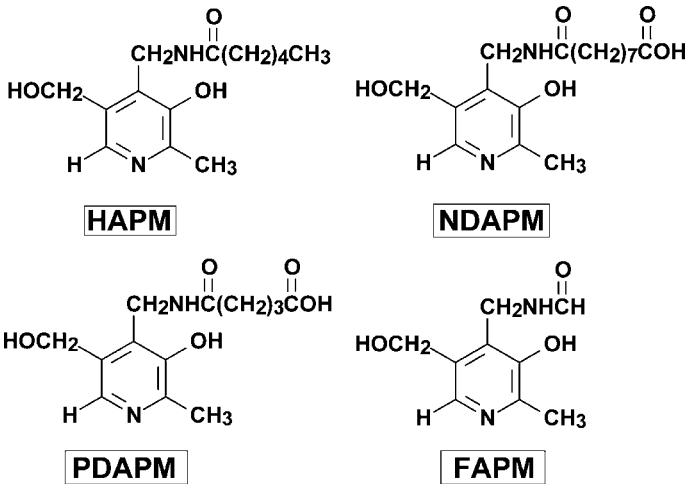

Fig. 4. Structure of various pyridoxamine adducts detected in reactions in vitro and in urine of diabetic and obese rats treated with pyridoxamine. HAPM = *N*-hexanoyl-pyridoxamine, derived from the ω-terminal end of linoleate and arachidonate; NDAPM = *N*-nonanedioyl-pyridoxamine monoamide, derived from the α-terminal end of linoleate or linolenate; PDAPM = *N*-pentanedioyl-pyridoxamine monoamide, derived from the α-terminal end of arachidonate; and FAPM = *N*-formyl-pyridoxamine, derived from polyunsaturated fatty acids, in general.

carboxymethyl, carboxyethyl, MDA or HNE derivatives of PM in either in vitro reactions or in rat urine; and second, the major PM derivative detected both in vitro and in vivo was the formic acid amide derivative of PM (FAPM) (T.O. Metz, et al., submitted). Although Glomb and Pfahler [38] detected carboxymethyl-PM in model reactions, the fact that the CM-, CE-, MDA-, and HNE-PM analogs of AGE/ALEs have not been detected either during lipid peroxidation reactions in vitro [7] or in urine suggests that PM reacts primarily with early intermediates in AGE/ALE formation. Voziyan et al. [39] and Miyata et al. [19] have reported that PM is not as reactive as AG with aldehydes and small dicarbonyl compounds, suggesting that PM is trapping precursors of GO, MGO, MDA, and HNE, perhaps enolate or epoxide intermediates, although their structure is not yet apparent. The fact that only lipid-derived PM-adducts have been detected in the urine of either diabetic or hyperlipidemic rats and that no products derived exclusively from carbohydrates have yet been identified in vivo is consistent with the conclusions from studies in STZ-diabetic and Zucker obese rats that lipids are a major source of chemical modification of proteins in vivo.

The detection of FAPM as the major PM derivative in model systems and in urine has provided a novel insight into the mechanism of action of PM. How are amides, in general, and FAPM, in particular formed from peroxidizing lipids? In previous work [5], we had proposed that PM cleaved carbon chains asymmetrically at sites of hydroperoxide formation on unsaturated fatty acids. Although this mechanism explained the origin of HAPM and NDAPM, from the α- and ω-termini of the linoleate, respectively, we were unable to identify the complementary cleavage products predicted by this mechanism. The dominance of FAPM among the reaction products now suggests that PM acts to cleave between vicinal dicarbonyl groups of intermediates in lipid (and carbohydrate) peroxidation chemistry. As shown in Fig. 5, we propose that HAPM and FAPM are formed in equal yield by cleavage of α-ketoaldehydes such as 2-ketoheptanal, the product of autoxidation of 2-hydroxyheptanal, a known product of peroxidation ω-6 fatty acids [40]. Similar pathways would lead to the formation of PDAPM from arachidonate and NDAPM from linoleate. *N*-Propanoyl-

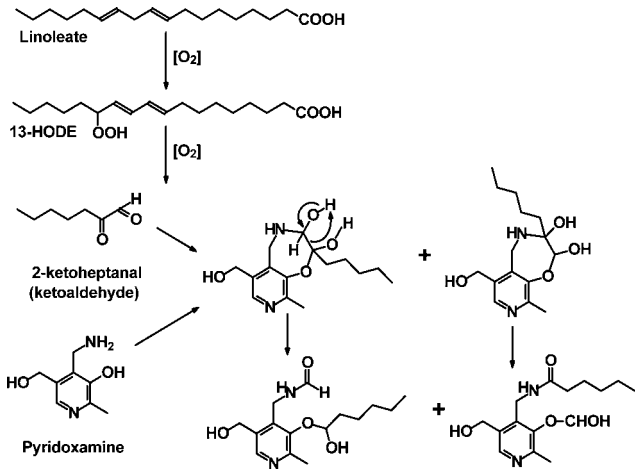

Fig. 5. Proposed mechanism for the formation of pyridoxamine adducts during inhibition of advanced glycation and lipoxidation reactions. Ketoaldehyde intermediates react with PM, forming a seven-membered ring, which undergoes a ring-opening rearrangement to form amide and hemiacetal adducts to PM. The hemiacetal hydrolyzes in aqueous solution to yield the stable amide adducts that are detected in model reactions and in urine. The two products are formed, not necessarily in equal proportions, depending on the manner of addition of PM to the ketoaldehyde.

PM, a predicted product from arachidonate oxidation, is also readily detected in rat urine, but its origin is less certain, since it may be formed by endogenous detoxification reactions, analogous to enzymatic N-acetylation reactions. For mechanistic reasons, the reaction sequence outlined in Fig. 5 is unlikely to be a major pathway of PM action. However, FAPM is formed as a minor product during reaction of PM with GO (T.O. Metz, unpublished), providing some support for the proposed mechanism. The total quantity of PM adducts, 100–200 nmol/24 h in urine of the diabetic and obese rats, is also small, suggesting that there are alternate mechanisms of action of PM or that entrapment of this small quantity of intermediates is sufficient to limit the cascade of reactions that leads to increased formation of AGE/ALEs and/or AGE/ALE-induced signal transduction processes [41].

The proposed mechanism of action of PM during lipid peroxidation reactions suggests that it may operate by a similar mechanism in its role as an Amadorin, i.e., that PM may react with other ketoaldehydes, such as glucosone (GLO), 1-deoxyglucosone (1DG) or 3DG, formed from glucose during Maillard reactions. These compounds are early intermediates, derived from the Amadori adducts by oxidative and non-oxidative pathways. FAPM would be one product of reaction of these reactions with PM and has, in fact, been detected in glycoxidation reactions in vitro (T.O. Metz, unpublished). The 5-carbon adducts to PM, theoretically derived from the non-reducing end of GLO and 1- and 3-DG, have not yet been detected in vitro or in rat urine. It is possible, that PM may react with protein-bound dicarbonyl compounds that are formed from the Amadori adduct and are precursors of AGEs.

The proposed mechanism of reaction of PM with ketoaldehydes suggests that PM would also cleave other α-dicarbonyl compounds. Indeed, we have recently observed that PM cleaves phenylpropanedione, a model for AGE crosslinks in protein, suggesting that it might also function like an AGE-breaker in vivo. However, in studies of PM or other AGE-breakers, we have been unable to confirm their proposed mechanism of action in reversing pre-existing AGE crosslinks in tissue proteins [42]. More likely, these compounds, like PM, intercept dicarbonyl intermediates in the formation of AGE/ALEs. The pathway for the formation of amide derivatives is also consistent with the recent work of Glomb and Pfahler [38] and Hasenkopf et al. [43] identifying amides as novel derivatives formed during glycoxidation reactions.

Antioxidant activity of pyridoxamine

Onorato et al. [5] showed that PM was a potent inhibitor of chemical modification of proteins by polyunsaturated fatty acids in vitro, inhibiting the formation of CML and CEL, and MDA-Lys and HNE-Lys during autoxidation of arachidonate in the presence of RNase and during metal-catalyzed oxidation of low density lipoprotein (LDL). However, the inhibition of AGE/ALE formation was not the result of inhibition of lipid peroxidation. PM did not inhibit the oxidative loss of linoleate or arachidonate in phosphate buffer, even at 1 mM concentration. At 100 μM concentration, the plasma concentration achieved in diabetic rats, PM increased the lag phase for oxidation of LDL by about 50%, but did not prevent the formation of diene conjugates, measured by absorbance at 234 nm. Thus, the lipid peroxidation proceeded, but modification of lysine residues or formation of AGE/ALEs in RNase or LDL was inhibited. These data, in addition to the recovery of numerous lipid-derived PM adducts in vitro and in urine, argue that PM is not functioning primarily as an antioxidant.

In studies in cell culture, Jain and Lim [44] observed that both PM and pyridoxine (Pyridoxine is not an AGE inhibitor [25,26].) decreased superoxide generation and protected red cells against oxidative stress during exposure to high glucose concentration in vitro. Both compounds provided modest protection against membrane lipid peroxidation, measured as MDA, and inactivation of Na^+, K^+-ATPase by high glucose. Kinae and co-workers [45] also observed that PM protected against oxidative damage to DNA during growth of human umbilical vein endothelial cells (HUVECs) in high glucose medium. In the HUVEC system, however, PM did not inhibit the glucose-induced increase in the generation of intracellular peroxides, arguing against an antioxidant activity. These authors concluded that PM probably acted by inhibiting the increase in AGE/ALE intermediates and indicators of carbonyl stress [46], such as 3-deoxyglucosone (3DG) and GO, which were considered the primary source of damage to DNA. Overall, the role of antioxidant activity in the mechanism of action of PM is unlikely, but deserves further study.

Overview

Understanding cause and effect in biological systems is a difficult challenge. Sometimes, processes are bi-directional, e.g., hypertension and hyperlipidemia are risk factors for renal disease, while renal disease is a risk factor for hypertension and hyperlipidemia. Often, multiple effects are observed simultaneously, so that it is impossible to identify the primary site of action of a drug. PM, for example, inhibits AGE and ALE formation in vitro, and also inhibits the chemical modification of proteins in both the hyperglycemic and hyperlipidemic animals. However, these data do not establish the fact that inhibition of AGE/ALE formation is the

primary mechanism of the action of PM in vivo. It can be argued, for example, that PM's primary effect is on preservation of renal function, thereby reducing the concentration of AGE/ALE precursors in plasma and tissues, leading to a decrease in AGE/ALEs in tissue proteins. Hyperlipidemia is associated with nephrotic syndromes, independent of diabetes [47,48], so that the preservation of renal function could also explain how PM inhibits hyperlipidemia. Alternatively, PM's primary effect may be on hyperlipidemia, secondary to some unidentified effect on lipid metabolism, and the reduction in AGE/ALE formation may be a direct consequence of the decrease in plasma lipids in diabetic and obese rats; in this case, hyperlipidemia is a recognized risk factor for renal disease [33–36], so that the preservation of renal function could be viewed as secondary to the reduction in hyperlipidemia. It is also possible that PM, although it is a weak chelator [23] that does not directly inhibit lipid peroxidation [5], promotes excretion of excess metal ions from tissue stores into urine—both the diabetic and obese rats are hyperphagic and may accumulate redox-active metal ions in tissues. At this point, although there are alternative interpretations of the data from pre-clinical studies, it is clear that PM provides protection against a range of pathology in diabetic and obese rats and that it clearly functions, at least in part, as an AGE/ALE inhibitor in vivo, based on the recovery of PM adducts in urine. The fact that similar effects on AGE/ALE formation, hyperlipidemia, and nephropathy in the STZ-diabetic rat were observed with structurally unrelated compounds, AG and PM, makes it unlikely that PM's activity is in any way related to its role as a B_6 vitamer and coenzyme. All of the data at this point are consistent with both a role for AGE/ALEs in the pathogenesis of disease and the proposed mechanism of the action of PM as an AGE/ALE inhibitor.

Other applications of PM

AGEs and ALEs are implicated in tissue damage in a number of diseases, including diabetes, atherosclerosis, dialysis-related amyloidosis, neurodegenerative diseases, and chronic inflammation. To the extent that these diseases may be exacerbated by AGE/ALE formation, PM may find use in the treatment of a wide range of chronic diseases in which oxidative stress, inflammation, and tissue damage lead to increased chemical modification of protein. By trapping chemical intermediates that may not only modify proteins, but also enhance oxidative damage, PM may protect against cycles of oxidative stress and tissue damage. While it may not inhibit the chemical modification of proteins during normal aging, PM may delay pathology associated with a number of age-related diseases and thereby contribute to a healthier lifespan. Experiments are now in progress to evaluate the influence of PM on the vitality and lifespan of rodents.

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