The Metabolism of Malondialdehyde

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Interest in malondialdehyde (MDA) metabolism stems from its formation as a product of lipid peroxidation in the diet and in the tissues; its reactivity with functional groups of nucleic acid bases, proteins and phospholipids; its mutagenicity in bacteria, and its reported skin and liver carcinogenicity in animals. Administration of the Na enol salt of MDA in the drinking water of mice over a range of 0.1–10.0 μg/g/day for 12 mo produced dose-dependent hyperplastic and neoplastic changes in liver nuclei and increased mortality at the highest level but produced no gross hepatic tumors. Addition of MDA to the medium of rat skin fibroblasts grown in culture caused nuclear abnormalities at concentrations as low as 10^{-6} M despite an uptake of only 4%. [1,3-^{14}C]MDA was rapidly oxidized to [^{14}C]acetate in rat liver mitochondria and to $^{14}CO_2$ in vivo; however, ~10% of the radioactivity was recovered in the urine. Chromatographic analysis of rat urine revealed the presence of several compounds which yield MDA on acid hydrolysis. Total MDA excretion increased in response to conditions which stimulate lipid peroxidation in vivo, including vitamin E deficiency, Fe or CCl_4 administration, and enrichment of the tissues with PUFA. N-acetyl-e-(2-propenal)lysine was identified as a major urinary metabolite of MDA in rat and human urine. This compound is derived primarily from N-α-(2-propenal)lysine released in digestion as a product of reactions between MDA and the ε-amino groups of N-terminal lysine residues in food proteins. However, its presence in the urine of animals fasted or fed MDA-free diets indicates that it is also formed in vivo. Identification of the metabolites of MDA excreted in the urine may provide clues to the mechanisms of cellular damage caused by this compound in the tissues.
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Interest in the metabolism of malondialdehyde (MDA) stems from its reactivity with the functional groups of a variety of cellular compounds, including the amino groups of proteins (1) and nucleic acid bases (2), the N bases of phospholipids (3), and the SH groups of sulfhydryl compounds (4). Its affinity for the N atoms of DNA bases, crosslinking action, nucleotoxicity at low concentrations in animal cells grown in culture and mutagenic activity in bacteria also have created an interest in its potential carcinogenicity.

MDA occurs in foods in amounts ranging from <0.1 to about 10 ppm, depending upon their fatty acid composition and conditions of storage (5,6). It arises predominantly from the oxidation of polyunsaturated fatty acids (PUFA) with three or more double bonds, and therefore generally occurs at higher concentrations in foods of animal origin. It is also formed in vivo, both nonenzymatically as a product of lipid peroxidation and enzymatically as a product of the cyclooxygenase reaction in prostaglandin metabolism.

The following is a summary of recent work on the occurrence, toxicity and metabolism of MDA conducted in this and other laboratories.

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CHRONIC TOXICITY OF MDA IN ANIMALS

Although application of a large dose of MDA to the skin of mice followed by daily treatment with croton oil has been reported to produce a high incidence of skin tumors, and daily application of MDA alone to produce internal tumors (7), chronic oral administration has failed to produce clear evidence of tumorigenesis (8,9). Nevertheless, administration of MDA as the Na enol salt in the drinking water at a level of 10 μg/g body weight/day for 12 mo significantly increased mortality from other causes (9). There was also a dose-dependent increase in hyperplastic and neoplastic changes in the liver from 0.1 to 10.0 μg/g/day (anisokaryosis, changes in cytoplasmic volume, architectural derangements, necrosis and neoplastic changes). Three animals (6%) given the highest dose developed the only stomach neoplasms observed. A further 22-mo study again yielded an increase in liver lesions but no increase in tumor incidence.

While these studies are reassuring with respect to the carcinogenicity of dietary MDA, the occurrence of hepatic nucleotoxicity in mice at an intake of free MDA as low as one μg/g/day is noteworthy. The results also do not necessarily reflect the carcinogenicity of MDA which may be formed in proximity to DNA in nuclear membranes.

MDA TOXICITY FOR CELLS GROWN IN CULTURE

Addition of MDA to the medium of rat skin fibroblasts grown in culture causes concentration-dependent abnormalities over a range of 10^{-6} M to 10^{-3} M (10) (Table 1). The lesions are predominantly nuclear: micro- and multinucleation, karyorrhexis, chromosomal fragments, chromatid breaks and increased DNA repair synthesis. Small and irregular nuclei are observed at 10^{-5} and 10^{-6} M MDA. The results imply that MDA formed in the nuclear

TABLE 1

Effects of Malondialdehyde on Rat Skin Fibroblasts Grown in Culture[a]

Conditions	Effects
10^{-3} M, 120 hr	Altered morphology, cytoplasmic vacuolization, karyorrhexis, micro- and multinucleation, < mitotic index, < DNA, RNA and protein synthesis
10^{-4} M, 12 hr	Chromosomal fragments, achromatic lesions, chromatid breaks
10^{-4} M, 120 hr	Mitotic aberrations, micronucleation, < mitotic index, < DNA synthesis
10^{-5} M, 10^{-6} M, 3 hr	> DNA repair synthesis
10^{-5} M, 10^{-6} M, 120 hr	Small and irregular nuclei

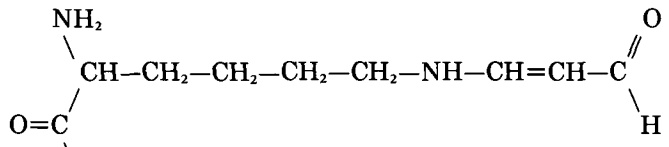
[a]Bird and Draper (10).

membranes of mammalian cells in vivo may be genotoxic. Studies on bacterial cells have demonstrated that MDA is a weak mutagen (11). Quantitative evaluation of MDA toxicity for cells grown in culture is complicated by its rapid binding to proteins and other ingredients of the medium.

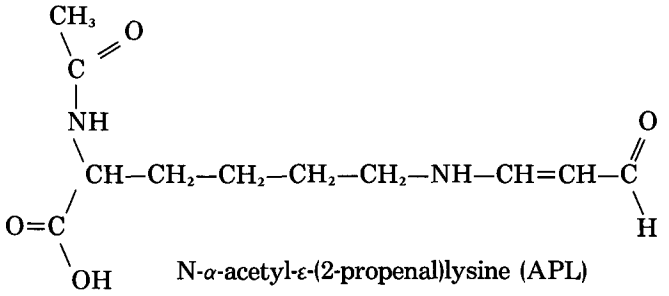
FORMS OF MDA IN THE DIET

A controversy which has persisted for many years over the specificity of the spectrophotometric method for the estimation of MDA in foods and other biological materials as the thiobarbituric acid (TBA) derivative (12) appears to have been satisfactorily resolved by the development of HPLC methods which clearly separate the TBA-MDA complex from other TBA reaction products. There remains a problem of determining the optimal conditions for release of MDA from its "bound forms," which differ from one material to another and require different conditions for hydrolysis. It is difficult, for example, to hydrolyze all the MDA bound to a meat sample without using strongly acidic conditions which endanger the stability of the TBA-MDA complex. Also, MDA may be formed from polyunsaturated fatty acids, endoperoxides and other compounds in the course of the TBA assay procedure. Thus, although MDA can be estimated satisfactorily as the TBA-MDA complex by HPLC, this method gives no indication of the forms in which MDA originally was present in the sample or how much may have been formed from precursors during the procedure.

To evaluate the possible pathogenicity of MDA in the diet, it is necessary first to determine the form(s) in which it occurs in foods and is absorbed from the gastrointestinal tract. Based on the observation that MDA reacts with the ε-amino group of terminal lysine residues in proteins (13), beef muscle protein was exposed to MDA in solution and then digested sequentially with pepsin and hog intestinal juice. Thin layer chromatography of the digesta revealed the presence of a TBA-reactive compound which was purified and shown to be the lysine-MDA adduct N-ε-(2-propenal)lysine (Scheme 1) (Piche, L. A., and Draper, H. H., unpublished results). If this adduct is the main form of dietary MDA released during digestion and



N-ε-(2-propenal)lysine



N-α-acetyl-ε-(2-propenal)lysine (APL)

SCHEME 1

absorbed into the blood stream, its metabolic fate, including the extent, if any, to which it undergoes scission to release free MDA, is of obvious toxicological interest.

OXIDATION OF MDA IN VITRO AND IN VIVO

MDA is rapidly oxidized by rat liver mitochondria through the action of the low specificity mitochondrial aldehyde dehydrogenase. In vitro experiments using [1,3-^{14}C]MDA yielded an apparent K_m and V_{max} of 0.5 mM and 9.3 nmol/min/mg protein for O_2 uptake, respectively, and 2.0 mM and 2.4 nmol/min/mg protein for $^{14}CO_2$ production (14). The biochemical sequence involves oxidation to malonic semialdehyde and decarboxylation to form acetaldehyde which is converted to acetate by the same dehydrogenase enzyme.

[1,3-^{14}C]MDA administered to rats by stomach intubation was extensively oxidized to $^{14}CO_2$, but at a slower initial rate than that for ^{14}C-acetate (14) (Table 2). About 65% of the ^{14}C administered was recovered in expired $^{14}CO_2$ within 12 hr, ca. 13% in the urine and 10% in the feces. The amounts of ^{14}C recovered in the urine and feces were greater than those for ^{14}C-acetate and were present in reaction products formed either in the intestinal lumen or in the tissues.

URINARY MDA AS AN INDICATOR OF LIPID PEROXIDATION IN THE DIET AND TISSUES

Based on reports of the presence of MDA in urine, confirmation of these reports by an HPLC procedure, and the finding of labeled metabolites in rat urine after oral administration of ^{14}C-MDA, the urinary excretion of MDA was evaluated as a possible reflection of lipid peroxidation in the diet and in the tissues (15).

MDA excretion was found to be responsive to its oral administration as the Na enol salt, ingestion of MDA-treated serum albumin and feeding a diet containing a highly peroxidizable oil (cod liver oil) (Table 3). Urinary MDA also increased in response to the increase in lipid peroxidation in vivo produced by vitamin E deficiency and administration of iron nitrilotriacetate. Administration of DPPD, a biologically active lipid antioxidant, but not BHA, a nonbiologically active antioxidant, reversed the increase in MDA excretion by vitamin E-deficient animals. However, urinary MDA was not responsive to the Se- and vitamin E-deficient hepatonecrogenic Torula yeast diet.

TABLE 2

Percent Recovery of Radioactivity from Rats 12 hr after Intubation with [1,3-^{14}C]MDA or [1,2-^{14}C]Acetate[a]

	^{14}C-MDA	^{14}C-Acetate
$^{14}CO_2$	75	65
Urine	2.5	13
Feces	1.5	10
Plasma	0.1	0.1
Total	79.1	88.1

[a]Siu and Draper (14).

TABLE 3

Influence of Various Conditions on Urinary Malondialdehyde in Rats[a]

Condition[b]	Urinary MDA μg/24 hr[c]
+E diet	1.2 ± 0.2
−E diet	3.8 ± 0.7
−E diet + 0.1% DPPD	1.2 ± 0.1
10% Coconut oil + 5% corn oil diet	1.1 ± 0.1
10% Corn oil + 5% cod liver oil diet	10.7 ± 1.8
Low PUFA diet + 48 hr fast	1.3 ± 0.2
High PUFA diet + 48 hr fast	2.9 ± 0.2
NTA injection	1.4 ± 0.2
FeNTA injection (9 mg Fe/Kg)	7.4 ± 0.8

[a]See ref. 15 for details.

[b]E, vitamin E; DPPD, N,N'-diphenyl-p-phenylene diamine; PUFA, polyunsaturated fatty acid; NTA, nitrilotriacetate; FeNTA, iron nitrilotriacetate.

[c]Mean ± SEM.

It is noteworthy that rats chronically fed a high PUFA diet exhibit an increase in urinary MDA when subsequently fasted or fed an MDA-free diet (Table 3), indicating that enrichment of the tissues with highly unsaturated fatty acids results in an increase in lipid peroxidation in vivo even in the presence of normal concentrations of vitamin E. Fasting for more than 24 hr also results in an increase in MDA excretion, implying that lipolysis is associated with peroxidation of the fatty acids released.

The diet appears to be the main source of urinary MDA under most conditions, and it is therefore necessary to employ fasting or an MDA-free diet to evaluate MDA excretion as an indicator of lipid peroxidation in vivo. While urinary MDA may be useful as an index of generalized lipid peroxidation in the tissues caused by such factors as vitamin E deficiency or iron administration, it is probably of little value in detecting peroxidative effects on specific target organs, such as the effect of adriamycin on cardiac tissue.

IDENTITY OF MDA COMPOUNDS IN URINE

Identification of the excretory forms of MDA is of interest as a possible clue to its reaction products in the diet and in the body. Ion exchange chromatography of rat urine revealed the presence of several compounds which yield MDA under the acidic conditions of the TBA reaction. There is a notable absence of free MDA. The main metabolite in the urine of rats fed Purina Chow® has been isolated and identified as N-α-acetyl-ε-(2-propenal)-lysine (APL) (Scheme 1) (McGirr, L. G., Hadley, M., and Draper, H. H., unpublished results). This compound appears to be derived mainly from MDA bound to the lysine residues of protein in the diet, from which it is released as a lysine-MDA adduct during digestion and acetylated prior to excretion in the urine. N-acetylation in the liver is a well-known detoxification reaction. Oral administration of lysine-MDA led to excretion of 28% of the dose in the urine as the acetylated derivative, indicating that only a portion of the adduct undergoes acetylation in the tissues.

The presence of APL in the urine of fasting animals and animals fed a saturated fat diet and its excretion in an increased amount after MDA injection indicates that it also is synthesized from MDA formed in vivo. However, its excretion does not increase following the intraperitoneal administration of iron nitrilotriacetate (Hadley, M., and Draper, H. H., unpublished data), despite the fact that there is a several-fold increase in total urinary MDA (Table 3). The varied responses in MDA excretion to different peroxidative stimuli, including the ability of CCl_4 to increase total MDA excretion and the failure of iron to increase APL excretion while increasing the excretion of other metabolites, is evidence for the presence of multiple pathways for the degradation of lipid peroxides. Identification of the MDA derivatives excreted in the urine under various conditions of peroxidative stress may provide a better understanding of the mechanisms and pathophysiology of lipid peroxidation in vivo.

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