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Volatile carbonyl levels in tissues of transgenic mice with nerve sheath tumors

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Abstract

Volatile carbonyl compounds in homogenates prepared from various tissues of tumor-bearing transgenic mice were determined. Formaldehyde and acetaldehyde were derivatized to thiazolidines. Malonaldehyde was derivatized to 1-methylpyrazole. The derivatives were quantified by gas chromatography with a highly sensitive and specific nitrogen–phosphorus detector. The limits of quantitation of formaldehyde and malonaldehyde were 2 μg/ml of homogenate and 27 ng/ml of homogenate, respectively. Levels of malonaldehyde in the erythrocytes and gastrocnemius of tumor-bearing transgenic mice were elevated as compared to the same tissue in control non-transgenic mice. Brain, liver, kidney, heart, and spleen tissues of the tumor-bearing mice exhibited decreased malonaldehyde levels. Similar results were obtained for formaldehyde and acetaldehyde.

1. Introduction

Numerous studies have shown that lipid peroxidation is significantly decreased in cancerous tissues as compared to similar non-cancerous tissues of animals and humans [1–5]. On the other hand, some studies reported that an increase in lipid oxidation levels of the host's non-cancerous tissue is frequently associated with a decrease in lipid oxidation in the cancerous tissue [6,7]. For example, a recent study showed an almost 6-fold increase in malonaldehyde levels in serum and plasma of patients with cancer as compared to healthy persons [8]. It is clear that the role of lipid peroxidation in the pathogenesis of cancer is complex. Secondary products of lipid peroxidation, in particular carbonyl compounds, may play a role in the promotional phase of carcinogenesis via interactions with DNA, cellular membranes and enzymes [9–12]. Lipid peroxidation and subsequent production of carbonyls also occurs in all cells as a function of normal cellular metabolism [6,13,14].

The individual carbonyl products of lipid peroxidation have potent biological consequences; however, the actual carbonyls produced by *in vivo* lipid peroxidation, their distribution and quantity during the disease process are unknown. This is due in part to the lack of specific analytical methods. HPLC methods

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which separate aldehydes derivatized with TBA or 2,4-dinitrophenylhydrazine have frequently been used, but they suffer from poor resolving power and/or low sensitivity [15]. Recently, gas chromatographic methods for the analysis of reactive carbonyls including formaldehyde, acrolein, malonaldehyde, and acetaldehyde have been developed [16]. These methods have been shown to be effective for quantifying carbonyls in a variety of fatty acid model systems [17], foods [18,19], and rat liver homogenates [20]. The distribution and concentration of the individual aldehydes in tumor-bearing and non-tumor-bearing mice can provide information on the role of these biologically active compounds in carcinogenesis.

In the present study, a specific and sensitive gas chromatographic method was applied for quantifying the individual carbonyl products of lipid peroxidation in tissues of control mice and mice with nerve sheath tumors. The overall aim of the present study was to utilize these analytical methods to better characterize the role of specific carbonyls and lipid peroxidation in carcinogenesis in a mouse model with well-characterized tumor kinetics.

2. Experimental

2.1. Reagents

Thiazolidine, 2,4,5-trimethylthiazole, 2-methylpyrazine, and cysteamine were obtained from Aldrich (Milwaukee, WI, USA). Reagent grade chloroform (containing 0.75% ethanol) and dichloromethane were purchased from J.T. Baker (Phillipsburg, NJ, USA). Ammonium sulfate, sodium chloride, and pesticide grade methanol were from Fisher Scientific (Fair Lawn, NJ, USA). N-Methylhydrazine (NMH, 98% pure) was obtained from Fluka (Ronkonkoma, NY, USA). Formaldehyde (37%), 2-thiobarbituric acid, and butylated hydroxytoluene (BHT) were obtained from Sigma (St. Louis, MO, USA). Protein dye (Coomassie Blue) was purchased from Bio-Rad Labs (Richmond, CA, USA). Solid-phase extraction (SPE) cartridges (6 ml, 0.5 g resin, C_{18} bonded phase) were bought from Varian Sample Preparation Products (Harbor City, CA, USA). Sodium salt of malonaldehyde (MA) was prepared according to the established method [21]. 1-Methylpyrazole (1-MP) was synthesized according to the method reported previously [22].

2.2. Sample preparations from transgenic mice

Transgenic mice used in the present study were derived from mice carrying the human T-lymphotrophic virus Type 1 (HTLV-1) [23] and were maintained as breeding colonies at the University of California-Davis (Davis, CA, USA).

Male and female mice were housed individually in wire-bottom cages in a controlled environment at 23°C and 95% relative humidity. The light was on at 7:00 am and off at 10:00 pm. The mice were fed a cereal-based, closed-formula diet (Mouse Chow, Ralston Purina, St. Louis, MO, USA) and ranged in age from 126 to 206 days at the time of analysis. The blood was collected in a sterile, 3.5-ml tube containing 0.06 ml of 7.5% K_3-EDTA solution (4.5 mg K_3-EDTA). Plasma and red blood cells (RBC) were separated following centrifugation (1000 *g*, 5 min, 4°C) and immediately frozen on dry ice. Tissues of muscle, testes, brain, tumor, liver, kidney, heart, and spleen were removed, weighed, and immediately frozen on dry ice. The tissues were stored at − 80°C until analysis. Tumor burden is expressed as tumor mass/body weight times 100.

2.3. Tissue homogenates preparation

Tissues were weighed and 100 μl of ice cold 0.2% BHT in ethanol was added to each sample. Ice cold 1% NaCl was added and the mixture homogenized for approximately 30 s (Polytron Homogenizer, Brinkman Instruments, Westbury, NY, USA) to yield a 33% homogenate (w/w). Two aliquots, a 10-μl and a 100-μl, were removed for protein and thiobarbituric acid (TBA) assay, respectively. The TBA aliquot was immediately frozen on dry ice and stored at − 80°C

overnight before analysis. The remaining homogenate was weighed and divided into two equal portions by weight. For liver tissue, the amount of homogenate used for each analysis was limited to 0.5 g to avoid overloading the SPE cartridge. Tissues were held in an ice bath throughout preparation to minimize oxidation.

2.4. Malonaldehyde analysis

MA in the samples was analyzed according to the method reported previously [20] with a slight modification. Tissue homogenates (33%, w/w) were stirred with 2 ml of 3 *M* HCl at room temperature for approximately 10 min. After 25 μl of NMH was added, the mixture was covered with parafilm and heated at 50–55°C for 15 min. Following the heat treatment, the samples were rapidly cooled to room temperature in an ice bath. An additional 75 μl of NMH was added, the pH of the sample adjusted to 7–8 with NaOH, and the mixture was covered and allowed to react, with stirring, for an additional 45 min at room temperature. Proteins and other particulates were removed by centrifugation at 4400 *g* for 20 min (10–15°C). The resulting pellet was resuspended in 1 ml of 1% NaCl and recentrifuged using the same conditions. The supernatants were combined and extracted using C_{18} SPE cartridges. The SPE cartridges were conditioned with dichloromethane, methanol, and water, respectively, prior to addition of the sample mixture. MA, as the 1-MP, was eluted with approximately 3 ml of dichloromethane. The dichloromethane eluate was adjusted to 3.0 ml and a 50 μl dichloromethane solution of the 2-methylpyrazine (50 μg/ml) was added as a gas chromatographic internal standard. The sample was analyzed by a Hewlett-Packard (HP) 5890 Series II gas chromatograph (Avondale, PA, USA) equipped with a nitrogen–phosphorus detector (NPD) and a 30 m × 0.25 mm I.D. ($d_f = 0.25$ μm) DB-WAX bonded phase fused-silica capillary column (J and W Scientific, Folsom, CA, USA). The detector and injector were operated at 220°C and 200°C, respectively. The linear velocity of helium carrier gas was 33 cm/s with a split ratio of 20:1. The oven temperature was held at 60°C for 2 min, programmed to 90°C at a rate of 4°C/min, and then programmed from 90°C to 180°C at 10°C/min with a 10 min hold at 190°C. Peak areas were integrated on an HP 3396 Series II reporting integrator.

The GC calibration curve for 1-MP analysis was prepared according to the method reported previously [18]. The 1-MP has been previously characterized by GC–MS [17].

2.5. Extraction efficiency test on 1-MP from tissues

Tissue homogenate samples were divided into two equal aliquots by weight. One aliquot was spiked with 300 μl of an aqueous 1-MP standard (100 nmol/ml) and the other aliquot was analyzed without spiking. Spiked reagent blanks, prepared in triplicate, were prepared by spiking 300 μl of aqueous 1-MP (100 nmol/ml) into a solution containing 1 ml of 1% NaCl and 100 μl of 0.2% BHT. 1-MP in the samples was analyzed by GC as described above. Background values in the unspiked samples were subtracted from the spiked homogenate samples to give corrected peak-area ratios. Extraction efficiency was defined as the ratio of the corrected spiked homogenate concentration to the mean spiked reagent blank concentration.

2.6. Recovery efficiency test on MA from tissues

Tissue homogenates (33%, w/w) were divided into two equal portions by weight. One portion of the homogenate was spiked with 300 μl of an aqueous MA standard (100 nmol MA sodium salt/ml) and the other was analyzed without spiking. Spiked reagent blanks were prepared, in triplicate, by spiking 300 μl of the aqueous MA into a solution containing 1 ml of 1% NaCl and 100 μl of 0.2% BHT. The samples were analyzed for 1-MP by GC with a NPD. Recovery efficiency was defined as the ratio of the corrected spiked homogenate concentration to the mean spiked reagent blank concentration.

2.7. Analysis of saturated aldehydes

Tissue homogenates and reagent blanks (containing 1 ml of 1% NaCl and 100 μl of 0.2% BHT) were stirred with 2 ml of 1 *M* NaOH at room temperature for *ca.* 10 min. After 1 ml of aqueous cysteamine (0.03 *M*, pH 8.5) was added, the mixture was covered with parafilm and heated at 50–55°C for 15 min. Following the heat treatment, the samples were rapidly cooled in an ice bath and allowed to react for an additional 45 min at room temperature while stirring. Each sample was adjusted to pH 8.3–8.5 with the addition of 2 ml of saturated $(NH_4)_2SO_4$. Proteins and other particulates were removed by centrifugation at 4400 *g* for 20 min at 10–15°C. The resulting pellet was resuspended in 1 ml of 1% NaCl and then recentrifuged using the same conditions. The supernatants were combined and extracted two times by adding 1.5 ml of chloroform, vortex-mixing, centrifuging, and removing the lower organic layer. The chloroform extracts were combined, brought to 3.0 ml, and 15 μl of 2,4,5-trimethylthiazole (5 mg/ml) was added as GC internal standard. Thiazolidine derivatives were analyzed using an HP 5890 GC (Hewlett-Packard) equipped with a NPD and a 30 m × 0.25 mm I.D. ($d_f = 0.25$ μm) DB-1 bonded phase fused-silica capillary column (J and W Scientific) according to the method reported previously [24]. The detector and injector were operated at 250°C and 260°C, respectively. The linear velocity of helium carrier gas was 30 cm/s with a split ratio of 33:1. The oven temperature was held at 80°C for 1 min, programmed to 115°C at 4.5°C/min, and then programmed from 115°C to 180°C at 15°C/min with a 10 min hold at 190°C. Peak areas were integrated on a Spectra-Physics SP4290 reporting integrator (Spectra-Physics, San Jose, CA, USA). The small amount of acetaldehyde or 2-methylthiazolidine that was present as a contaminant in reagent blanks was subtracted from the tissue values.

The physical properties and spectral data of the thiazolidine derivatives were reported previously [25].

2.8. Extraction efficiency test on thiazolidine from tissues

Preparation of tissue homogenates (33%, w/w) is described above. Following homogenization, the samples were divided into two equal aliquots by weight. One aliquot of the homogenate was spiked with 300 μl of an aqueous thiazolidine standard (100 mg/ml) and the other aliquot was analyzed without spiking. Spiked reagent blanks, prepared in triplicate, were prepared by spiking 300 μl of aqueous thiazolidine (100 μg/ml) into a solution containing 1 ml of 1% NaCl and 100 μl of 0.2% BHT. Samples were prepared and analyzed by GC as described above.

2.9. Recovery efficiency of formaldehyde from tissues

Tissue homogenates (33%, w/w) were divided into two equal portions by weight. One portion was spiked with 300 μl of an aqueous formaldehyde standard (34 mg/ml) and the other was analyzed without spiking. Spiked reagent blanks were prepared, in triplicate, by spiking 30 ml of the aqueous formaldehyde into a solution containing 1 ml of 1% NaCl and 100 μl of 0.2% BHT. Samples were prepared and analyzed by GC as described above.

2.10. Protein analysis

The Coomassie Blue dye-binding assay (Bio-Rad Labs) was used to determine tissue protein concentrations. A 100-μl aliquot of appropriately diluted tissue homogenate was added to the dye reagent, mixed, and absorbance at 594 nm measured *versus* a reagent blank. A standard curve using bovine serum albumin was used to calculate protein concentration.

2.11. Thiobarbituric acid assay

The TBA assay was performed using a modification of the method reported previously [26]. A 100-μl aliquot of a 33% tissue homogenate

was mixed with 3 ml of 1% phosphoric acid and 1 ml of 0.6% TBA. The mixture was stoppered, heated in a boiling water bath for 45 min, cooled on ice, and extracted with 4 ml of *n*-butanol. Absorbance of the organic layer at 520 and 534 nm was measured *versus* a reagent blank. Standards, ranging in concentration from 0.08 to 8 nmol MDA, were prepared using MA sodium salt. A standard curve was obtained using the difference in absorbance at 534 and 520 nm and sample TBA reactive substances (TBARS) concentrations calculated correspondingly.

2.12. Statistical analysis

Statistical significance of the mean differences in tissue aldehyde levels, TBARS, and protein concentrations between the control and tumor mice were evaluated by an unpaired two-tailed Student's *t*-test using the StatView software package (Abacus Concepts, Berkeley, CA, USA) on a Macintosh IIci computer. Linear regression equations were developed to describe the relationship between tissue aldehyde levels (*y*-variable) and tumor burden (*x*-variable).

3. Results and discussion

MA was easily separated and quantitated in the tissues of liver, kidney, spleen, muscle, brain, heart, plasma, RBC, tumor, and testis in the present study (Fig. 1A). The limit concentration of 1-MP for quantitation in the present study was 46 ng/ml of homogenate, equivalent to 27 ng/ml. Its detection limit was previously reported as 8.9 pg, equivalent to 7.8 pg MA [20].

In biological systems, carbonyls frequently exist bound to cellular nucleophiles such as proteins. The extraction efficiency of the 1-MP in biological tissues ranged from 60.6 ± 15.2% to 88.4 ± 1.5% while that for MA ranged from 55.5 ± 7.0% to 88.9 ± 9.6% (Table 1). Recovery of MA in the liver tissue was maximized by limiting the tissue mass loaded onto the SPE cartridge to 0.5 g.

Although formaldehyde and acetaldehyde are known to have potent biological and toxicological consequences, their formation *in vivo* has not been adequately explored due to their high volatility and reactivity. In the present study, formaldehyde and acetaldehyde were identified and quantified satisfactorily in biological tissues as their thiazolidine derivatives (Fig. 1B). Liver was the only tissue that contained consistently measurable amounts of formaldehyde; however, low levels were occasionally observed in kidney,

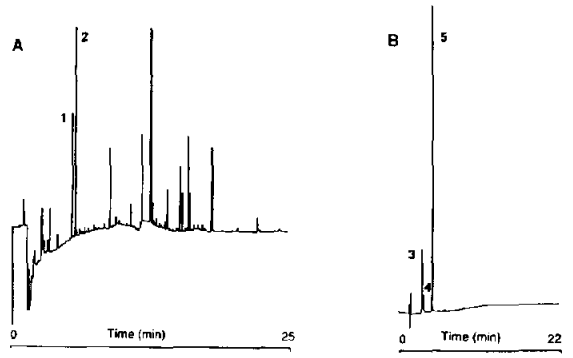

Fig. 1. Gas chromatograms of (A) derivatized MA and (B) derivatized saturated aldehydes from mouse muscle and liver tissues, respectively. Gas chromatographic conditions described in text. Peaks: 1 = 1-MP; 2 = 2-methylpyrazine (I.S.); 3 = thiazolidine; 4 = 2-methylthiazolidine; 5 = 2,4,5-trimethylthiazole (I.S.).

Table 1
Recovery of malonaldehyde from biological tissues: derivatization and extraction efficiency

Tissue	Spiked 1-MP extraction recovery (%)	Spiked MA extraction and derivatization recovery (%)
Spleen	80.2 ± 2.8	88.6 ± 11.5
Brain	74.0 ± 3.9	67.2 ± 5.0
Tumor	71.6[a]	82.1 ± 4.6
Kidney	88.4 ± 1.5	55.5 ± 7.0
Liver	60.6 ± 15.2	71.0 ± 1.9
RBC	81.7 ± 2.4	75.9 ± 11.6
Plasma	82.9 ± 2.0	87.3 ± 4.4
Muscle	85.3 ± 0.9	85.6 ± 4.7
Heart	74.6 ± 4.4	88.9 ± 9.6

See Experimental section for details on procedures. Values are means ± S.D. of $n = 3$ except for [a] where $n = 1$.

RBC, muscle, heart, and brain tissues. The limit of quantitation for the present study was 2 μg thiazolidine/ml, equivalent to 0.67 μg formaldehyde/ml. A detection limit of formaldehyde previously reported using this derivatization procedure was 5.8 pg [24]. All tissue samples were corrected for the small amount of acetaldehyde or 2-methylthiazolidine (0–2.6 mg/ml) that was present as a contaminant in reagent blanks. Basic hydrolysis conditions were adapted for the analysis of bound formaldehyde and acetaldehyde in biological tissues [24]. Using these conditions, in conjunction with a simple liquid–liquid continuous extraction of the thiazolidine derivative into chloroform, thiazolidine extraction efficiencies ranged from 65.2 ± 6.6% to 97.9 ± 8.4% for liver and heart tissues, respectively (Table 2). Recovery efficiencies for spiked formaldehyde ranged from 85.0 ± 19.3% to 113.9 ± 8.0% in kidney and brain tissues, respectively. Thus, the method provides an accurate procedure for quantification of formaldehyde and acetaldehyde in the tissues studied.

The transgenic mice employed in the present study provided an opportunity to study the cancer process in a well-defined animal system without the use of exogenous chemicals to induce tumor formation. In addition, control animals could be easily matched for age and sex using non-transgenic siblings. The nerve sheath tumors that develop in the transgenic mice are similar to those that occur in human neurofibromatosis and provide an excellent model for studying this disease [27]. While many previous studies have examined lipid peroxidation in hepatomas, little emphasis has been placed on other cancers, especially those of the nervous system.

Table 2
Recovery of formaldehyde from biological tissues: derivatization and extraction efficiency

Tissue	Spiked thiazolidine extraction recovery (%)	Spiked formaldehyde extraction and derivatization recovery (%)
Spleen	96.1 ± 0.8	112.7 ± 7.7
Brain	77.7 ± 10.4	113.9 ± 8.0
Tumor	86.5[a]	
Kidney	72.7 ± 13.44	85.0 ± 19.3
Liver	65.2 ± 5.6	102.9 ± 18.2
RBC	90.0 ± 6.4	92.6 ± 4.0
Plasma	82.3 ± 3.6	103.9 ± 11.7
Muscle	85.6 ± 7.2	105.5 ± 8.1
Heart	97.9 ± 8.4	104.9 ± 12.5
Testis	79.2 ± 1.4[b]	104.8 ± 12.0

See Experimantal section for details on procedures. Values are means ± S.D. of $n = 3$ except for [a] where $n = 1$ and [b] where $n = 2$.

MA levels in control mice varied widely with tissue type, with plasma having the lowest levels and spleen tissue having the highest levels of MA per gram of tissue (Fig. 2A). Previous studies have reported that rapidly dividing tissue, such as testis, has lower levels of lipid peroxidation products than the more slowly dividing brain tissue [28–30]. However, the specific measurement of MA did not reflect this difference (Fig. 2A). The method used in this study, while specifically measuring MA levels, also reflects actual *in vivo* carbonyl levels at the time of analysis rather than susceptibility to lipid peroxidation under oxidative stress conditions *in vitro* as is commonly measured. These distinctions may provide for a more accurate estimation of the biological effects of lipid peroxidation *in vivo*.

When MA levels in tissues of tumor-bearing and control mice are compared, the effect is again dependent on tissue type (Fig. 2A). Elevated levels of MA were observed in the RBC, muscle, and testis tissue of tumor-bearing mice. Brain, liver, kidney, heart, and spleen tissues of the tumor-bearing mice exhibited decreased MA levels as compared to the corresponding tissue of control non-tumor-bearing mice. While the elevated levels of MA in the RBC, muscle, and testis are in agreement with reports of elevated levels of lipid oxidation in non-tumor tissues of the host animal [6–8], the decreased levels in brain, liver, kidney, heart, and spleen contrast with these reports. In all cases, the animals in the present study were examined after the development of visible tumors comprising approximately 0.5–9% of the total body weight. The extent of *in vivo* lipid peroxidation may be

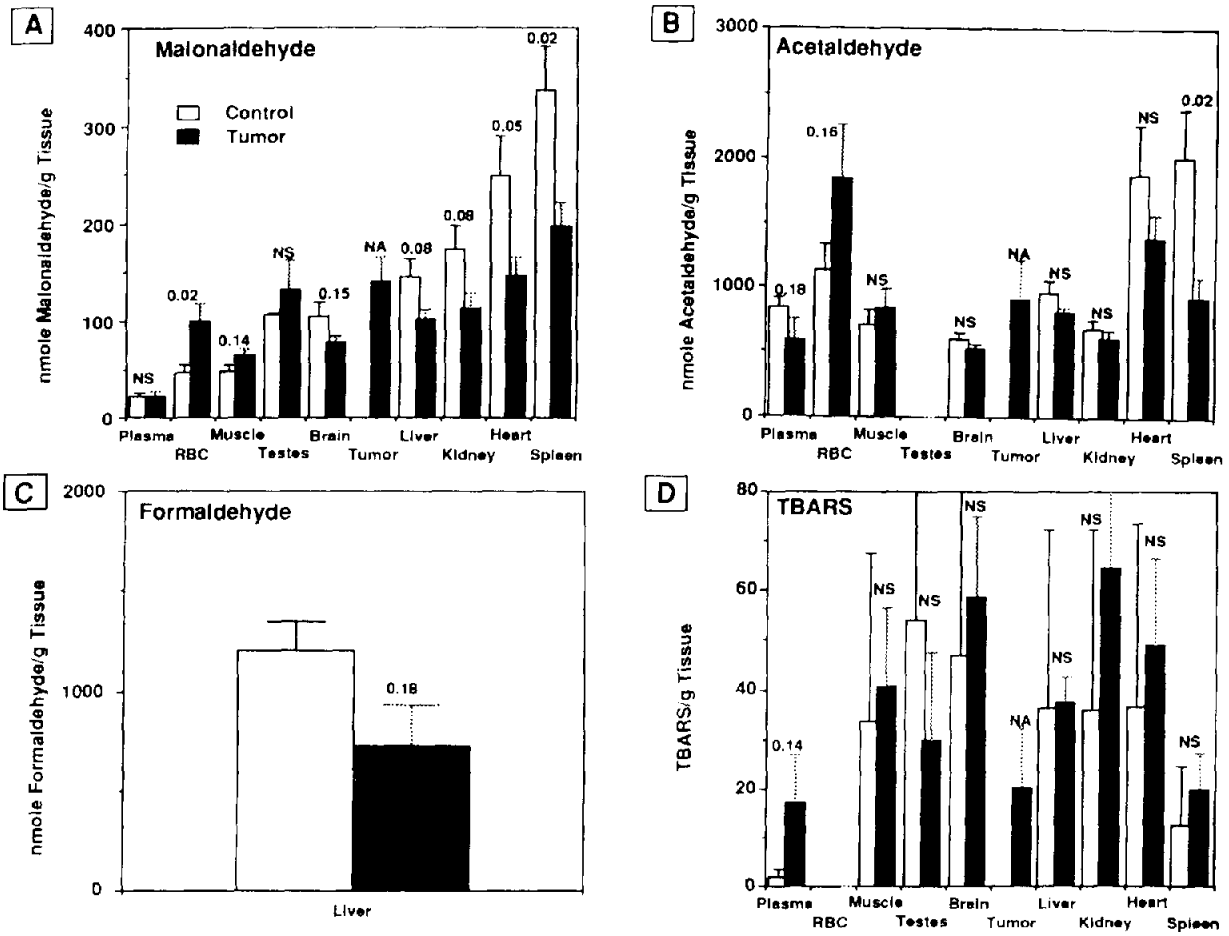

Fig. 2. Aldehyde and TBARS levels in tissues from control and tumor-bearing transgenic mice. Values plotted are means ± S.E.M. for $n = 6$ (4 females and 2 males per treatment group). Significance levels for unpaired Student's t-statistic are indicated. N.S. = $p > 0.2$, NA = not applicable. See text for details.

dependent on the time course of the disease process [6]. In addition, results from the present study may indicate that blood and muscle tissues undergo lipid oxidation preferentially in order to protect the tumor tissues and other large organs such as brain, liver, kidney, heart, and spleen. Similarly, the blood and muscle tissues may provide a mechanism for removal of the potentially toxic MA from the other vital organs.

In general, acetaldehyde levels were higher than MA levels in the control tissues (Fig. 2B). The lowest acetaldehyde levels were detected in the testis tissue and were below the limit of quantitation. This may reflect the low level of lipid peroxidation previously reported in the literature for rapidly dividing tissues.

Comparison of acetaldehyde levels between control and tumor-bearing animals showed trends similar to those observed for MA (Fig. 2B): elevated acetaldehyde levels were observed in RBC and muscle tissues of tumor-bearing animals, while brain, liver, kidney, heart and spleen tissues exhibited decreased acetaldehyde levels. Formaldehyde was similarly decreased in the liver tissue of tumor-bearing animals (Fig. 2C).

Because the TBA test has been the most commonly employed method for measuring lipid oxidation, the above results were compared with measurements of TBARS in the same tissues (Fig. 2D). A comparison of rapidly dividing testis tissue with more slowly dividing brain tissue shows little difference in TBARS; however, tumor tissue has one of the lowest TBARS levels, *e.g.* lower than corresponding control brain tissue levels. While central nervous system tissue and peripheral nervous system tissues are different, these results appear to agree with previous findings of decreased levels of lipid oxidation in tumor tissue. Except for testis tissue, TBARS were elevated in all other tissues of tumor-bearing mice compared to controls. These results are consistent with previous literature reports of elevated lipid peroxidation in non-tumor tissues of the host animal but are in sharp contrast with the variable levels of specific aldehyde products, such as MA, acetaldehyde, and formaldehyde, observed in this study.

Matrix components present in the tissues which have strong absorptions near the absorbance maximum of the TBA–MA complex can interfere with quantification by the TBA method. Using the difference in absorption at 545 and 532 nm minimized these interferences [26]; however, the interference was still strong enough to prevent accurate measurement of TBARS in red blood cells. Use of capillary GC to separate interfering components in conjunction with specific detectors to eliminate interferences is a major advantage of the derivatization procedures presented in this study, allowing accurate determination of specific carbonyls in all tissue types.

Body weights, hematocrit, and tissue protein levels were not different for the two treatment groups, except in the case of heart tissue where the tumor-bearing animals exhibited lower levels of protein in the heart tissue than control animals ($p < 0.05$). Therefore, alterations in lipid peroxidation observed in the present study did not appear to be related to reduced dietary intake.

In the present study, spleen tissue weights of tumor-bearing and control mice were significantly different ($p = 0.0096$) with mean values of 0.40 ± 0.02 g and 0.10 ± 0.01 g, respectively. There were no significant differences in the weights of the other tissues studied. Because spleen weight accounts for a larger percentage of total body weight in tumor-bearing animals, MA and acetaldehyde levels per gram spleen tissue were multiplied by the ratio of spleen weight to body weight for each mouse. The resulting value allows comparison of the total amount of aldehyde per gram body weight (Table 3) and indicates that tumor-bearing mice have significantly elevated total body levels of MA and acetaldehyde (Table 3).

The relationship between tumor burden or tumor growth and aldehyde levels may provide valuable predictive or diagnostic potential in monitoring the cancer process. MA and acetaldehyde levels were not related to tumor burden in any of the tissues studied; however, formalde-

Table 3
MA and acetaldehyde levels in spleen of control and tumor bearing mice expressed relative to total spleen and body weights

Compound	Sample	Level (mean ± S.E.M., $n = 6$) (nmol/g body weight)	T-statistics p-value
MA	Control	0.63 ± 0.17	0.002
	Tumor	2.97 ± 0.54	
Acetaldehyde	Control	6.56 ± 0.97	0.007
	Tumor	12.51 ± 1.18	

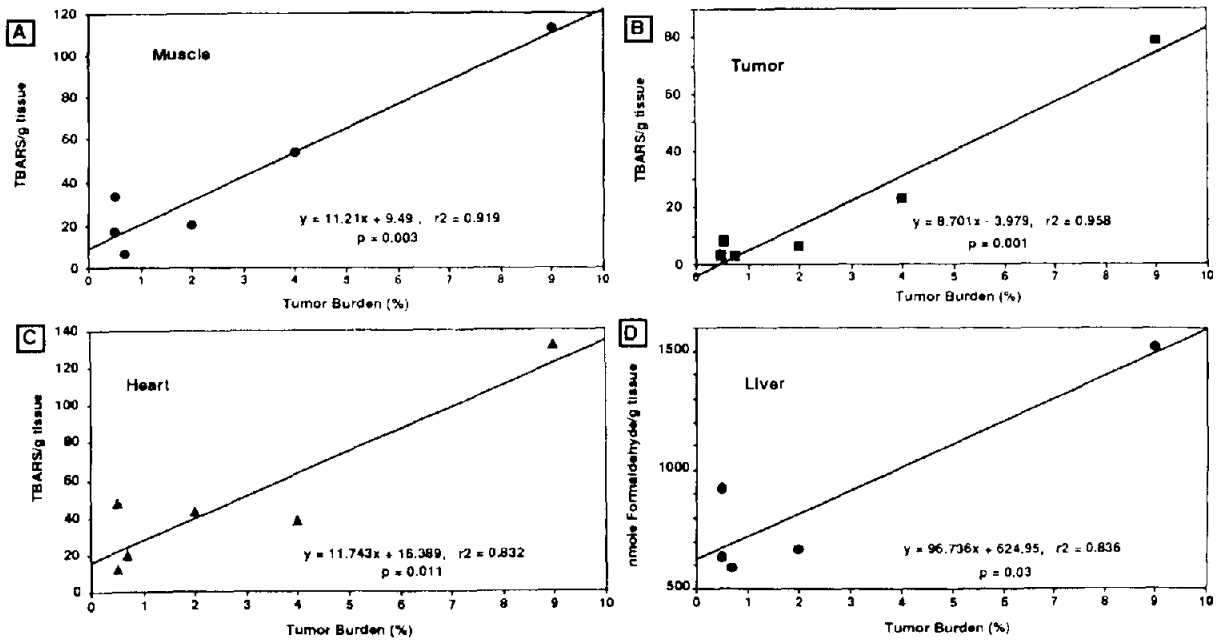

Fig. 3. Correlation between TBARS or aldehyde levels and tumor burden in selected tissues from tumor-bearing transgenic mice ($n = 6$; 4 females, 2 males).

hyde levels in liver tissue and TBARS levels in tumor, heart, and muscle tissue were significantly correlated with tumor burden (Fig. 3). Therefore, in these tissues, formaldehyde and TBARS may serve as biomarkers for monitoring the growth of the tumor.

Results from this study do not indicate whether the observed differences in carbonyl tissue levels in tumor-bearing mice are related to changes in peroxidation substrates, alterations in rate of aldehyde removal from the tissue, or modifications in antioxidant status which can increase or decrease tissue susceptibility to lipid peroxidation. However, the presence of elevated levels of MA and acetaldehyde in RBC and muscle tissues in tumor-bearing animals may indicate that these tissues are being preferentially oxidized to protect the tumor tissues and other large organs such as brain, liver, kidney, heart, and spleen. Similarly, the blood and muscle tissues may provide a mechanism for removal of the potentially toxic aldehydes from the other organs.

4. Conclusions

Tissue levels of MA, acetaldehyde, and formaldehyde were specifically and accurately quantified as stable derivatives by GC with a NPD. Their levels increased in RBC and muscle tissues of tumor-bearing mice while levels in brain, liver, kidney, heart, and spleen tissue decreased as compared to non-tumor-bearing controls. This contrasted with an almost uniform elevation of TBARS in all tissues of tumor-bearing animals. These results emphasize the non-specificity of the TBA method and the need for estimates of individual carbonyl levels which may have important biological activity. Results from this study do not prove or disprove any hypothesis regarding a role for individual carbonyls in tumor promotion or the regulation of cell growth. However, they emphasize the need for understanding the role that specific aldehydes may have in the regulation of cell growth and the cancer process. The analytical methods and the transgenic animal model described in this paper

provide the opportunity to evaluate these relationships.

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