PROTECTION FROM GLUTATHIONE DEPLETION BY A GLYCONUTRITIONAL MIXTURE OF SACCHARIDES

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ABSTRACT

A complex glyconutritional (GN) mixture of mono-, di- and polysaccharides was investigated to assess its capacity to protect two different types of rodent cells, rat hepatocytes and mouse splenocytes, from depletion of glutathione by a sulfhydryl-reactive mycotoxin, patulin, or by coxsackievirus B3 (CVB3) infection, respectively. Rat hepatocytes were treated with the GN mixture *in vitro* or received carrier medium only prior to treatment with patulin. When treated with the GN mixture prior to patulin exposure hepatocytes demonstrated protection against depletion of intracellular reduced glutathione (GSH). Cells treated with the GN for up to 15 hours prior to patulin exposure showed no increase in protection of GSH above that demonstrated by cells treated for 3 hours. Mice were infected with CVB3 and one treatment group was injected intraperitoneally with the GN once a week. Animals were splenectomized each month over a ten month treatment for analysis of spleen monocytic cells. Splenocytes from mice treated with the GN mixture did not show the virally-associated depletion of intracellular GSH or damage to pancreatic acini observed in CVB3 inoculated but non-GN-treated mice. Animals from which spleen cells were taken for analysis showed no decrease in anti-CVB3 antibodies and no decrease in viral titers to accompany or explain the normal levels of intracellular GSH. These data strongly suggest that a complex mixture of exogenous saccharides exerts a protective effect on liver cells *in vitro* in that the cells are protected from chemically initiated depletion of intracellular GSH, and on spleen cells *in vivo* in that the cells are protected against a CVB3-initiated decrease in intracellular GSH and increase in pancreatic acini damage.

INTRODUCTION

Free radical-initiated damage to cell membranes and macromolecules was initially proposed by Harman (1956)

to be involved in the aging process and has been implicated as a pathophysiological mechanism in a variety of human diseases (Maxwell, 1995; Stohs 1995). For centuries dietary intake of fresh fruits and vegetables has been known to be a necessary part of a healthy lifestyle. One factor in fruits and vegetables, the naturally occurring anti-oxidant, vitamin C, was identified some 70 years ago as being essential to good health. Modern findings have shown that dietary supplementation with a variety of antioxidants, including α -lipoic acid (Low et al., 1997), and vitamins C and E (Stahl and Sies, 1997; vanDam et al., 1998), is important to prevent or retard the onset of a group of diseases with origins in the interaction of oxidative radicals and other reactive chemicals with cellular components. These diseases typically exhibit an increased incidence in aged human populations, and include atherosclerosis, bursitis, transplant rejection phenomena, diabetes, some types of cancer, Parkinson's disease, hypertension, trauma, bacterial sepsis, hypoxia, stroke, rheumatoid arthritis, inflammatory bowel disease, cataract, senile macular degeneration, and chronic obstructive pulmonary disease (Maxwell, 1995). In some instances these disorders have been difficult to characterize as to whether the disease initiated elevated levels of free radicals resulting in damage to macromolecules, or oxidative damage to macromolecules by reactive chemicals resulted in the onset of disease.

Incomplete reduction of oxygen during aerobic metabolism leads to the production of reactive oxygen species (ROS), including superoxide radicals ($O_2^{\cdot-}$) which interact with transition metal ions to produce reactive hydroxyl radicals ($\cdot OH$) and H_2O_2 (Halliwell and Gutteridge, 1985; Pacifici and Davis, 1991; Pacifici and Davis, 1991; Maxwell, 1995). ROS are inherently highly reactive, initiating spontaneous interactions with other charged molecules. Sources of naturally occurring ROS include immune system cells, such as phagocytic monocytes and neutrophils, involved in inflammatory processes. These cells produce reactive chemicals during the oxidative burst essential to mounting an immune attack against bacteria and viruses. Microbes may be effectively killed by the reactive molecules; however,

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other cells and tissues are damaged by them as well, resulting in the types of injury typically observed in severe inflammatory episodes.

Intracellular antioxidants and antioxidative enzyme systems effectively inactivate reactive chemicals and inhibit their damage to target molecules. Some physiological antioxidants that occur naturally and/or may be ingested as dietary components include vitamins C and E, β -carotene, bilirubin, serum proteins with sulfhydryl groups, ubiquinol, flavonoids, uric acid, salicylates, selenium, mannitol, dimethylthiourea, α -lipoic acid, and specific steroids (Maxwell, 1995). Cellular enzyme systems that counter the effects of oxidative radicals, and that decline in function in some disease states or with increased age, include superoxide dismutase (SOD), catalase, GSH synthetase and GSH peroxidase (Cand and Verdeti, 1989; Hussain et al., 1995). Of these, the antioxidant enzyme system GSH peroxidase differs in function from other scavenging antioxidants in that it catalyzes the reduction of oxidants utilizing the sulfhydryl-containing protein as the reducing equivalent donor, resulting in GSH oxidation [$2\text{GSH} + \text{GSH peroxidase} + \text{H}_2\text{O}_2 \rightarrow \text{GS-SG} + 2\text{H}_2\text{O}$]. Cellular functions initiating the synthesis of GSH, reducing oxidized glutathione (GS-SG), or sparing GSH oxidation may significantly increase the protection of cells from damage in an environment rich in reactive chemicals. As long as the target cell has adequate endogenous mechanisms to eliminate reactive chemicals cellular macromolecules are damaged to a minimal degree (Burkart et al., 1995). However, when intracellular antioxidants, such as vitamins C and E, which function coordinately to reduce oxidized GSH, are depleted, or when endogenous antioxidant systems such as SOD, catalase, GSH transferase and GSH peroxidase are depleted or fail to function properly, damage to macromolecules increases dramatically (Ames, 1989; Low et al., 1997).

The mycotoxin, patulin, was used to deplete GSH because it is thought to cause oxidative injury perturbing a number of cellular processes through modification of protein and non-protein sulfhydryl groups (Phillips and Hayes, 1978; Hinton et al., 1989; Riley and Showker, 1991). GSH depletion appears to be a very early event, if not the initial event, in the chronology of cell injury cause by patulin (Barhoumi and Burghardt, 1996). In this study we evaluate the effectiveness of treating cells or intact animals with a complex nutritional mixture of mono-, di- and polysaccharides (Ambrotose®) as a mechanism to protect cells, both *in vitro* and *in vivo*, from GSH depletion. We discuss some of the similarities between oxidation-associated pathologies of aging and insulin dependent diabetes mellitus. Future studies will further evaluate the ingestion or injection of this carbohydrate mixture as a mechanism to protect cells *in vivo* from oxidative damage.

MATERIALS AND METHODS

Culture media, Dulbecco's phosphate buffered saline (PBS), serum, patulin, and all general chemical reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Tissue culture flasks were obtained from Corning (Oneonta, NY) and LabTek Coverglass chamber slides were purchased from Nunc, Inc. (Naperville, IL). Monochlorobimane (mBCI) was purchased from Molecular Probes, Inc. (Eugene, OR). A proprietary gluconutritional (GN) mixture of mono-, di- and polysaccharides, Ambrotose®, was obtained as a gift from Mannatech, Inc. (Coppell, TX). A stock solution of 100 mM patulin was prepared in dimethylsulfoxide (DMSO), and stored at -20°C until used at a final concentration of 40 μM . For use in cell cultures, patulin was dissolved in Ham's F-12 medium for serial dilution (1.0-1000 μM , <1.0% DMSO). Stock mBCI was prepared in ethanol (50 mM) and diluted to 50 μM in medium (0.1% ethanol).

Clone 9 (ATCC, CRL 1439, passage 17) normal rat liver cells were used between passages 25-35. Cells were grown in Ham's F-12 Medium with 10% fetal bovine serum and plated onto 2 well Lab-Tek Chambered Coverglass slides (Nunc, Inc., Naperville, IL) at a density of 25,000 cells/cm² 48 hr prior to laser cytometry. During incubations with patulin, mBCI, and/or the GN, Ambrotose®, cells were kept in medium without serum or phenol red.

Cellular GSH levels were evaluated with mBCI using a laser scanning Meridian Ultima confocal microscope (Meridian Instruments, Inc., Okemos, MI). The cell-permeant mBCI probe is non-fluorescent, but forms a fluorescent conjugate with GSH in a reaction catalyzed by glutathione S-transferase (Rice et al., 1986; Shrieve et al., 1988). The Meridian Ultima was used at an excitation wavelength of 351-363 nm, and emitted fluorescence at 461 nm was detected using a barrier filter (BP 485/45). Excitation and detection parameters were kept constant for all GSH experiments. For image capture, the laser-excited fluorescence in cells was determined from a two-dimensional raster pattern (scan parameters were optimized for maximum detection of fluorescence with minimum cellular photobleaching). Kinetic analysis of mBCI-GSH conjugation was performed at room temperature in the presence or absence of the GN on the Ultima stage by recording changes in fluorescence intensity at 15 sec intervals for approximately 15 min (Barhoumi et al., 1995).

Both long term and transient effects of the GN on GSH levels were evaluated. In the initial *in vitro* study one group of rat liver cells was incubated for 3 and 15 hr in medium without serum or phenol red. Another set of cells was incubated for 3 and 15 hr in the absence of the GN in medium without serum or phenol. Cells were subsequently washed and an area of the culture dish was scanned once to determine background fluorescence intensity of untreated cells. Patulin (40 μM) and mBCI were then added to both sets of cells and increases

in fluorescence intensity resulting from the conjugation of mBCl with GSH were recorded.

In a second study the transient effect of the GN on cellular GSH levels was evaluated *in vitro*. Clone 9 cells were again scanned once to determine background fluorescence intensity. Cells were then treated with the GN alone, patulin alone, or a combination of the GN plus patulin. Increases in fluorescence intensity resulting from the conjugation of mBCl with GSH were monitored as above.

In vivo effects of the GN were also evaluated using 20 g male CD-1 mice. Mice were treated with the GN, 10 mg/kg i.p. in 0.2 ml Dulbecco's PBS, on days -7, 0, and every 7 days thereafter through day 241 post-inoculation. Control mice received i.p. injections of 0.2 ml PBS only. All mice were i.p. inoculated with 5×10^6 plaque-forming units of purified coxsackievirus B3 (CVB3) on day 0. Spleens were aseptically removed at days 28, 49, 70, 133, and 248 post-inoculation and spleen cells were prepared by maceration and centrifugation of expressed cells. Splenocytes were plated onto FBS-coated Lab-Tek Chambered Coverglass slides (see above) and allowed to adhere overnight. Cells were washed twice, treated with mBCl, and evaluated for fluorescence by laser cytometry to determine levels of mBCl-GSH. For each treatment group 8 samples with an average of 10 cells per sample were evaluated. Statistical analysis of the GSH data was performed using ANOVA, and Tukey's test was used to compare the different treatment groups. Differences were considered significant at $P < 0.05$.

Pancreata were removed from CVB3-inoculated CD-1 mice treated with GN as given above. Coronal sections were stained with hematoxylin and eosin, and histopathological evaluations of pancreatic preparations were independently completed on duplicate slides to determine the degree of CVB3-associated acinar cell destruction in infected animals in the presence or absence of GN treatment.

RESULTS

The effects of a complex glyconutritional mixture on intracellular GSH levels were first evaluated *in vitro* using Clone 9 cells. Cells pretreated with the GN over a period of 3 hr exhibited an increase in mBCl-GSH fluorescence intensity that reached saturation (about 14-15 fIU) by about 100 seconds after addition of mBCl. Cells treated with 40 μ M patulin alone revealed a much lower GSH level (a normalized level of about 3-4 fIU). However, cells treated with the GN prior to the addition of 40 μ M patulin exhibited a fluorescence intensity (about 7 fIU), indicative of an intermediate level of GSH. This suggests that the GN had a protective effect on GSH levels in cells exposed to patulin (Fig. 1). Comparison of Clone 9 cells treated with 50 μ g/ml of the GN for 3 hr to control cells held without the GN provides additional evidence for a direct protective effect of the GN on intracellular GSH levels (Fig. 2).

Figure 1. A 200 sec evaluation of control GSH levels in rat hepatic cells under three differing sets of conditions. A. Cells were pre-incubated with 50 μ g/ml Ambrotose® for 3 hr prior to the determination of glutathione levels. B. Cells were not pre-incubated for a 3 hr period after being removed from growth medium. C. Cells were removed from growth medium and pre-incubated for 3 hr in detection medium in the absence of Ambrotose®. The quantitation of GSH was dependent on formation of a fluorescent mBCl-GSH complex (fluorescent units; fIU) with detection by laser scanning cell imaging.

Figure 2. A 600 sec determination of GSH levels in rat hepatic cells. Cells were either held in detection medium for 3 hr in the absence (A) or presence (B) of Ambrotose® at 50 μ g/ml. At 600 sec the detection of glutathione had plateaued and was stable. The quantitation of GSH was dependent on formation of a fluorescent mBCl-GSH complex with detection by laser scanning cell imaging.

The transient effect of exposure of Clone 9 cells to the GN was also significant. When 40 μ M patulin was added to cells, a normalized value of about 3.5 fIU was detected (Fig. 3). However, GSH levels in Clone 9 cells immediately after addition of both 50 μ g/ml GN and 40 μ M patulin to cells had a normalized value of approximately 6.5 fIU. These data indicate that the GN mixture added simultaneously with patulin addition protected cells from GSH depletion, with about twice as much intracellular GSH remaining unbound to patulin.

Figure 3. An evaluation of GSH depletion by the sulfhydryl-binding agent, patulin, in control cells or control cells pre-treated with Ambrotose®. Rat hepatic (Clone 9) cells grown to subconfluence were incubated in the presence or absence of 50 μ g/ml Ambrotose®. Cells with Ambrotose® but no patulin were compared with cells treated with patulin, 40 μ M, with or without prior exposure to Ambrotose®. The quantitation of GSH was dependent on formation of a fluorescent mBCL-GSH complex with detection by laser scanning cell imaging.

An evaluation of splenocyte GSH levels in uninfected CD-1 mice, either untreated or treated by ip injection with GN at 10 mg/kg, showed that GN-treated mice had splenocyte GSH levels which did not differ from those detected in splenocytes of untreated mice out to 248 days (Table 1). CD-1 mice were CVB3-inoculated and either GN-treated at 10 mg/kg or untreated prior to splenocyte preparation and GSH determination. Splenocytes from non-CVB3-infected mice treated with GN exhibited GSH levels that were not statistically different from those of control cells at every test point. If changes from control cells were noted, they showed GSH levels as high or higher than those of cells from non-CVB3-inoculated mice. Cells from CVB3-infected mice exhibited a greater than 20% decline in GSH at all test days post-inoculation out to 248 days, except for day 70. At day 70 the GSH levels appeared to be normal for CVB3-treated mice. The maximal decrease in intracellular GSH, 33.25%, in cells from CVB3-infected mice

was seen at day 248. Cells from infected mice treated with GN at -7, 0 and every 7 days thereafter showed no decreased intracellular GSH out to day 248.

A histopathological evaluation of acinar cell destruction in pancreata following CVB3 inoculation showed that more than 70% of mice had acinar cell destruction by day 28. Approximately 87% of mice demonstrated acinar destruction at day 248 (Table 2). While CVB3-inoculated mice that were GN-treated also showed more than 70% destruction of acinar cells by day 28, only 40% of infected mice treated with GN showed acinar cell destruction at day 248. At days 49, 70, 105, and 133 post-inoculation, mice treated with GN also demonstrated approximately 40% acinar cell destruction. Thus, GN treatment appeared to preserve or restore acinar cells in about 30% of CVB3 infected mice.

Table 1. Effect of Ambrotose® Treatment on Free Glutathione [GSH] Levels in Splenic Monocytes/Macrophages from Coxsackievirus B3-Inoculated CD-1 Mice

Day post-inoculation of CVB3	Mean Units ($\pm$ SD) of Free GSH per Group:			
	normal	normal+ Ambrotose®	virus-infected	virus-infected+ Ambrotose®
28	26.0 $\pm$ (7)	19.8 $\pm$ 6.8 (7)	17.4 $\pm$ 7.6 (7)	34.2 $\pm$ 9.6** (7)
49	1181 $\pm$ 536 (108)	1184 $\pm$ 579 (144)	794 $\pm$ 380 (101)	1527 $\pm$ 688** (114)
70	1364 $\pm$ 523 (226)	1344 $\pm$ 409 (250)	1314 $\pm$ 217 (458)	1431 $\pm$ 548 (310)
105	1601 $\pm$ 271 (216)	1475 $\pm$ 342 (217)	1279 $\pm$ 512 (181)	1506 $\pm$ 321** (242)
133	1116 $\pm$ 300 (179)	1115 $\pm$ 295 (221)	875 $\pm$ 280 (156)	1194 $\pm$ 300** (203)
248	1251 $\pm$ 572 (117)	1428 $\pm$ 643 (101)	835 $\pm$ 435 (118)	1621 $\pm$ 565** (117)

Adolescent male CD-1 mice were intraperitoneally inoculated with 5×10^5 plaque-forming units of purified CVB3 on day 0. Mice were injected with Ambrotose® at 10 mg/kg body weight on days -7, 0, and approximately every 7 days thereafter through day 241 post-infection. Free GSH levels, fluorescence units determined as a function of the fluorescence of GSH bound to mBCL, were measured 5-20 times for each of the splenic cell samples from 7 individual mice per sample through days 133 post-infection. On day 248 post-infection there were 8, 9, 8 and 10 mice in the N, N+A, V, and V+A groups respectively. Low mean units reported from samples taken on day 28 were due to a technical problem of cell plating and were consistent for all four samples.

** Significantly different from the virus control group ($p < 0.05$) by Tukey's Multiple Comparison Test.

DISCUSSION

The ability of serum antioxidants to delay the peroxidation of lipids has been expressed as the total peroxyl radical-trapping antioxidant parameter (TRAP). This has been determined primarily by the serum content of the "scavenging" antioxidants vitamins C and E, uric acid, and reduced protein sulfhydryl groups such as glutathione (Wayner et al., 1987). Natural mechanisms preventing damage to cells by ROS and other reactive molecules are typically quite effective and, in some disease states, are known to have been compromised prior to cellular injury and the onset of symptoms. The concept that

Table 2. Effect of Ambrotose™ (GN) on Coxsackievirus B3-Induction of Pathology in Pancreata of CD-1 Mice*

Day p.i.	Treatment with GN	number of sections examined	Histopathology of the pancreas, number of sections presenting with extent of acinar cell destruction (% destroyed)					Proportion of sections with:		Average % of acinar cells destroyed in all sections per group (mean ± S.E.M.)
			(≥90)	(90-50)	(50-10)	(≤10)	(none)	any extent acinar cell destruction	destruction of <10% of cells or none	
28	NO	14	0	0	7	4	3	11/14	7/14	29.4 ± 6.5
	YES	14	0	1	4	7	2	12/14	9/14	12.6 ± 4.2 ^a
49	NO	14	2	2	4	2	4	10/14	6/14	66.3 ± 14.0
	YES	14	0	0	0	10	4	10/14	14/14	2.6 ± 0.6 ^c
70	NO	14	1	0	9	4	0	14/14	4/14	26.9 ± 6.0
	YES	14	4	2	0	0	8	6/14	8/14	39.6 ± 12.4
105	NO	14	3	0	3	4	3	10/13	7/13	27.2 ± 11.3
	YES	14	2	0	2	6	4	10/14	10/14	19.1 ± 9.1
133	NO	14	0	3	6	3	2	12/14	5/14	25.3 ± 6.0
	YES	14	0	0	2	5	7	7/14	12/14	4.2 ± 1.8 ^b
248	NO	16	0	1	7	7	1	15/16	8/16	16.6 ± 4.8
	YES	19	1	1	3	5	9	10/19	14/19	13.4 ± 5.4

Mice were inoculated with 10mg/kg of Ambrotose™ in DPBS or DPBS only by intraperitoneal route 7 days prior to challenge with CVB3_m on day 0. On days -7, 0 and approximately every 7 days thereafter, mice were inoculated with Ambrotose™ or DPBS through day 241. Two coded sections, 40-60 microns apart, of each pancreas were examined for acinar cell destruction by two observers at 40 and 100X magnification. The data presented represent a conservative consensus of the outcomes, i.e., where there was disagreement on the % cells destroyed, the lower number was used.

[†] Fisher's exact test; p = superscript indicating a significant difference between groups of mice on Ambrotose™ supplementation and those in the virus control.

[‡] Student's t test comparative analysis of average percentages of acinar cells destroyed in all sections in the virus control group vs the group of virus-challenged mice given Ambrotose™: p<0.05^a, p<0.01^b or p<0.001^c

aging might be manifested as a collection of diseases resulting from cell and tissue damage due to the decreased function of naturally occurring mechanisms by which cells protect themselves from free radicals has gained significant credibility from its initial proposal more than 40 years ago (Harmon, 1956; Halliwell and Gutteridge, 1985; Sohal, 1987; Sohal et al., 1990; Pacifici and Davis, 1991; Harris, 1992; de Hann et al., 1992). Inflammatory diseases in general exhibit the occurrence of damage resulting from reactive chemicals binding and initiating destruction of cells and tissues. In other disease states oxidative damage to cells and tissues has been thought to be a potential mechanism of pathology occurring subsequent to onset of the disease. It is possible that some disorders result in increased levels of oxidative radicals, while in others elevated levels of reactive chemicals may initiate the disease.

Insulin dependent diabetes mellitus (IDDM) results in a progressive sequence of physiological pathologies that appear to mimic many of the vascular and neurological phenomena of aging. Angiopathy and neuropathy are initiated by oxidative stress subsequent to the onset of a prediabetic state and coincident with hyperglycemia, autooxidation of glucose, and a decrease in antioxidant levels (Asayama et al., 1993; Tiedge et al., 1998). This finding is supported by studies showing an abnormal serum and cellular antioxidant status, with increased lipid peroxidation, decreased levels of GSH and vitamins C and E, and diminished function of SOD and catalase enzyme systems, in persons with impaired

glucose tolerance and hyperglycemia (Vijayalingam et al., 1996). These studies strongly suggest that decreased capacity to counter the membrane-damaging effects of free radicals may be a factor predisposing the onset of symptomatic IDDM. Evidence demonstrates that antioxidant supplementation, currently accepted as a mechanism that appears to counter some of the effects of aging, might retard progression of angiopathy and neuropathy as a part of the accepted IDDM pathophysiology (Vijayalingam et al., 1996; Stahl and Sies, 1997; Bermejo and Hidalgo-Correas, 1997).

Asayama et al., (1993) evaluated preventive antioxidant activity, total peroxy radical-trapping antioxidant activity, and major component antioxidants in the serum of IDDM children, and found them to be significantly decreased. Their data strongly suggest that defective serum antioxidant mechanisms in IDDM children contributed to increased oxidative stress and tissue damage leading to peripheral vascular disease and neuropathy that are a hallmark of overt diabetes and occur frequently in the non-diabetic elderly. Maxwell (1995) gives eight general principles that should apply to antioxidant therapy. (1) Therapies functioning to decrease serum oxidant levels associated with specific disorders would be most effective if the disorder is associated with oxidative damage to proteins or DNA. (2) The disorder should demonstrate the interaction of oxidants as a mechanism of disease pathophysiology. (3) Do persons with the disease exhibit known defects in serum antioxidant levels or in the antioxidant enzyme systems? (4) Is

the site of oxidant damage known for the specific disease? (5) Is it possible for a supplemental antioxidant to effectively reach the site of oxidant activity? (6) Will supplemental antioxidants have an impact on the oxidative process? (7) Can the supplementary antioxidant be provided in a dose that is effective and also well tolerated? (8) Is the therapy safe? Vijayalingam et al., (1996) provided support for the proposal of Asayama et al., (1993), reporting that a variety of antioxidant mechanisms, including the GSH peroxidase system, are defective in both IDDM and impaired glucose tolerance (IGT) children who are at significant risk for developing diabetes mellitus. They further proposed that studies of the effectiveness of antioxidant therapy for IGT children without overt diabetes should be undertaken to determine whether such therapy could retard the development of symptomatic diabetes mellitus.

Data presented here from an *in vitro* determination of bioavailable GSH in rat hepatic cells indicate that *in vitro* supplementation of cells with a complex mixture of mono-, di- and polysaccharides, Ambrotose®, prevents chemical depletion of GSH. In addition, an *in vivo* study of coxsackie virus B3-induced disease in mice showed that the CVB3-initiated depletion of GSH in mouse splenocytes was significantly diminished by i.p. treatment with the GN, Ambrotose®. Along with the protective effect of GN on intracellular GSH, there was a clear indication that CVB3-associated oxidative damage to pancreatic acini was decreased in GN-treated mice. This is consistent with data from Rabinovitch et al., (1996) showing that antioxidant treatment inhibited the autoimmune destruction of islet β -cells typical of IDDM.

At this time the mechanism(s) of intracellular GSH protection by the GN, Ambrotose®, is unknown. Further study of this phenomenon is warranted to determine a mechanism(s) of action of the GN, whether the GN functions to protect experimental animals from the onset of angiopathy and neuropathy characteristic of both IDDM and aging, and to determine whether the multiple pathologies associated with both diabetes and aging are effectively countered by ingestion or injection of the GN.

ABBREVIATIONS

Glyconutritional, GN; coxsackievirus B3, CVB3; glutathione, GSH; fluorescence units, FIU; total peroxy radical-trapping antioxidant parameter, TRAP; insulin dependent diabetes mellitus, IDDM; reactive oxygen species, ROS; monochlorobimane, mbCl.

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