Synergistic protection by *S*-adenosylmethionine with vitamins C and E on liver injury induced by thioacetamide in rats

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Abstract

Free radicals are involved in the pathogenesis of acute liver injury induced by thioacetamide (TAA). We investigated the effects of *S*-adenosylmethionine (SAME) combined with/without vitamins C and E on TAA-induced acute liver injury in rats. TAA was given intraperitoneally (200 mg kg⁻¹). Antioxidant treatments (SAME, 25 mg kg⁻¹; vitamin C, 100 mg kg⁻¹; vitamin E, 200 mg kg⁻¹, intraperitoneal) were given 1 h later. Liver histology, enzymology, and ability to release hepatic insulin-sensitizing substance (HISS) were assessed. TAA caused liver tissue injury, increased liver enzymes, and decreased insulin sensitivity ($p < 0.01$). Blockade of HISS release by atropine did not further decrease insulin sensitivity in rats with TAA insult, indicating that the decrease in insulin sensitivity was HISS dependent. Treatment with SAME alone or vitamins C+E slightly improved liver histology but not the changes in liver enzymes and insulin sensitivity. Combined treatment with SAME plus vitamins C+E greatly protected the liver from tissue injury, the increase in liver enzymes, and the decrease in insulin sensitivity. In conclusion, acute liver injury causes HISS-dependent insulin resistance (HDIR). There are synergistic antioxidative effects among the antioxidants, SAME and vitamins C and E, that protect the liver from TAA-induced HDIR, suggesting that antioxidant treatment may best be done using a balanced “cocktail.”

Keywords

S-Adenosylmethionine; Vitamin C; Vitamin E; Antioxidant; Oxidative stress; Liver; HISS; Thioacetamide; GSH; Free radical

Introduction

Oxidative stress has been implicated in the pathogenesis of acute and chronic liver injury in a variety of pathophysiological conditions such as hepatotoxin exposures, intrahepatic cholestasis, alcoholic liver injury, liver ischemia/reperfusion injury, and viral hepatitis [1–4]. Overproduction of reactive oxygen species (ROS) and nitrogen species (RNS), along with

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significant decrease of antioxidant defense in these pathological conditions, impairs various cellular functions through the processes of lipid peroxidation, protein oxidation, and nucleic base oxidation. Lipid peroxidation, for example, causes changes in the physical and chemical properties of cellular membranes, thus altering their fluidity and permeability, leading to impairment in membrane signal transduction and ion exchange, resulting in swelling, cytolysis, and finally cell death. The oxidation of proteins and DNA also relates directly to cellular dysfunction and death [5].

Accordingly, effects of antioxidants or free radical scavengers have been widely tested for the prevention and treatment of acute and chronic liver injuries. In some of those studies, antioxidants have shown beneficial effects, specifically for prevention and treatment of chronic liver injury [6–12]. However, the efficacy of antioxidant treatment in acute liver injury with a single antioxidant is less clear [13]. We suggest that the major reason is that, while the production of the free radicals with various chemical properties in these diseases is widely spread throughout the different tissue and cellular components, the chemical property of an individual antioxidant can only allow it to scavenge the free radicals located in a specific cellular component, e.g., lipid or aqueous phase. Moreover, the efficacy of an antioxidant substance is also dependent on the redox state of the cell. In situations that an imbalanced redox state preexisted, antioxidant treatment will be less, or none, effective [14–16]. It has been suggested recently that the therapeutic strategy for protecting against oxidative stress will be to target simultaneously the free radicals in both the lipid and the aqueous phases, in extracellular and intracellular spaces [17].

Based on this background, we hypothesized that the combined antioxidative treatment with *S*-adenosylmethionine (SAME), vitamin E, and vitamin C would achieve a significant synergistic antioxidative effect, thus greatly protecting the liver from injury induced by oxidative stress.

All three compounds play an important and different role in scavenging free radicals [5]. The water-soluble property of vitamin C makes it the first-order antioxidant to protect cell components from free radical-induced damage by quenching various water-soluble radicals, e.g., superoxide anion, in the aqueous phase. Vitamin E is a lipid-soluble molecule and can transfer its phenolic hydrogen to a peroxyl free radical of a peroxidized polyunsaturated fatty acid, thereby breaking the radical chain reaction, thus preventing the lipid peroxidation in cellular and subcellular membrane phospholipids, especially those of mitochondria and microsomes. SAME is a natural, nontoxic regulator of glutathione (GSH) with good bioavailability [18]. GSH is the main intracellular defense against free radicals. GSH stores are significantly depleted in liver injury induced by oxidative stress. Administration of SAME represents an effective way to restore intracellular GSH stores, especially in mitochondria, thus improving the cellular ability to scavenge free radicals [18].

The experiments were conducted in a rat model of acute liver injury induced by thioacetamide (TAA), which has been demonstrated to cause liver damage through oxidative stress [19,20]. The liver damage was assessed by the changes in liver histology and enzymology (aminotransferase alanine (ALT) and aspartate aminotransferase (AST)), as well as by a rapid insulin sensitivity test (RIST) to evaluate the ability of the liver to produce

hepatic insulin-sensitizing substance (HISS). HISS is responsible for approximately 55% of the glucose disposal effect of an injection of insulin in the fed state and this portion of insulin response is termed HISS-dependent insulin action [21–23]. It has been demonstrated that the ability of the liver to release HISS is severely impaired in situations such as hepatic parasympathetic dysfunction and chronic bile duct ligation. The insulin resistance caused by bile duct ligation is due to absence of HISS action; mimicking the normal parasympathetic nerve function with intraportal acetylcholine restores insulin action to normal levels [24]. The physiology, pharmacology, pathophysiology, and therapeutic potential of HISS recently have been reviewed [23,24].

The results revealed that combined treatment of SAME with vitamin E plus vitamin C greatly decreased thioacetamide-induced liver damage including induction of HISS-dependent insulin resistance. The mechanism may be related to the synergistic antioxidative effect among these three endogenous antioxidants.

Material and methods

The experimental procedures were approved by an ethics committee on animal care at the University of Manitoba and performed in accordance with the Guidelines of the Canadian Council on Animal Care.

Animal groups

Six groups of animals were established under the following treatments: (1): normal control rats, treated with saline and corn oil injection at the dose of 2 and 1 ml kg⁻¹, respectively, $n = 12$; (2) normal rats, treated with vitamin C, vitamin E, and SAME, $n = 8$; the doses for vitamin C and vitamin E were 100 and 200 mg kg⁻¹, respectively; for SAME 25 mg kg⁻¹; (3) TAA rats, given intraperitoneal injection of TAA at the dose of 200 mg kg⁻¹, $n = 9$; (4) TAA rats treated with vitamin C + vitamin E, $n = 11$. (5) TAA rats with SAME treatment, $n = 7$; (6) TAA rats treated with vitamin C + vitamin E + SAME, $n = 8$. All compounds were administered by intraperitoneal injection (ip). TAA was administered 24 h before the start of surgery, and all treatment with vitamins and SAME were given 1 h post-TAA administration.

Surgical preparation

Male Sprague-Dawley rats weighing 260–300 g from the University of Manitoba colony were maintained in the animal house under controlled conditions ($22 \pm 1^\circ\text{C}$, 12-h light/12-h dark cycle). They were fed a standard rat chow diet with free access to water. All rats underwent a fasting period of 8 h and a refeeding phase of 2 h immediately before the start of surgical preparation. As HISS is only released in the fed state, the fasting-refeeding protocol assured a high level of HISS release in response to insulin. The rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (65 mg kg⁻¹; Somnotol, MTC Pharmaceuticals, Ontario). Maintained anesthesia was achieved throughout the experiment by a continuous infusion of pentobarbital sodium (0.5 mg ml⁻¹ saline given at 50 $\mu\text{l min}^{-1}$) through a cannula in the jugular vein, supplemented with a 0.65 mg (0.01 ml) bolus injection when required. The rats were placed on a temperature-controlled surgical

table (Harvard Apparatus, Kent, England) and rectal temperature was monitored and held at 37–37.5°C. Spontaneous respiration was allowed through a tracheal tube.

An arterial-venous shunt was established, as previously described [21], for monitoring mean arterial blood pressure (MAP) and blood glucose level and for drug delivery. Briefly, two catheters (polyethylene tubing PE60), one inserted into the right femoral artery and the other into the right femoral vein, were connected to the two openings of a three-way vascular circuit consisting of a T tube connected with silicon tubing. The third opening of the circuit was connected to a pressure transducer for the recording of the shunt pressure which, when the silicon tubing toward the venous side of the circuit was closed by clamping, represented the systemic arterial blood pressure. Blood samples were taken from the arterial side of the shunt for the glucose measurement. Flowing blood within the shunt assures the real time measurement of the blood glucose concentration, which is essential for the euglycemic clamp test as noted below. An infusion line was inserted into the venous side of the loop for drug delivery. Another infusion line connected to the jugular vein was established for glucose infusion. Animals were heparinized (100 IU kg⁻¹) to prevent clotting in the vascular loop.

Rapid insulin sensitivity test

The RIST was performed as previously described [21]. Briefly, following completion of surgery, animals were allowed a 30-min stabilization period. The baseline glucose levels were then determined by samples taken at 5-min intervals and continued until three successive stable determinations were made. The mean of these three data points is regarded as the baseline for the RIST. To perform the RIST, human insulin (50 mU kg⁻¹ in 0.5 ml saline) was infused into the femoral vein at the rate of 0.1 ml min⁻¹ for 5 min. After 1 min of insulin infusion, the first test glucose sample was determined and a variable glucose infusion (10%) was initiated. Blood samples were taken every 2 min and the glucose infusion rate was adjusted accordingly to maintain euglycemia. The RIST index is the amount of glucose (mg kg⁻¹) infused, to maintain euglycemia, over the test period that terminated when no further glucose infusion was required (approximately 30 min). At the end of a RIST, the animal is at its pretest glycemic level.

Protocol

Two RIST tests were performed for each rat. Following the establishment of the glucose baseline, the first RIST index was determined and this was regarded as the control RIST. The rats were allowed to achieve stabilized glucose baseline again and the second RIST was performed after blockade of HISS formation with intravenous infusion of atropine (0.1 mg kg⁻¹ in 0.5 ml saline, 0.1 ml min⁻¹). The first RIST index includes the effects of both HISS-dependent and HISS-independent components, and the second RIST index represents only the HISS-independent component (22,23). The percentage contribution of HISS-dependent component to total insulin sensitivity was calculated as: %HISS = (RIST index in control–RIST index after atropine)/RIST index in control. At the end of the experiment, 1 ml blood was withdrawn from the femoral artery. The blood was centrifuged (1.4 × 10⁴ rpm for 5 min; Eppendorf Centrifuge 5414C, Brinkmann Instruments, NY) and the plasma was kept at –20°C for further hepatic enzymes analysis.

Hepatic enzymes analysis

Serum samples from rats in groups 1 to 6 were analyzed for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities spectrophotometrically utilizing an Abbott analyzer and Diagnostic Chemicals Limited assay reagents (Charlottetown, P.E.I, Canada).

Liver histopathological examination

The left liver lobe was excised and embedded in paraffin, sliced 7 μm in thickness, and stained with hematoxylin-eosin. The tissue slices were then analyzed with light microscopy. Histopathological analysis of the injured area was performed with a computerized video-imaging system (Image-Pro Plus. Media Cybernetics, MD) by a pathologist (Y.F.) who was not aware of sample assignment to experimental group. The degree of injury, including inflammation, necrosis, and bridging necrosis, was expressed as the mean of 5 different fields for each slide scaled as 0–3: normal, 0; mild, 1; moderate, 2; and severe, 3.

Data collection, instruments, and chemicals

A PowerLab recording unit, together with a computer application software (AD Instruments Pty Ltd, Australia), was used to record and analyze the mean arterial blood pressure. Blood glucose concentration was measured by a glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH). The infusion pumps were from Kent Scientific Corporation, Torrington, CT (Model RS 232).

Human insulin was purchased from Eli Lilly and Company (Toronto, Ontario). Vitamin C (L-ascorbic acid), vitamin E (($\pm$)- α -tocopherol), SAME, and TAA were all purchased from Sigma. Insulin, vitamin C, and TAA were dissolved in saline. SAME was dissolved in cold saline. Vitamin E was dissolved in corn oil.

Statistical analysis

Values are presented as means $\pm$ SE. The data were analyzed by paired or unpaired *t* test where appropriate. A one-way ANOVA followed by Tukey's test was employed when the multiple means from different groups were compared. Statistical significance was taken at *p* < 0.05.

Results

RIST in normal rats

Normal rats had a RIST index of $168.2 \pm 9.2 \text{ mg kg}^{-1}$ (Fig. 1A). Atropine at the dose of 0.1 mg kg^{-1} did not change the basal glucose level (112.2 ± 2.5 in control and $110.9 \pm 2.2 \text{ mg\%}$ postatropine) but significantly decreased the RIST index to $82.7 \pm 3.2 \text{ mg kg}^{-1}$ (Fig. 1B), representing a $50.0 \pm 2.1\%$ inhibition of insulin sensitivity.

RIST in TAA rats

TAA at the dose of 200 mg kg^{-1} significantly impaired insulin sensitivity. The RIST index was reduced to $75.5 \pm 2.8 \text{ mg kg}^{-1}$, significantly lower than in normal rats (*p* < 0.01, Fig. 1). Atropine did not further decrease the RIST index in TAA 200 rats as demonstrated by a

similar RIST index of $69.0 \pm 2.1 \text{ mg kg}^{-1}$, suggesting that the impaired insulin sensitivity by TAA treatment resulted from damage in the HISS-dependent component. The basal glucose level was lower ($96.5 \pm 3.0 \text{ mg\%}$) in TAA 200 rats compared to their normal partners; however, the MAP ($101 \pm 4.0 \text{ mm Hg}$) remained in the same range as compared to the normal rats ($106 \pm 3.5 \text{ mmHg}$).

Effect of combined treatment with SAME + vitamin C + vitamin E on RIST in normal and TAA rats

Combined treatment of SAME + vitamin C + vitamin E did not significantly influence the insulin sensitivity in normal rats, as demonstrated by a similar RIST index ($185.6 \pm 9.2 \text{ mg kg}^{-1}$, Fig. 1) compared to normal rats without treatments. However, this treatment significantly improved insulin sensitivity in TAA rats. As shown in Fig. 1, the treatment increased the RIST index to $134.6 \pm 4.6 \text{ mg kg}^{-1}$. Although it was still lower than the RIST index compared to the normal rats, it was significantly higher than what was observed in TAA rats without treatment, suggesting a partial recovery in insulin sensitivity after the treatment. Further, blockade of HISS formation with atropine decreased the RIST index to $74.9 \pm 3.2 \text{ mg kg}^{-1}$ in this group, or a decrease in insulin sensitivity by 44.4% (Fig. 2), which was close to the percentage inhibition seen in normal rats after atropine (50%). The results suggest that the improved insulin sensitivity by combined treatment of SAME + vitamin C + vitamin E is mainly due to the improvement in the HISS-dependent component.

RIST in TAA rats treated with SAME alone or a combination of vitamin C + vitamin E

TAA rats treated with SAME alone had a RIST index of $88.0 \pm 4.1 \text{ mg kg}^{-1}$, which was not different compared to the index from TAA rats without treatment (Figs. 1 and 2). The RIST index postatropine ($72.1 \pm 3.5 \text{ mg kg}^{-1}$) in this group was also similar to TAA rats without treatment (Figs. 1 and 2).

Similarly, TAA rats treated with a combination of vitamin C + vitamin E had a RIST index of $81.7 \pm 3.1 \text{ mg kg}^{-1}$, which was not different compared to the index from TAA rats without treatment. The RIST index postatropine ($70.7 \pm 3.4 \text{ mg kg}^{-1}$) in this group was also similar to TAA rats without treatment (Figs. 1 and 2).

Serum ALT and AST activities

As shown in Fig. 3, Animals treated with TAA showed a significant increase in ALT and AST activities. These increases were partially prevented when rats were treated with SAME + vitamin C + vitamin E ($p < 0.05$). In contrast, treatment with SAME alone or vitamin C + vitamin E did not prevent the increases in these enzymes.

Liver histopathology

Histological examination of livers in the TAA-treated rats revealed massive hepatocytic necrosis, including bridging necrosis, with influx of inflammatory cells. Treatment with SAME alone or vitamin C + vitamin E showed a slight reduction in histological injury; in contrast, combined treatment with SAME + vitamin C + vitamin E produced great reduction in histological injury (Table 1, Fig. 4).

Discussion

Rats treated with TAA showed severe liver injury as demonstrated by significant increases in liver enzymes, massive hepatocytic necrosis with inflammatory cell influx, and severely impaired HISS-dependent insulin sensitivity. Combined treatment of antioxidants, SAME plus vitamins E and C, greatly protected liver function against damage induced by TAA. In contrast, these antioxidants did not show significant beneficial effect in protecting liver function when used separately. To the best of our knowledge, the present study is the first to provide data to suggest a significant synergistic antioxidative and liver protective effect for these three chemicals. It is also the first study to show that a hepatotoxin causes insulin resistance attributable to HISS-dependent insulin resistance.

Liver injury by TAA and oxidative stress

TAA is a potent selective hepatotoxin, which has been widely used to induce acute and chronic liver injury [11,12,19,20]. Oxidative injury has been recognized as the major mechanism in TAA-induced liver damage. The oxidative metabolism of TAA through the hepatocyte FAD–mono-oxygenase system and cytochrome P-450 monooxygenase systems produces reactive oxidative agents, especially the very reactive compound thioacetamide-*S*-dioxide, which targets tissue macromolecules, lipids, protein, and DNA, leading to tissue oxidative injury and necrosis [11,12]. It has been observed that TAA causes tissue injury and necrosis/apoptosis through the processes related to lipid, protein, and DNA peroxidation [11,12,26–29]. Depletion of the endogenous antioxidants vitamin C, vitamin E, and GSH has been observed in animals treated with TAA [27,29,30] and beneficial effects with antioxidant treatment have been observed [11,12]. We demonstrated in the present study that combined antioxidative treatment with SAME plus vitamins E and C effectively protected the liver from injury induced by TAA.

Synergistic effect of SAME with vitamin E and vitamin C

SAME and vitamins C and E are three important endogenous antioxidants with distinguishing chemical properties in scavenging various free radicals. The efficacy of these compounds in the treatment of diseases related to production of free radicals, specifically diseases with long-term oxidative stress, has been well established in the literature [6–10]. These compounds, however, are less effective in the treatment of liver injury caused by severe oxidative stress when used alone [13,14,31]. Consistent with these observations, the present study has also found that treatment with SAME or vitamin C plus vitamin E only showed a slight reduction in histological injury induced by TAA but did not significantly improve associated severely deteriorated liver function evaluated either by the changes in liver enzymes or by HISS-dependent insulin sensitivity, suggesting that the treatments with SAME alone or vitamin C plus vitamin E were insufficient to protect the liver from severe oxidative stress induced by TAA. In contrast, combined treatment with SAME with vitamin C plus vitamin E not only greatly reduced liver inflammation and necrosis but also significantly prevented the increases in liver enzyme activities and, more importantly, improved impaired HISS-dependent insulin sensitivity caused by TAA. The results suggest that synergistic protective effects among these compounds exist.

Two major hypotheses can be proposed to explain the mechanisms for the synergistic protective effects provided by the combined treatment of SAME with vitamins E plus C. First, the specific chemical properties of these three compounds enable them to simultaneously scavenge the overproduced free radicals following TAA insult from various tissue and cellular components. Vitamins E and C are the most important antioxidants in the lipid and aqueous phase, respectively. SAME is the key regulator of GSH, which is known as the most important cellular thiol to maintain cellular redox potential [32]. GSH plays a crucial role in protecting mitochondria from membrane lipid peroxidation by scavenging hydrogen peroxide, thus preventing the production of the most active radical, $\text{OH}^\bullet$. Free radical overproduction, following TAA attack, initiates from mitochondria and microsomes in liver cells and spreads out quickly to all cellular and tissue components, causing dysfunction in cellular energy metabolism, membrane transportation, and finally cell death. Another potent source of free radicals in the liver can be Kupffer cells and circulatory neutrophils being activated by inflammatory reactions secondary to initial liver cell injury and death. In response to sustained oxidative stress induced by TAA, endogenous antioxidants are quickly consumed and depleted, specifically the key antioxidants vitamins E and C, SAME, and associated GSH, resulting in continuously decreasing tissue and cellular antioxidative capacity that leads to progressive oxidative injury [27,29,30]. In addition to depletion, endogenous synthesis of SAME is also decreased due to the reduction of hepatic SAME synthetase activity that is related to the decrease in GSH [33–35]. Cosupplement of SAME with vitamins E and C, therefore, could simultaneously improve the overall ability of scavenging free radicals from various tissue and cellular components, thus providing a synergistic effect to protect the liver from TAA-induced oxidative injury.

Second, the interaction among these three antioxidants may maintain them in their reduced form to rebuild the endogenous antioxidant balances that could have been impaired by elevated oxidative stress following TAA attack. Oxidative vitamin E is recycled to its reduced form by vitamin C; oxidized vitamin C is recycled to its reduced form by GSH; the reduced form of vitamin C/vitamin E contributes to maintain local GSH/GSSH ratio, especially in the regions of mitochondria and microsomes [5]. In certain circumstances, vitamins E and C and GSH or its precursor could serve as prooxidants that restrict their antioxidant properties. For example, it has been observed that in liver microsomes isolated from rats fed a diet deficient in vitamin E, treatment with GSH induces lipid oxidation and this response is prevented by additional vitamin E [16]. Likewise, prooxidative functions of vitamin E have been demonstrated in the absence of vitamin C, but in the presence of vitamin C, vitamin E does not have a prooxidant function [36]. Furthermore, in the presence of redox-active transition metal ions with hydrogen peroxide, vitamin C can also act as a prooxidant to induce cellular oxidative injury [37]. The prooxidant functions of these three compounds may explain why in some circumstances they do not show significant antioxidant effects when used alone. Cotreatment of SAME with vitamins E and C could prevent each compound from becoming prooxidants, thus improving their antioxidative ability.

Liver injury and RIST

Traditionally, evaluation of liver function is achieved by assessing the activities of hepatic enzymes in plasma, such as the activities of ALT and AST. This is an indirect method since the change in enzyme activities in plasma is only reflecting the amount of enzymes that leak out from the membrane of damaged hepatocytes, rather than a change in “real liver function.” In the present study, in addition to assessing hepatic enzyme activities, the rapid insulin sensitivity test is performed to evaluate liver function.

In response to insulin administered in the fed state, the liver is suggested to release a putative hormone, hepatic insulin sensitizing substance, to stimulate glucose uptake in skeletal muscle [21–24]. HISS is responsible for approximately 55% of the glucose disposal effect of an injection of insulin in the fed state and this portion of insulin response is termed HISS-dependent insulin sensitivity. Blockade of HISS action results in HISS-dependent insulin resistance (HDIR). HDIR is seen in a wide variety of pathological models including the spontaneously hypertensive rat, sucrose-fed rats, adult offspring of fetal alcohol exposure, acute stress and ageing, physical interruption of hepatic parasympathetic nerves, and pharmacological blockade of hepatic muscarinic cholinergic receptors or nitric oxide production or cyclooxygenase (for review, see Refs. [22–24]). It has also been demonstrated that the HISS action is dramatically decreased in rats that underwent chronic bile duct ligation, highlighting the correlation of liver function to its ability to release HISS [25].

Acute liver injury by TAA also severely reduced HISS-dependent insulin sensitivity, resulting in a decreased RIST index in TAA-treated rats. The decrease in RIST index was due to decreased action of HISS since atropine, which blocks HISS action but not HISS-independent direct insulin action [22–24], did not further influence the RIST index in TAA-treated rats. More importantly, it has been observed that combined treatment with SAME plus vitamins C and vitamin E greatly protects against HDIR induced by TAA. Thus, the RIST index could be used as an index to evaluate liver function in injured liver and may have more advantage than hepatic enzyme alterations in the assessment of liver function since the latter is relatively quiescent in chronic liver diseases. The mechanism that causes the HISS-dependent insulin resistance is currently unclear but may be related to the decrease in hepatic GSH content and to the unbalanced production of nitric oxide and O_2^- . It has been demonstrated by Guarino et al. [38] that reduction in HISS-dependent insulin sensitivity can be caused by depletion of hepatic GSH and reduced production of *S*-nitrosoglutathione. The acute effect of alcohol also results in HISS-dependent insulin resistance that is associated with reduced hepatic GSH [39].

In conclusion, we have shown in the present study that, while SAME alone or vitamin E plus C did not show significant therapeutic efficacy, combined treatment of SAME with vitamins E plus C greatly improved liver function in a rat model of acute liver injury induced by TAA. In association with histological and enzymological alterations, HISS-dependent insulin sensitivity was severely impaired in rats that encountered oxidative liver injury induced by TAA and this impairment was greatly prevented by combined treatment of SAME with vitamins E plus C. These are the first data to suggest that free radical imbalance can cause HISS-dependent insulin resistance. Additional studies are required to test this hypothesis

and to fully understand the mechanism of synergism of SAdMe with the antioxidant vitamins to explore their therapeutic possibilities and possibly to understand the etiology of insulin resistance caused by HDIR.

Acknowledgments

The study was supported by operating grants from the Canadian Institutes of Health Research and the Heart and Stroke Foundation of Manitoba. The authors gratefully acknowledge Dallas Legare and Dianne Kropp for excellent technical support and Karen Sanders for secretarial assistance with the manuscript.

Abbreviations

ALT	alanine aminotransferase
AST	aspartate aminotransferase
GSH	glutathione
HISS	hepatic insulin-sensitizing substance
HDIR	HISS-dependent insulin resistance
MAP	mean arterial blood pressure
RIST	rapid insulin sensitivity test
RNS	reactive nitrogen species
ROS	reactive oxygen species
SAdMe	<i>S</i> -adenosylmethionine
TAA	thioacetamide

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Fig. 1.

RIST index in control (A) and after atropine (B) in rats with different treatment. Normal, normal rats; N-SCE, normal rats treated with SAmE + vitamins E and C; TAA, rats with thioacetamide-induced liver damage; TAA + SCE, TAA + S, and TAA + CE, rats with thioacetamide-induced liver damage treated with SAmE + vitamins C and E, with SAmE alone, and with vitamins alone, respectively. RIST index decreased by thioacetamide insult. In contrast, RIST index after atropine did not differ among different groups, suggesting that the decrease in RIST index by thioacetamide is due to impairment of the HISS-dependent component. While treatment with SAmE alone or vitamins alone did not show significant improvement, combined treatment with SAmE + vitamins significantly improved the RIST index. $n = 7-12$ for each group. * $p < 0.05$ vs normal rats; # $p < 0.05$ vs TAA rats.

Fig. 2.

% HISS component in rats with different treatment. Normal, normal rats; N-SCE, normal rats treated with SAmE + vitamins E and C; TAA, rats with thioacetamide-induced liver damage; TAA + SCE, TAA + S, and TAA + CE, rats with thioacetamide-induced liver damage treated with SAmE + vitamins C and E, with SAmE alone, and with vitamins alone, respectively. Percentage HISS component was decreased by thioacetamide insult. While treatment with SAmE alone or vitamins alone did not show significant improvement, combined treatment with SAmE + vitamins improved significantly percentage HISS component. $n = 7-12$ for each group. * $p < 0.05$ vs normal rats.

Fig. 3. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in rats with different treatment. Normal, normal rats; N-SCE, normal rats treated with SAME + vitamins E and C; TAA, rats with thioacetamide-induced liver damage; TAA + SCE, TAA + S, and TAA + CE, rats with thioacetamide-induced liver damage treated with SAME + vitamins C and E, with SAME alone, and with vitamins alone, respectively. Combined treatment with SAME and vitamins significantly decreased ALT and AST activity in rats with thioacetamide insult. $n = 7-12$ for each group. * $p < 0.05$ vs normal rats; # $p < 0.05$ vs TAA rats.

Fig. 4. Histological section of the liver from rats in six different groups as described under Materials and methods. (A) Normal rat; (B) normal rat treated with SAME + vitamins C and E; (C) TAA rat; (D) TAA rat treated with SAME + vitamins C and E; (E) TAA rat treated with SAME alone; (F) TAA rat treated with vitamins C and E. Severe inflammation and necrosis induced by TAA is shown. Combined treatment with SAME and vitamins E and C shows a greater effect in reducing liver damage than the treatment with SAME or vitamins alone (Hematoxylin-eosin; original magnification 80 $\times$).

Table 1

Effect of different treatments with SAdMe, vitamin C, and vitamin E on TAA-induced liver pathology

Rat groups	Inflammation	Necrosis	Bridging necrosis
Normal	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Normal + SCE	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
TAA	1.50 ± 0.22	2.33 ± 0.17	1.17 ± 0.36
TAA + CE	1.12 ± 0.17 [#]	1.53 ± 0.13 ^{**,##}	0.08 ± 0.08 ^{**}
TAA + SAdMe	0.93 ± 0.13 [*]	1.79 ± 0.15 ^{*,#}	0.09 ± 0.09 ^{**}
TAA + SCE	0.36 ± 0.05 ^{**}	0.74 ± 0.06 ^{**}	0.00 ± 0.00 ^{**}

^{*} or ^{**} $p < 0.05$ or 0.01 vs. TAA.

[#] or ^{##} $p < 0.05$ or 0.01 vs. TAA + SCE.

Normal, normal rat; SAdMe, *S*-adenosylmethionine; TAA, thioacetamide; TAA + CE, TAA + SAdMe, TAA + SCE, liver injury induced by TAA treated by vitamins C and E, by SAdMe, and by SAdMe plus vitamins C and E.