Vitamin E prevents cold wrap restraint stress-induced intestinal fluid transport alterations in rats

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S BURDICK, N CUI, LR EMPEY, RN FEDORAK. Vitamin E prevents cold wrap restraint stress-induced intestinal fluid transport alterations in rats. Can J Gastroenterol 1994;8(7):417-421. Psychological stress may alter gastrointestinal absorptive function by increasing the quantity of intestinal free radicals or by lowering endogenous intestinal free radical scavenging capacity. Vitamin E has been shown to be a potent endogenous antioxidant and free radical scavenger under both physiological and pathological conditions. The purpose of this study was to determine whether cold wrap restraint stress altered in vivo intestinal fluid absorption in rats, and whether vitamin E administration prior to the induction of cold wrap restraint stress could prevent such changes in intestinal secretion. Jejunal, ileal and colonic fluid and electrolyte transport rates were measured in vivo using an isolated loop technique. Cold wrap restraint stress reduced in vivo fluid absorption in the ileum and colon, but not in the jejunum. Administration of vitamin E prior to the cold wrap restraint stress procedure completely prevented this alteration of ileal and colonic fluid absorption.

Key Words: *Absorption, Fluid and electrolyte, Free radical, Intestine, Stress, Transport, Vitamin E*

La vitamine E prévient l'altération du transport des liquides dans l'intestin de rats soumis à un stress par enveloppement froid

RÉSUMÉ : Le stress psychologique peut altérer l'absorption gastro-intestinale en augmentant la quantité de radicaux libres ou en abaissant la capacité endogène de l'intestin à les piéger. La vitamine E s'est révélée être un puissant anti-oxydant endogène piégeur des radicaux libres dans des conditions, tant physiologiques que pathologiques. Le but de cette étude était de déterminer si un stress provoqué par un enveloppement froid modifiait *in vivo* l'absorption liquidienne de l'intestin chez le rat et si l'administration de vitamine E avant l'induction du stress par enveloppement froid pouvait prévenir de tels changements au niveau de la sécrétion intestinale. Le taux de transport des liquides et des électrolytes au niveau jéjunal, iléal et colique ont été mesurés *in vivo* à l'aide d'une technique de loupe isolée. Le stress induit par l'enveloppement froid a réduit l'absorption des liquides *in vivo* au niveau de l'iléon et du côlon, mais non pas au niveau du jéjunum. L'administration de vitamine E avant l'enveloppement froid a complètement empêché cette altération de l'absorption de liquide au niveau de l'iléon et du côlon.

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THE EFFECTS OF PSYCHOLOGICAL stress on gastrointestinal functions have been known for more than a century and a half (1). Stress can alter gastric acid secretion, intestinal motility, and fluid and electrolyte absorption rates in nondiseased intestine as well as in the intestine displaying inflammatory changes, irritable bowel syndrome or celiac sprue (2-4). While an endogenous mediator regulating and coordinating gastrointestinal secretory and motor responses following stress has not been identified, recent evidence has shown that stress is capable of upregulating the generation of reactive oxygen metabolites and that these reactive oxygen metabolites have physiological consequences (5,6).

Reactive oxygen metabolites exert a multitude of biological effect in the gastrointestinal tract, ranging from cell death to nontoxic alterations in the intestinal fluid and electrolyte transport, motility and mutagenic activity (7-11). Application of physiological, noncytotoxic concentration of hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) or monochloramine (NH_2Cl) to the serosal surface of rat colon mounted in Ussing Chambers elicits marked increases in net water and chloride sercetion (11,12). Furthermore, the H_2O_2- and NH_2Cl-mediated increases in chloride secretion are inhibited by atropine, tetradotoxin and piroxicam, suggesting that the cholinergic nervous system and prostaglandins may be important in this response (11,13). Taken to-

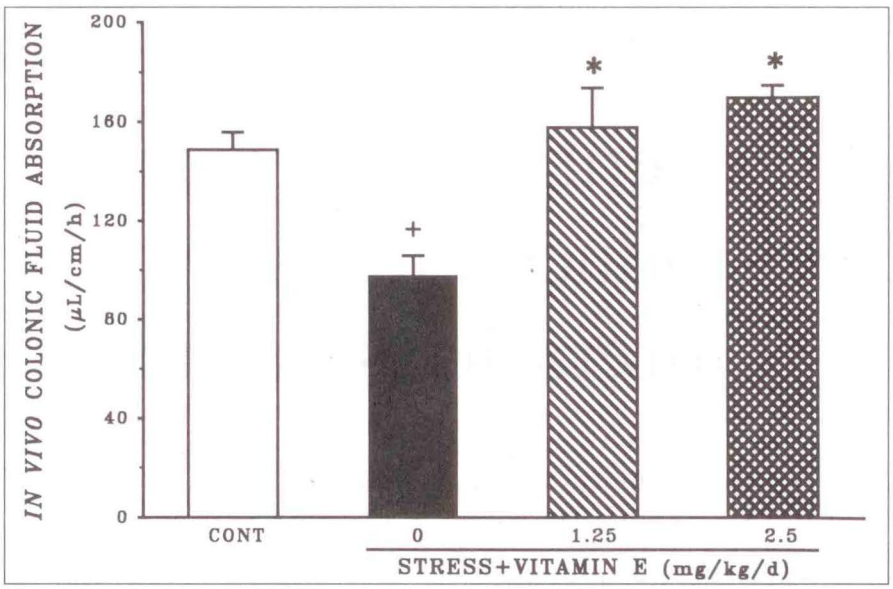

Figure 1) *Effect of vitamin E on in vivo colonic fluid absorption in rats with cold wrap restraint-induced stress. Values are mean ± SEM for n=6 rats. $^{+}P<0.05$ compared with nonvitamin E (sham)-treated nonstressed group. $^{*}P<0.05$ compared with the nonvitamin E-treated cold wrap restraint stressed group*

gether, these data suggest that some of the alterations in intestinal fluid and electrolyte transport associated with stress may be mediated by reactive oxygen species.

Reactive oxygen species are continuously produced as metabolic by-products by virtually all tissues in relatively small amounts. Under basal conditions, oxidant-generating systems in the intestine are compensated for by complex sets of protective mechanisms that prevent or limit oxidative damage. These mechanisms include antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) and nonenzymatic free radical scavengers (14,15). A free radical scavenger represents one type of antioxidant that is defined as any substance that will donate an electron to a free radical, thus inactivating the radical species. Alpha-tocopherol (vitamin E) has recently been identified as one of a number of biologically significant, endogenous, nonenzymatic free radical scavengers (15).

This study investigated whether alterations in functional intestinal fluid and electrolyte absorption occurred as a result of the induction of cold wrap restraint stress in rats and whether the administration of vitamin E, perhaps through its free radical scavenging effect, prevented the occurrence of these fluid transport abnormalities.

MATERIALS AND METHODS

L-alpha-tocopherol (vitamin E), Lot 86F-0370, purity 95%, was purchased from Sigma Chemical Company (Missouri). Alpha-tocopherol standard was purchased from Eastman Organic Chemicals (New York). Xylazine, 20 mg/mL, was purchased from the Bayvert Division of Chemagro Limited. Ketamine hydrochloride, 100 mg/mL, was purchased from MTC Pharmaceutical. The remainder of the chemicals used were reagent grade and were purchased from Sigma Chemical Co. The L-alpha-tocopherol was diluted with 25 mL of methyl cellulose to a final concentration of 5 μg/μL.

Animals: Male Sprague-Dawley rats, 250 to 300 g, were randomly divided into four groups: group 1, nonvitamin E (sham)-treated nonstressed controls; group 2, nonvitamin E (sham)-treated cold wrap restraint stressed animals; group 3, vitamin E-treated (1.25 mg/kg/day) cold wrap restraint stressed animals and; group 4, vitamin E-treated (2.5 mg/kg/day) cold wrap restraint stressed animals. All animals were given access to standard rat chow (Wayne Rodent Blox, Continental Grain Co, Illinois) and water ad libitum. A 12-h light-dark cycled animal care facility was used to hold the animals. Animals were allowed seven days to acclimatize to the environment before the studies began.

Vitamin E administration: Vitamin E (1.25 or 2.5 mg/kg/day in 1% methyl cellulose) was given once daily by intraperitoneal injection for seven consecutive days prior to stress administration. The control group received intraperitoneal injections of 1% methyl cellulose for the same length of time.

Cold wrap restraint stress: Cold wrap restraint stress was carried out as previously described (5). Briefly, nonfasting rats were lightly anesthetized with ether, and the forelimbs and shoulders were attached to the thoracic trunk with a harness of paper masking tape. The hind limbs were then taped to the body in similar fashion. The animals were put in the cage in a prone position and kept at 4°C for 2 h. While restrained, the animals were completely immobilized and remained in one position without struggling. Care was taken during the restraint stress process to prevent injury and to allow the limbs to rest in a neutral position in order to avoid providing a painful stimulus. These studies were carried out in accordance with the guidelines established by the University of Alberta Animal Health Sciences Committee.

In vivo intestinal fluid transport studies: Immediately following restraint stress the animals were anesthetized with a mixture of ketamine and xylazine (2:1, volume:volume) and kept warm with a thermostatic heat lamp. In vivo intestinal fluid absorption studies were then performed as previously described (16). The intestinal tract was exposed through a midline incision. An occluding ligature was placed at the cecal ascending junction, and the colonic luminal contents were flushed out the rectum with a warm 154 mM sodium chloride solution instilled via a cannula inserted though an incision just distal to the occluding ligature. Residual saline was emptied by gentle manual expression. A colonic loop approximately 12 cm long, beginning 2 cm distal to the

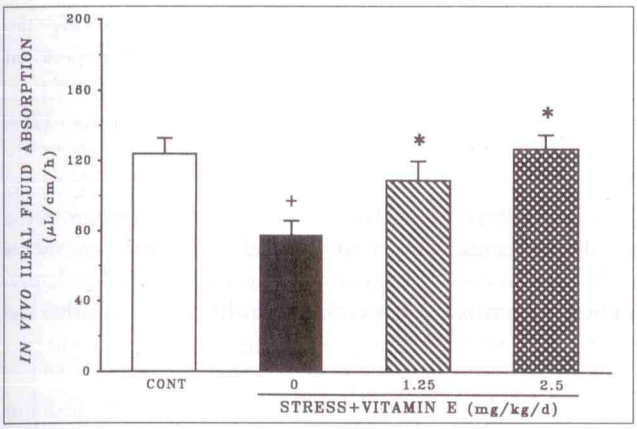

Figure 2) *Effect of vitamin E on in vivo ileal fluid absorption in rats with cold wrap restraint-induced stress. Values are mean ± SEM for n=6 rats. $^{+}P<0.05$ compared with nonvitamin E (sham)-treated nonstressed group. $^{*}P<0.05$ compared with nonvitamin E-treated cold wrap restraint stressed group*

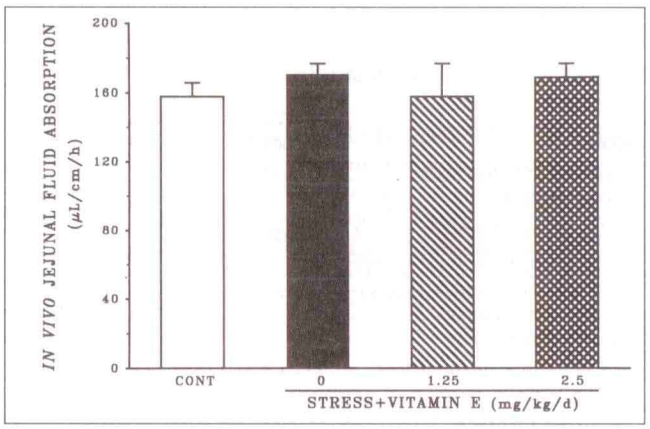

Figure 3) *Effect of vitamin E on in vivo jejunal fluid absorption in rats with cold wrap restraint-induced stress. Values are mean ± SEM for n=6 rats. $^{+}P<0.05$ compared with nonvitamin E (sham)-treated nonstressed group. $^{*}P<0.05$ compared with nonvitamin E-treated cold wrap restraint stressed group*

cecal-colonic junction and extending to the peritoneal reflection, was created with ligatures. In a similar fashion, the small intestine was occluded with ligatures placed at the ligament of Treitz and the ileocecal valve. A cannula was inserted distal to the ligature, and the contents were flushed with warm 154 mM sodium chloride. Loops approximately 12 to 15 cm long were created with ligatures in the jejunum (beginning 2 cm distal to the ligament of Treitz) and in the ileum (beginning 2 cm proximal to the ileal-cecal valve). Care was taken not to compromise mesenteric, vascular or neural continuity. A 26-gauge needle was inserted obliquely through the outer muscle layer along the antimesenteric border, and 2 mL of 154 mM sodium chloride prewarmed to 37°C was instilled into each empty loop. The loops were checked for leakage. Previous studies using dye markers had confirmed that leakage does not occur from the intestinal loops over the 1 h duration of the experiment (16). The viscera were returned to the abdominal cavity and the incision was closed. The rats then were placed in a recovery chamber for 1 h. Animals were given an intraperitoneal overdose of pentobarbitol (250 mg/kg), and the loops were removed. The length of each loop was measured, and the weight of the loop (both full and empty) was recorded to determine the residual intraluminal volume. Results were expressed as the difference between initial and residual luminal volume per centimetre of bowel per hour.

Serum vitamin E determination: Blood samples were taken at the time of pentobarbitol overdose and serum vitamin E levels were determined using method established by Catignani and Bieri (17). Briefly, serum was deproteinized with ethanol that contained the internal standards (retinyl acetate and alpha-tocopheryl acetate), and the lipid was extracted with hexane. After an aliquot of the solvent phase was evaporated, the residue was dissolved in diethyl ether and diluted with methanol. A portion of this solution was injected onto a C18 reversed-phase chromatographic column, and absorbance of the vitamin and internal standards measured at 280 nm. Peak-height ratios were used to quantify vitamin concentration.

Statistics: Statistical analysis of the data was completed using the Student-Newman-Keuls multiple range test. Values are presented as mean ± SEM.

RESULTS

Animal groups: Cold wrap restrained rats were completely immobilized and remained in the prone position at the bottom of the cage without moving throughout the restraint period. Body temperature remained constant at 37°C. Following the period of restraint, stress animals were immediately anesthetized and in vivo fluid transport measurement carried out. Cold wrap restraint stress has previously been shown to cause gastric ulceration (5); therefore, the authors carefully searched for gross ulceration along the small and large intestine. There was no evidence of macroscopic ulceration in any animal used in these experiments.

Administration of vitamin E to sham-operated control animals: Administration of vitamin E to sham-operated control animals did not alter in vivo fluid and electrolyte absorption in the jejunum (148±15 μL/cm/h), ileum (118±12 μL/cm/h) or colon (163±14 μL/cm/h).

Colonic in vivo fluid absorption: As shown in Figure 1, in vivo net colonic fluid absorption was significantly decreased in the nonvitamin E (sham)-treated cold wrap restraint stressed group of rats relative to the level found in nonstressed controls. In contrast, cold wrap restraint stressed animals that received vitamin E supplementation for seven days prior to the period of stress did not show a decrease in colonic fluid absorption. This protective effect of vitamin E was seen at both doses (1.25 and 2.5 mg/kg/day) examined.

Ileal in vivo fluid absorption: Figure 2 shows that in vivo net ileal fluid absorption was significantly decreased in the nonvitamin E (sham)-treated cold wrap restraint stressed group. Similar to the results seen in the colon, supplementation with vitamin E for seven days prior to the period of stress prevented the ileal fluid absorption from decreasing.

Jejunal in vivo fluid absorption: As shown in Figure 3, in vivo net jejunal

TABLE 1
Serum vitamin E levels after seven days of vitamin E administration

Group	Vitamin E (mmol/L)
Nonvitamin E-treated nonstressed	10.6±1.2
Nonvitamin E-treated cold wrap restraint stressed	10.8±0.9
Vitamin E (1.25 mg/kg/day)-treated cold wrap restraint stressed	18.1±0.8*
Vitamin E (2.5 mg/kg/day)-treated cold wrap restraint stressed	22.9±1.2*

*Values are mean ± SEM for n=6 rats. *P<0.02 compared with nonvitamin E-treated animals*

fluid absorption was not altered by either cold wrap restraint stress or supplementation with vitamin E.

Serum vitamin E levels: Table 1 shows the serum vitamin E levels for each group. Serum vitamin E levels were significantly elevated, in a dose dependent fashion, in each vitamin E supplemented group.

DISCUSSION

Under physiological conditions free radicals are continuously being produced. In uncontrolled states, the free radicals are able to induce membrane lipid peroxidation. As a consequence of lipid peroxidation, intestinal membrane functions (including transport processes) are markedly impaired. The lipid hydroperoxides, which are formed during lipid peroxidation, can form clusters which create pores in the membrane through which ions can diffuse into the cells; enzymes are inactivated, membrane fluidity decreases, signal transduction is decreased and cytotoxic aldehydes are formed (18).

In order to prevent or minimize free radical-induced tissue damage, antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase mop up excess free radicals. In addition, antioxidants can prevent the formation of free radicals or interfere with the propagation steps of the lipid peroxidation process.

Vitamin E is the major lipid-soluble, peroxyl radical-trapping, chain-breaking antioxidant in human blood (19,20). Vitamin E consists of four tocopherols and four tocotrienols which are able to interfere with the propagation steps on the lipid peroxidation process. L-alpha-tocopherol is, in vivo, the most abundant and the most bioactive tocopherol (21). The tocotrienols have an unsaturated side-chain which likely functions to retain the molecule in the membrane, while the tocopherol, with its chroman head-group, is responsible for the majority of the antioxidant activity (6,22,23).

Cold stress has been shown to alter both the production of free radicals and the function of the free radical-trapping defence system (24). In rats exposed to cold stress for short intervals of time, physiological free radical-trapping is reduced as a consequence of diminished superoxide dismutase activity (6). In rats exposed to cold stress for longer intervals of time, the activity of glutathione-S-transferase, a major antioxidant, is decreased in liver and adipose tissue, while glutathione levels are markedly reduced in plasma (24).

Physical and psychological stresses have been shown to alter gastrointestinal physiological function in humans and animals (25-29). Psychological stress experienced during dichotomous listening significantly reduced human jejunal fluid absorption through a function of the cholinergic parasympathetic nervous mechanism (25). In animal studies, restraint-based stress-induced changes in intestinal transit and transport were partially mediated by endogenous central nervous system corticotrophin-releasing factor (28,29). Recently, psychological stress has been shown to reduce intestinal bloodflow in animals (30). Indeed, oxygen free radicals represent a critical factor in the development of gastrointestinal injury after intestinal ischemia and reperfusion have occurred (31).

In the present study, cold wrap restraint stress reduced basal in vivo intestinal fluid absorption in the ileum and colon, but not in the jejunum. This regionalization of beneficial effects by mucosal protective agents in the intestine are similar to those previously described in other experimental models of intestinal injury (16). In addition, divergent effects of stress on the small intestine have been previously described and emphasize the independent regulation that exists in different regions of the gastrointestinal tract (29). The decrease in ileal and colonic absorption in the stressed animals occurred in the absence of gross macroscopic ulceration, and thus likely represents changes at the membrane level; perhaps related to alterations in either the transport carrier or in membrane permeability.

Table 1 shows that cold restraint stress does not alter the vitamin E content in serum, and previous investigations have confirmed that cold stress does not alter vitamin E levels in the rat small intestine (24). This result suggests that, under the conditions of cold restraint stress, the vitamin E concentration is maintained at sufficient levels to ensure the integrity of the cellular membrane against the action of basal antioxidant enzymes. Whether a functional disturbance in vitamin E antioxidant activity exists or whether an elevated level of oxidant activity is present under conditions of restraint stress remains to be determined. Vitamin E administration for seven consecutive days prior to the administration of cold wrap restraint stress resulted in a significant elevation in serum vitamin E levels. It is thus possible that this elevated level of vitamin E might have overridden any functional deficit or elevated oxidant activity induced in the stressed rats.

In the present study, intraperitoneally administered vitamin E reversed the net intestinal secretory response in the ileum and colon of cold wrap restraint stressed rats to control levels. This effect was seen at doses of both 1.25 and 2.5 mg/kg/day over seven days. The doses are equivalent to those that might be administered to humans under physiological conditions. It is unlikely that the vitamin E directly stimulated intestinal sodium and chloride absorption because vitamin E alone administered to control rats did not alter intestinal fluid absorption rates. It is possible that, during the cold wrap restraint stress, the antioxidant defence mechanisms provided by endogenous levels of normally functioning vitamin

E are inadequate to scavenge increased levels of oxygen free radicals or, alternatively, that vitamin E antioxidant activity is functionally impaired and cannot scavenge baseline levels of oxygen free radicals. Oxygen free radicals in the intestine under these conditions could thus be responsible for the observed alterations in fluid and electrolyte transport. Nevertheless, the exact mechanism responsible for the stress-related effects and the protective effects of vitamin E remain to be determined.

In summary, cold wrap restraint-induced stress reduced in vivo fluid absorption in the ileum and colon, but not in the jejunum. Administration of vitamin E prior to the cold wrap restraint process prevented the alteration of fluid absorption rates in both the ileum and colon.

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