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Research Article

Diamine oxidase (DAO; EC 1.4.3.6) is an enzyme found in high activity in the mature cells of the upper villus of rat small intestinal mucosa and in very much lower activity in all other tissues in the nonpregnant rat. This study was designed to determine whether a provocative test for increasing the level of plasma DAO activity by heparin administration could be used to monitor the extent and severity of acute, severe, small intestinal mucosal injury. In adult rats, small intestinal loops of varying lengths were perfused with 2,100 mosM sodium sulfate solution for 60 min to produce selective damage to villus epithelium. Plasma postheparin DAO (PHD) activity (180 min after 400 U/kg i.p. heparin) was measured 7 h after initiation of perfusion. With increasing length of intestinal mucosal injury, there was a progressive decrease in both basal and plasma PHD activity. The decrease in plasma PHD activity closely reflected the length of intestinal mucosa injured (n = 128, r = 0.86, P less than 0.001), and it was much more sensitive (threshold limit of detection = 13% of total length, range = 67 U/ml for 100% length of injury) than unstimulated basal levels of plasma DAO (threshold = 40%, range = 2.1 U/ml). Our previous data have suggested that DAO is unique among intestinal mucosal enzymes in that circulating […]

Plasma Postheparin Diamine Oxidase

SENSITIVE PROVOCATIVE TEST FOR QUANTITATING LENGTH OF ACUTE INTESTINAL MUCOSAL INJURY IN THE RAT

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ABSTRACT Diamine oxidase (DAO; EC 1.4.3.6) is an enzyme found in high activity in the mature cells of the upper villus of rat small intestinal mucosa and in very much lower activity in all other tissues in the nonpregnant rat. This study was designed to determine whether a provocative test for increasing the level of plasma DAO activity by heparin administration could be used to monitor the extent and severity of acute, severe, small intestinal mucosal injury. In adult rats, small intestinal loops of varying lengths were perfused with 2,100 mosM sodium sulfate solution for 60 min to produce selective damage to villus epithelium. Plasma postheparin DAO (PHD) activity (180 min after 400 U/kg i.p. heparin) was measured 7 h after initiation of perfusion. With increasing length of intestinal mucosal injury, there was a progressive decrease in both basal and plasma PHD activity. The decrease in plasma PHD activity closely reflected the length of intestinal mucosa injured ($n = 128$, $r = 0.86$, $P < 0.001$), and it was much more sensitive (threshold limit of detection = 13% of total length, range = 67 U/ml for 100% length of injury) than unstimulated basal levels of plasma DAO (threshold = 40%, range = 2.1 U/ml). Our previous data have suggested that DAO is unique among intestinal mucosal enzymes in that circulating levels can serve as a marker of mucosal injury; this study illustrates that the addition of a low-dose heparin administration enhances the use of DAO even further as a sensitive, quantitative, circulating marker for monitoring the extent of small intestinal mucosal injury in the rat.

INTRODUCTION

The diagnosis and management of patients with limited, minimal, or mild intestinal[1] diseases can present a clinical challenge as there is often a paucity of intestinal signs and symptoms (1, 2). Even the use of clinical severity indices has not provided completely satisfactory methods for diagnostic and management studies in these patients (3–7). The development of a sensitive and reliable marker of intestinal mucosal disease would be important and useful.

Previous studies from our laboratory have shown that the activity of the mucosal enzyme diamine oxidase (DAO;[2] EC 1.4.3.6) might serve as a marker of intestinal mucosal maturation and integrity, and of mucosal injury and recovery (8, 9). DAO is an intracellular enzyme found in high activity in the intestinal mucosa of all mammalian species studied, including humans (10). In the rat, the intestinal mucosa is the tissue containing the highest activity of DAO (8, 11) and this activity is localized primarily within the cytoplasm (12) of the mature, differentiated upper villus cells (13, 14). Unlike the disaccharidases, DAO is measurable in peripheral blood; and unlike circulating alkaline phosphatase activity, which may emanate from multiple tissue sources, plasma DAO may be a relatively specific plasma marker of the maturity and integrity of the intestinal epithelium. All other tissues in the rat, except for the placenta in the pregnant animal, contain <5% of the DAO activity of the intestinal mucosa (8).

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[1] Intestine as used in this paper refers to small intestine only.

[2] *Abbreviations used in this paper:* ara-C, 1-β-D-arabinofuranosylcytosine; DAO, diamine oxidase; PHD, postheparin DAO.

Plasma DAO activity increases as the intestinal mucosa matures in the newborn rat, and DAO activity decreases in the adult rat as the mucosa is progressively injured with exposure to hyperosmolar solutions (8). Plasma DAO activity also decreases with progressive mucosal injury after administration of 1-β-D-arabinofuranosyl cytosine (ara-C), a chemotherapeutic agent, and increases in parallel with subsequent mucosal recovery (9). Circulating DAO changes in parallel with these changes in mucosal status, thus, this enzyme might provide a circulating marker for the status of the intestinal mucosa. However, our previous studies showed that unstimulated plasma DAO was not adequately sensitive to detect mild degrees of mucosal injury, as plasma DAO only showed a 12% reduction with moderate injury and no reduction with minimal injury (8).

In this study, we have sought to improve the sensitivity of plasma DAO as a monitor of intestinal mucosal integrity and injury. Heparin administration is known to increase plasma DAO activity in man and other mammalian species (11, 15–18). Several studies suggest that the source of this plasma postheparin DAO (PHD) in the rat is the intestine (11, 19, 20). We investigated whether heparin administration could serve to enhance the sensitivity of DAO as a marker of the extent of acute, severe mucosal injury produced by perfusions of increasing lengths of the intestine with hyperosmolar sodium sulfate solutions. Our studies show that plasma PHD activity can serve as a sensitive and quantitative marker of the extent or length of mucosal injury in the rat.

METHODS

Animals. Adult female Wistar-Lewis rats weighing 200–220 g were purchased from Charles River Breeding Laboratories, Inc., Wilmington, MA. These animals were allowed to acclimate to our animal facilities for 2 wk before using them in our studies. They were kept four to a cage and housed with 12-h light (7 a.m.–7 p.m.) and 12-h dark cycles, and given regular laboratory rat chow and water ad lib.

Hyperosmolar mucosal injury model. The hyperosmolar perfusion model was previously described (8). Animals were fasted overnight, and anesthetized between 7 and 8 a.m. with sodium pentobarbital, 45 mg/kg body wt i.p. After adequate anesthesia, the peritoneal cavity was opened and the small intestine exposed from the ligament of Treitz to the ileocecal junction. Total intestinal length (excluding duodenum) was measured; the average length was 75 cm. Intestinal loops of increasing lengths, beginning 2 cm proximal to the ileocecal junction and extending proximally for 10 cm to 75 cm (the entire intestine), with their blood supply intact, were prepared. Inflow and outflow catheters were inserted and the loops were returned to the peritoneal cavity, which was then closed. The distal end of the proximal nonperfused intestinal segment was vented with a catheter. The test loops were perfused with sodium sulfate solutions of 1,400 or 2,100 mosM concentrations for 30 or 60 min, at a rate of 30 ml/h and a pressure of 10 cm of water. At the end of the hyperosmolar perfusion period, the animals were kept anesthetized for another 6 h. The loops were then perfused with normal saline at the same rate of 30 ml/h and the same pressure of 10 cm of water. The perfusate was collected and frozen every 30 min. Hydration was maintained with parenteral normal saline, 2 ml/h. Sodium heparin, 400 U/kg body weight, was given intraperitoneally 4 h after the initiation of hyperosmolar perfusion, when sloughing of cells and loss of mucosal cellular contents into the lumen was complete. 3 h after heparin administration (or a total of 7 h after the initiation of hyperosmolar perfusion), when the increase in PHD activity was maximal, blood was removed by cardiac puncture, the intestinal loops were removed, and the animals were killed. 5-mm sections of the proximal segment of the perfused intestinal loop and the adjacent unperfused control segment were fixed in formalin and Hollande's fixative for histologic studies, and the adjacent sections were used for enzyme assays. For perfusion studies of the entire length of the intestine, the most proximal 5-mm segment (proximal jejunum) and the most distal 5-mm segment (distal ileum) as well as a 5-mm segment from the middle of the intestine, were used for histologic and enzyme assays.

Mucosal, basal, and PHD correlation. Studies were then done to correlate the dynamics of changes in unstimulated plasma DAO and plasma PHD activities with the changes in mucosal DAO activities during different degrees of injury. Normal untreated rats and rats that had their entire intestinal lumen perfused were used. Blood was obtained from the tail vein, and then sodium heparin at 4, 10, 40, 100, 400, 1,000, and 4,000 U/kg was given intraperitoneally to the animals. Blood was then obtained for DAO assay via the tail vein at 30-min intervals, up to 6 h after the heparin administration. Mucosa was then obtained at death for DAO assay. In another group of animals, heparin was given intravenously via the tail vein, to determine the differences in the levels of plasma DAO after intraperitoneal and intravenous heparin. Because the peak levels in PHD were similar for both routes of administration, heparin was given intraperitoneally for all other studies, and plasma PHD activities measured 180 min after intraperitoneal heparin administration, when the increase in PHD activity was maximal.

Assay methods. DAO activity was assayed by the method of Beaven and Jacobsen (21) as modified in our previous studies (8, 9), measuring tritiated water formed upon deamination of β[^{3}H]histamine, and results were expressed where 1 U represents 1 picomole of histamine deaminated per hour at 37°C. Disaccharidases were assayed by the method of Dahlqvist (22), measuring glucose formed upon hydrolysis of the appropriate substrates of sucrose, maltose, and lactose, and results were expressed as 1 U is equal to μmol of substrate hydrolyzed per minute at 37°C. Protein determination was done by the method of Lowry et al. (23); and DNA determination, by the diphenylamine reaction (24). Unpaired *t* test, linear and nonlinear regression was used to evaluate the correlation between length of intestinal segment injured and plasma DAO activities.

RESULTS

Mucosal injury model. We first extended our previous data to validate hyperosmolar perfusion as a graded intestinal mucosal injury model. Histologically, hyperosmolar sodium sulfate solutions produced different degrees of selective damage to the mature villus cells, while sparing the proliferative crypt cells (Fig. 1), similar to the previously described injury in rabbit

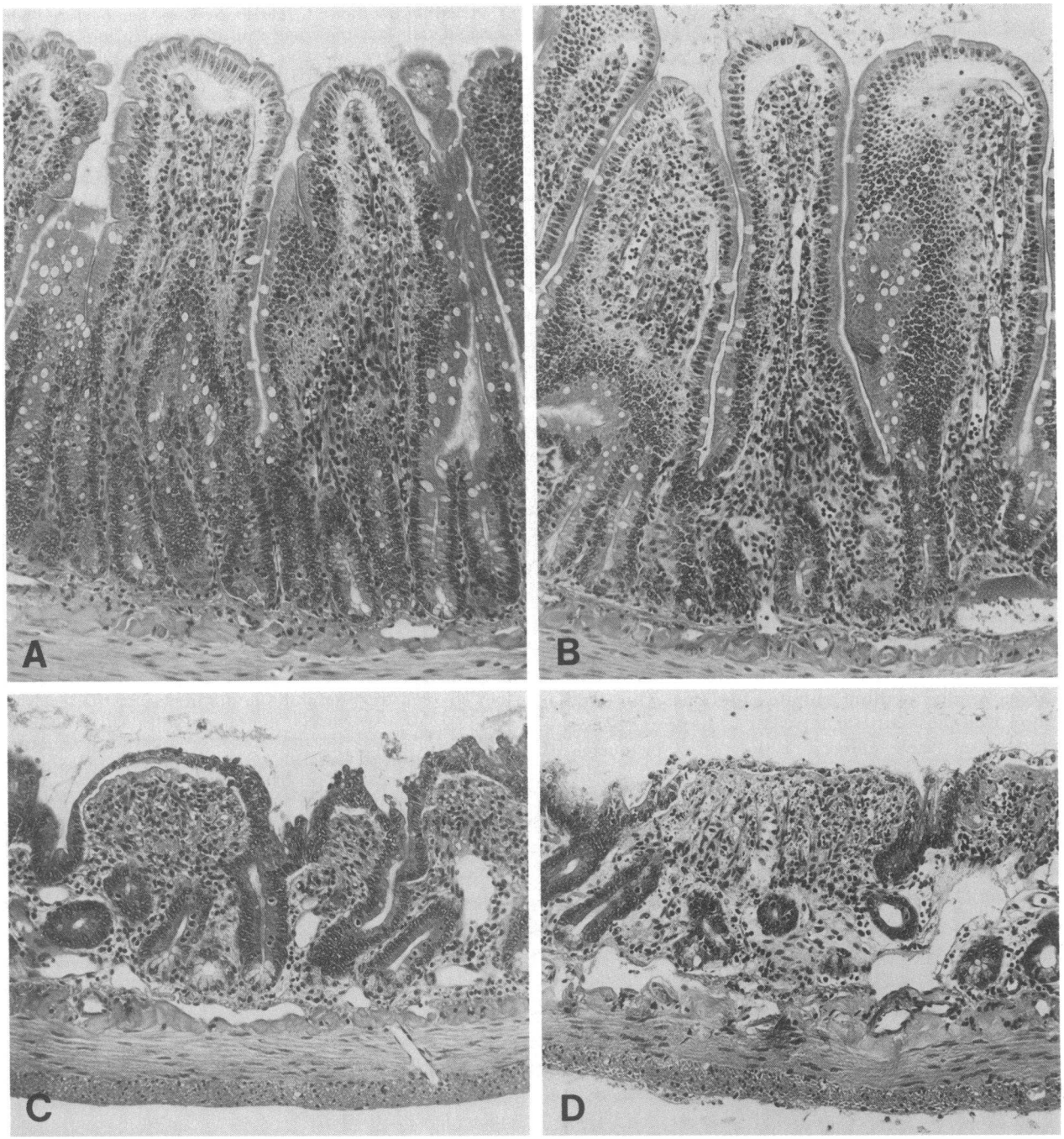

FIGURE 1 Histology of the mucosal injury produced by perfusion of the entire length of the intestine with hypertonic sodium sulfate in the proximal jejunum, just distal to the ligament of Treitz. A, normal control; B, minimal injury produced by 1,400 mosM perfusion for 30 min: columnar epithelial cells appear normal, with only a few pyknotic nuclei; C, moderate injury, produced by 2,100 mosM perfusion for 30 min: there is a loss of contour of epithelial cells, a moderate number of pyknotic nuclei, and lymphatic dilation; D, severe injury, produced by 2,100 mosM perfusion for 60 min: epithelial cells are markedly shortened and cuboidal, with dense-staining cytoplasm and pyknotic nuclei. Some sloughing of mucosal cells has occurred. The pattern of histological injury in the proximal, middle, and distal intestinal segments were similar in the eight animals studied.

jejunum (25). Increasing concentrations and perfusion times produced progressively more severe damage to the villus mucosa. The extent of injury in hyperosmolar perfusion studies of the entire length of the intestine was similar in the proximal, middle, and distal intestine for any given osmolarity, as examined histologically and morphometrically (Table I). With this increasing damage to the entire length of the intestine, there were progressive and statistically significant decreases in multiple enzyme activities; reduction first occurred in lactase, followed by maltase, sucrase, and finally mucosal DAO. The decreases in mucosal DAO were followed by a significant decrease in unstimulated basal plasma DAO and a marked decrease in plasma PHD (Fig. 2). The decreases in both basal plasma DAO and plasma PHD were similar when segments of equal length of either proximal, middle, or distal intestine were injured by hyperosmolar perfusion. Even though there were significant decreases in all enzyme activities, only plasma PHD showed a significant decrease with every increment of mucosal injury.

We then studied more directly the relation between the dynamics of mucosal injury and the loss of mucosal DAO activity. In the loops perfused with 2,100 mosM solution for 60 min, the luminal contents of protein and DNA increased by 30 min, was maximal at 60 min, and then declined, documenting an intraluminal loss of mucosal cellular material that was complete by 4 h (Fig. 3). Concurrently, luminal content of DAO activity also underwent a similar increase and decrease in parallel, documenting intraluminal loss of mucosal DAO enzyme activity (Fig. 3). The time course of this intraluminal loss of mucosal cellular material paralleled the time course of the histological injury observed: Both processes increased with time of hyperosmolar perfusion.

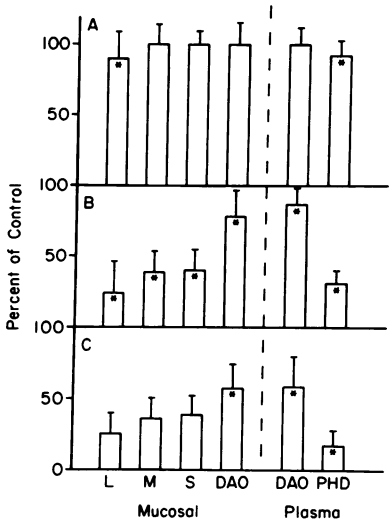

FIGURE 2 Effect of graded mucosal injury from hyperosmolar perfusion on mucosal disaccharidases and DAO activities, and plasma, unstimulated basal DAO and PHD activities, all measured 7 h after initiation of perfusion, A, Minimal injury after 1,400 mosM for 60 min; B, moderate injury after 2,100 mosM for 30 min; and C, severe injury after 2,100 mosM for 60 min. L, lactase; M, maltase; S, sucrase; DAO, and PHD (180 min after 400 u/kg heparin i.p.). Specific enzyme activities were used for calculation as percent of control, with mucosal activities expressed as units per milligram of protein and plasma activities as units per milliliter of plasma; n = at least 7 in each group; bars represent the SEM; *, statistically significant difference when A was compared with normal controls, B was compared with A, and C was compared with B.

Mucosal, basal, and plasma PHD correlation. We then sought to find how changes in plasma PHD activity compared with changes in basal plasma DAO as a measure of intestinal mucosal DAO content. Ini-

TABLE I
Effect of Hyperosmolar Perfusion of the Entire Length of the Intestine on the Mucosal Villus Height and Crypt Depth

		Perfusate and (injury)			
	Perfusate: / Injury:	None (none)	1,400 mosM × 30 min (minimal)	2,100 mosM × 30 min (moderate)	2,100 mosM × 60 min (severe)
Proximal jejunum (n = 8)	Villus height, μm	396±47	367±49	125±38	78±22
	Crypt depth, μm	187±52	192±48	188±52	194±61
Midintestine (n = 8)	Villus height, μm	343±39	321±42	108±29	67±21
	Crypt depth, μm	196±43	188±40	192±46	186±52
Distal ileum (n = 8)	Villus height, μm	294±32	272±39	94±32	57±23
	Crypt depth, μm	192±46	194±49	185±52	188±55

Histological injury is graded as for Fig. 1. Values given are means±SEM.

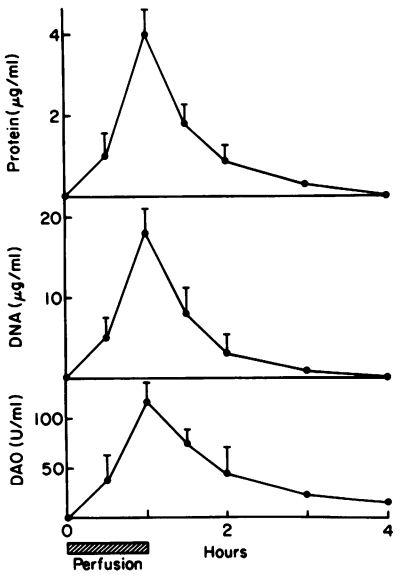

FIGURE 3 Effect of perfusion of 2,100 mosM sodium sulfate solutions for 60 min on luminal contents of protein, DNA, and DAO activity. n = 5; bars represent the SEM and are not drawn where SEM is less than twice the height of the symbol.

tially, we sought to confirm the dynamics of the heparin-mediated increase in plasma DAO activity in our normal animals. Parenterally administered heparin produced an increase in plasma DAO activity (Fig. 4). The peak DAO activity after intravenous heparin occurred at 30 min, with a rapid decline to base line by 90 min. The increase in DAO activity after intraperitoneal heparin was more gradual, with the peak at ~180 min, and the decline to base line only after 360 min. The maximal increase in plasma DAO activity after heparin administration was dose-dependent; there was a linear relationship between the maximal plasma PHD activity and the heparin dose for all heparin doses used, ranging from 4 to 4,000 U/kg. Even doses as low as 4 U/kg increased plasma DAO by two- to threefold. For subsequent studies, the dose of 400 U/kg was used because that was the highest dose used that still had only a minimal effect on the clotting time (20% higher than control) and the partial thromboplastin time (23% higher than control) of the rats used. As the levels of plasma DAO activity increased, the mucosal content of DAO enzyme activity declined progressively, again documenting that the site of release of DAO is the mucosa (Fig. 4).

We then studied the effects of heparin administration on mucosal and plasma DAO activities in individual animals in which the entire length of the intestinal mucosa was selectively damaged with hyperosmolar sodium sulfate perfusion. There was a correlation between mucosal and basal plasma DAO activity (n = 42, r = 0.79, $P < 0.01$) with varying degrees of injury (Figs. 2 and 5). The correlation held whether results were expressed as specific activity (Fig. 2) or as total enzyme activity. The correlation between mucosal and PHD activity at 180 min after 400 U/kg intraperitoneal heparin was even higher (n = 34, r

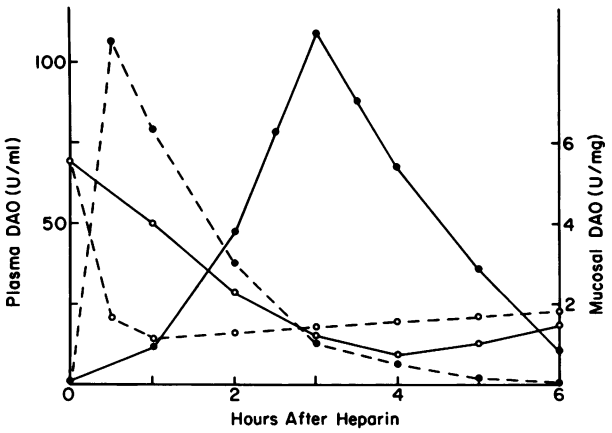

FIGURE 4 The time course of the effects of parenteral heparin on plasma and mucosal DAO activities. Heparin, 400 U/kg, was given either intravenously (- - -), or intraperitoneally (——), at time zero. Plasma DAO activities are denoted by ● and mucosal DAO activities by O. SEM was <10% of the mean for all values shown.

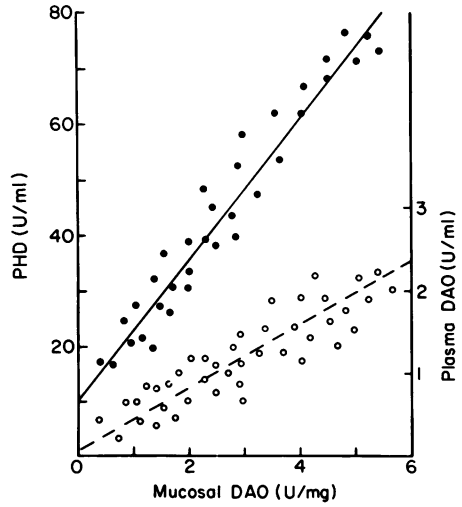

FIGURE 5 Correlation of basal mucosal DAO activity with plasma basal DAO and PHD activities 7 h after the initiation of mucosal injury by hyperosmolar sodium sulfate perfusion. Basal mucosal and plasma DAO activities were obtained before heparin DAO and PHD activity obtained 180 min after 400 U/kg heparin was given intraperitoneally. Basal plasma DAO activities are denoted by O (n = 42, r = 0.79, $P < 0.01$); PHD activities, by ● (n = 34, r = 0.89, $P < 0.01$).

= 0.89, $P < 0.01$), and the absolute difference in DAO activities was also much higher (Fig. 5). Thus, we continued our studies of plasma PHD activity by measuring the peak plasma activity at 180 min after 400 U/kg intraperitoneal heparin.

Length of injury. We then examined the correlation between plasma basal DAO and PHD activities and increasing length of injury of intestine. There was a correlation between plasma basal DAO activity and length of mucosal injury. However, the decrease in plasma basal DAO activity was statistically significant only when the length of injury was 45 cm or greater; therefore, the threshold limit of detection of plasma basal DAO activity is injury of >40% of the total length of intestinal mucosa. The correlation between plasma basal DAO activity and length of mucosal injury for lengths of 45 cm or greater was statistically significant ($n = 31$, $r = 0.67$, $P < 0.01$) and the total range of plasma basal DAO activity was 2.1 U/ml (Fig. 6).

The decreases in plasma PHD activity, however, were statistically significant for all lengths of intestinal mucosal injury studied (Fig. 6). The threshold limit of detection of a decrease in plasma PHD is injury of <13% of the total length of the intestinal mucosa. There was a highly significant correlation between the length of intestinal mucosa injured by hyperosmolar perfusion and the decrease in plasma PHD activity from control levels ($n = 136$, $r = 0.89$, $P < 0.01$), and the total range of plasma PHD was 67 U/ml (Fig. 6). Results were similar whether the extent of mucosal injury was expressed as absolute length of injury or as a percentage of total intestinal length in each individual animal.

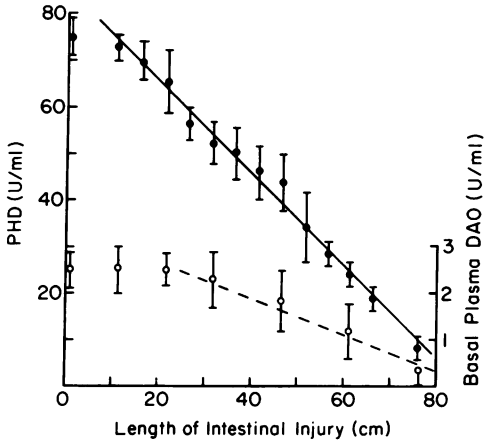

FIGURE 6 Effect of increasing length of intestinal mucosal hyperosmolar injury on plasma basal DAO (○) and PHD (●) activities 7 h after initiation of injury. (PHD activity was measured 180 min after 400 U/kg heparin i.p.). Bars denote±SEM. The mean basal DAO activities were statistically significantly different from control only when length of intestinal mucosal injury was 45 cm or greater ($P < 0.05$). The mean decrease in basal DAO activity was significantly correlated with the length of intestinal mucosal injury for lengths of 45 cm or greater ($n = 31$, $r = 0.67$, $P < 0.01$). The mean PHD activities for all lengths of intestinal mucosa injured were statistically significantly different from control ($P < 0.05$). The mean decrease in PHD activity was also significantly correlated with the length of intestinal mucosal injury ($n = 136$, $r = 0.89$, $P < 0.01$).

DISCUSSION

Our present data extend and confirm our previous report that in the rat, perfusion of the intestinal lumen with hyperosmolar sodium sulfate solutions produces a dose-dependent, selective mucosal villus cell damage that can be used as a model for study of intestinal mucosal injury (8). In addition, plasma DAO can serve as a monitor of the severity of mucosal injury. Our present results also confirm the results of other investigators (11, 19) and from our own laboratory (17, 18, 20) that heparin mobilization of DAO from the mucosa accounts for increases in plasma DAO activity seen after administration of this polyanionic compound. Our results further document that the vascular release of DAO by heparin (20) must involve the villus tip cells as these are the prime source of the enzyme. Most important, our present results show that a simple provocative test, using low doses of heparin, greatly enhances the use of circulating DAO as a sensitive and quantitative circulating marker of intestinal mucosal injury.

It is likely that plasma PHD activity might also reflect the severity and extent of mucosal injury in humans. In humans, the intestinal mucosa is again the tissue containing the highest DAO activity and the postheparin increase in plasma DAO activity is similar to that seen in rats (17, 18, 26). The doses of heparin required would be low, in the order of 10–50 U/kg for one dose, and are well within the prophylactic dosage of 5,000 U every 8–12 h used for prevention of deep vein thrombosis in humans; such doses have consistently been shown not to change coagulation test results (27, 28).

One of the many challenges in the diagnosis and management of intestinal diseases is that posed by the patient with minimal, limited, or subclinical disease. Often these patients present minimal signs and symptoms that are not obviously related to the intestinal tract. The patient with celiac disease limited only to the duodenum and proximal jejunum may have no intestinal symptoms and present only a hypochromic, microcytic anemia that is refractory to oral iron therapy (1). The patient with Crohn's disease with limited involvement may present only a low body weight (2).

After appropriate diagnosis, often by intestinal mucosal biopsies, their management is difficult because of the paucity of signs and symptoms to follow. The development of various clinical indices, which has helped in the stratification of patients with varying degrees of disease severity, has not been satisfactory in the management of patients with limited, mild, or minimal disease (3–7). The availability of a sensitive and quantitative index of limited mucosal disease, such as the plasma PHD activity, may prove useful in the monitoring and management of these patients.

Another challenge in clinical medicine is the monitoring of disease severity in the critically ill patient, in whom the present diagnostic tools of contrast radiography and mucosal biopsy are contraindicated (1, 2, 9). The availability of a simple, noninvasive test, such as the plasma PHD activity, which is easily measured and could serve as an index of the degree and extent of mucosal injury, would be extremely useful. This is especially important in the patient who is undergoing cancer chemotherapy. For these patients, optimal therapy is often limited by the intestinal mucosal toxicity of the drugs and agents used. The malnutrition, fluid and electrolyte imbalance, and colonization of the intestinal tract by pathogens, resulting in part from the mucosal injury, are all important causes of morbidity and mortality (For references, see reference 9). Preliminary data in our laboratory have already shown that basal plasma DAO is a useful marker of mucosal injury and subsequent recovery in patients with acute leukemia being treated with ara-C and may eventually prove helpful in the management of these severely ill patients (9). Preliminary results have also documented that plasma PHD is more sensitive than plasma basal DAO as an index of ara-C mucosal injury in the rat (unpublished results). PHD may eventually be clinically useful in determining the timing of oral refeeding and of subsequent courses of chemotherapy in man. Further studies on plasma PHD as a sensitive provocative test for quantitating the degree and extent of mucosal injury, based on the animal data presented in this paper, is now warranted.

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