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Alterations in Intestinal Permeability After Thermal Injury

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● Alterations in intestinal permeability have been postulated to occur after thermal injury. We evaluated the status of intestinal permeability during the first 2 postburn weeks in 15 subjects by measuring the differential excretion of enterally administered lactulose and mannitol. The mean age and burn size of the patients were 32.7 ± 3.6 years and $53.3\% \pm 5.1\%$ of the total body surface area, respectively. Ten healthy volunteers were also studied. The lactulose-mannitol excretion ratio was 0.159 ± 0.017 for the patients and 0.017 ± 0.003 for controls. The increased ratio did not correlate with burn size or postburn day. Patients who developed significant clinical infections during their first 2 postburn weeks had lactulose-mannitol ratios on postburn day 2 that were significantly higher than those of controls and patients who did not develop infections. This suggests a relationship between susceptibility to infection and early alterations in intestinal permeability.

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Sepsis and multiple-organ failure contribute significantly to morbidity and mortality after thermal injury. In recent years, the gastrointestinal tract has been implicated in the development of multiple-organ failures. It has been proposed that enteric organisms or their toxins translocate across the intestinal mucosa, enter the systemic circulation, and contribute either directly or indirectly to the development of the hypermetabolic and catabolic responses associated with injury and sepsis.

Berg¹ defined bacterial translocation as the passage of viable bacteria from the gastrointestinal tract to the mesenteric lymph nodes and other organs. Conditions that promote translocation include alteration of the indigenous gastrointestinal tract microflora leading to bacterial overgrowth, impaired host immune defenses, and physical disruption of the gut mucosal barrier.² Thermally injured patients may manifest all of these conditions during the course of their illness. Maejima et al³ documented

bacterial translocation within 2 days after injury in a rat model of thermal injury. Ziegler et al,⁴ using the lactulose-mannitol clearance assay, reported a threefold increase in intestinal permeability to lactulose in infected thermally injured patients but no alteration in permeability in uninfected burned patients. Patients in that study were examined an average of 15 to 18 days after injury. However, those authors could not determine whether the increased intestinal permeability was secondary to an infection superimposed on the thermal injury, or whether the infection was a result of altered intestinal permeability in the early postburn period.

In an attempt to define with more precision the temporal pattern of intestinal permeability changes in thermally injured patients, we measured the lactulose-mannitol clearance in 15 burned patients every 48 hours for the first 2 weeks after injury.

PATIENTS AND METHOD

Patient Population

Fifteen patients with burns covering more than 20% of their body surface who were admitted to the US Army Institute of Surgical Research, Fort Sam Houston, Tex, within 24 hours of injury during the period from April 8 to August 23, 1990, were enrolled in this study. The study patients had no evidence of preexisting renal dysfunction, gastrointestinal tract disease, chronic alcohol abuse, or diabetes mellitus. Ten healthy volunteers served as normal controls. The study protocol was approved by the US Army Surgeon General's Human Use Review Board, and informed consent was obtained from all subjects before the study.

Study Design

Intestinal permeability was assessed on postburn days 2, 4, 6, 8, 10, and 12 by simultaneous enteral administration of two sugars of different molecular weights, mannitol and lactulose.

On the day of study, a test solution consisting of 10 g of lactulose and 5 g of mannitol mixed in 60 mL of distilled water was instilled via the patient's nasogastric tube or, in the case of control subjects or patients without nasogastric tubes, was orally administered. All urine was then collected for a 6-hour period and refrigerated. At the completion of collection, the urine was divided into aliquots and frozen at -20°C before analysis.

Lactulose and Mannitol Assay

Urinary lactulose and mannitol concentrations were simultaneously determined by gas-liquid chromatography. This analytic method was employed because the urine from thermally

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	Patients (n=15)	Controls (n=10)
Age, y	32.7 ± 3.6	25.6 ± 1.9
Sex, No. M/F	13/2	8/2
Inhalation injury, No.	10	...
% of total body surface area	53.5 ± 5.1	...
Mortality, No.	2	...

Table 2. Lactulose and Mannitol Excretion for Entire Study Period		
	Patients	Controls
% lactulose excreted	0.96*	0.14†
% mannitol excreted	9.2	10.3
Lactulose-mannitol excretion ratio	0.159 ± 0.017	0.017 ± 0.003†

*Mean values for entire group.

†P<.001.

‡Mean ± SEM for each patient on each day.

Table 3. Mean ± SEM Lactulose-Mannitol Ratio in Controls, Uninfected Patients, and Infected Patients			
	Patients		
	Controls	Uninfected	Infected
Postburn day 2	0.017 ± 0.003	0.044 ± 0.01	0.153 ± 0.04*
Postburn days 2-14	0.017 ± 0.003	0.082 ± 0.02	0.208 ± 0.02*

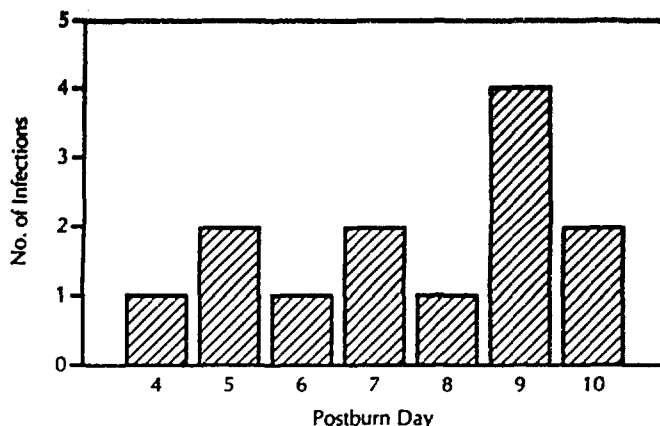
*P<.01 vs controls and uninfected patients by analysis of variance.

injured patients contains multiple fluorescent substances that interfere with the enzymatic assays usually employed for mannitol and lactulose measurement. We previously reported the application of gas-liquid chromatography for the measurement of these simple sugars.⁵

In brief, small diluted aliquots of urine were dried under nitrogen in a heating block at 75°C. After cooling, 100 µL of an oxime solution was added to convert the sugars to oximes before analysis. After 30 minutes of incubation at 75°C, the samples were allowed to cool, and 100 µL of trimethylsilyl imidazole derivatizing reagent was added, followed by a 15-minute incubation period at 75°C. Two microliters of the prepared sample was then injected into a DB-5, 15m × 0.53mm inside diameter capillary column installed in a gas-liquid chromatograph (Hewlett-Packard 5890 series II (Hewlett-Packard, Atlanta, Ga). Injection temperature was set at 220°C, with a detection temperature of 300°C and a flow rate of 9.7 to 9.9 mL/min.

Chromatographs of standard solutions containing mannitol and lactulose were run daily. Linearity of the standard curves for mannitol and lactulose was demonstrated. The minimum detectable concentrations for mannitol and lactulose in the urine were 5 and 1 nmol/L, respectively. Urine samples were routinely spiked with mannitol and lactulose, and recovery data indicated the accuracy of the assay. The test results were then expressed as milligrams of mannitol and lactulose excreted per 6-hour period.

The amount of each sugar excreted in the urine during 6 hours was then converted to a percentage of the amount of the sugar that had been enterally administered. The fraction of lactulose excreted was then indexed to mannitol excretion by dividing the lactulose excretion fraction by the mannitol excretion fraction, yielding a permeability index, the lactulose-mannitol ratio (L/M ratio).



The postburn day on which each infection was clinically diagnosed. No infection was clinically obvious before postburn day 4, with 69% of infections diagnosed in the second postburn week.

Study Design

Patients and control subjects were studied beginning at 8 AM. Patients were studied on postburn days 2, 4, 6, 8, 10, and 12. The control subjects were studied on 2 consecutive days. Control subjects and patients who were on an enteral oral diet fasted for 6 hours before the study. Continuous small-bowel enteral feedings were continued in those patients in whom this therapy was employed. Before administration of the test solution, the urinary bladder was emptied in all subjects.

Statistical Analysis

Statistical analysis was carried out with the BMDP Statistical Program package. Unpaired *t* tests, analysis of variance, and linear regression analyses were used as indicated. Differences were considered significant at *P*<.05. All values are expressed as the mean ± SEM unless otherwise specified.

RESULTS

Fifteen thermally injured patients and 10 control subjects were studied. Demographics of each thermally injured patient are given in Table 1. The mean age of the thermally injured patients was 32.7 ± 3.6 years, with a mean burn size of 53.5% ± 5.1% of total body surface area. Two patients died, for a mortality of 13.3%. The mean age of the 10 control subjects was 25.6 ± 1.9 years. The control subjects were healthy, without any history of recent or remote gastrointestinal tract, cardiovascular, or renal disease, and none was taking medication at the time of study.

The patients and control subjects excreted 9.2% and 10.3% of the mannitol administered, respectively. Overall, the patients excreted a mean of 0.96%, or 96.3 mg, of the lactulose administered. Control subjects excreted a mean of 0.14%, or 13.8 mg of lactulose. The mean L/M ratios for the patients and control subjects were 0.159 ± 0.017 and 0.017 ± 0.003, respectively (*P*<.001). There was no statistically significant difference in mannitol excretion between the two groups.

There was no correlation between the magnitude of increased intestinal permeability and burn size for the entire patient group (*r*<.1). Additionally, there was no correlation between postburn day and increased intestinal permeability for the patient group as a whole, or for patients individually (*r*<.1). On each postburn day of study, the L/M ratio was significantly higher in the patients than in the control subjects. There was no difference in the L/M

ratio for patients with inhalation injury compared with those without such injury.

During the 2-week study period, there were 13 diagnosed infections in nine patients, consisting of six pneumonias, two bacteremias, two cases of wound cellulitis, two cases of tracheobronchitis, and one urinary tract infection. The mean burn size for uninfected and infected patients was 52.7% and 53.7% of the body surface area, respectively. The postburn days on which infections were diagnosed are depicted in the Figure. No infection was clinically evident before postburn day 4.

The L/M ratio for the entire study period for controls, noninfected patients, and infected patients was 0.017 ± 0.003 , 0.082 ± 0.02 , and 0.208 ± 0.02 , respectively. The L/M ratio of the infected burned patients was significantly greater than that in the noninfected patients and normal controls ($P < .01$). There was no statistically significant difference between the ratios in the noninfected patients and the control subjects.

In an attempt to define whether or not the increased permeability occurred before or after the infection, the L/M ratio was examined on postburn day 2, which was before the infection episode in any patient in whom an infection was diagnosed. The mean L/M ratios for controls, noninfected patients, and infected patients were 0.017 ± 0.003 , 0.044 ± 0.01 , and 0.153 ± 0.04 , respectively. Once again, the patients who ultimately developed infections had a mean L/M ratio that was significantly greater than those of the noninfected patients and the control subjects ($P < .01$). The L/M ratio for patients who ultimately did not become infected during the first 2 postburn weeks and that of the control subjects did not differ significantly.

COMMENT

Altered intestinal permeability has been documented in many clinical states, including celiac disease, Crohn's disease, and other intestinal mucosal disorders.^{6,7} It was recently proposed that alterations in intestinal permeability may contribute to the hypermetabolic response after injury and to subsequent development of infection. To assess whether intestinal permeability is altered after thermal injury, we used two unmetabolized low-molecular-weight sugars: lactulose, a disaccharide with a molecular weight of 342, and mannitol, a monosaccharide with a molecular weight of 182. Normally, lactulose is poorly absorbed enterally. When absorbed, these sugars passively cross the gut, enter the circulation, remain unmetabolized, and are excreted by the kidney. Mannitol is reported to be absorbed via a transcellular pathway through aqueous pores in the cell membrane. Normally, 10% to 20% of an enteral load is absorbed. Lactulose, on the other hand, is larger, and its absorption occurs via pericellular pathways across damaged tight junctions. Lactulose is normally poorly absorbed enterally. Mucosal damage leading to altered intestinal permeability has a greater effect on lactulose absorption and subsequent renal excretion than on mannitol. Factors other than permeability may influence the absorption and subsequent excretion of both sugars. These include gastric emptying, intestinal transit time, mucosal surface area, cardiac output, and renal function. Since these factors affect each sugar equally, indexing excretion of one to the other controls for these factors unrelated to intestinal permeability.

We documented a 10-fold increase in intestinal permeability as measured by the L/M ratio in thermally injured

patients during the first 2 postburn weeks. The increased ratio did not correlate with postburn day or burn size. Deitch⁸ recently reported a threefold increase in intestinal permeability in thermally injured patients during the first 24 hours after injury with the use of the L/M ratio. The significantly larger increase in intestinal permeability documented in our patients may be explained by the methods used for analysis of urinary lactulose and mannitol concentrations. We employed gas-liquid chromatography to measure urinary lactulose and mannitol concentrations simultaneously. Previous work used the enzymatic method of Behrens et al⁹ to measure lactulose content. Our laboratory, as well as others, has found that urine from patients with severe burns frequently contains multiple fluorescent substances that interfere with enzyme assays of lactulose content.

Only one previous study has addressed the relationship between altered intestinal permeability and infection in thermally injured patients. Ziegler et al,⁴ who studied patients 2 weeks after injury by means of the L/M ratio, noted that only infected burned patients had altered permeability. However, due to the design of the study, no data were available for the first postburn week or for the period immediately preceding the infection episode. Our data showed a significant increase in permeability occurring on postburn day 2, before infection, in those patients who ultimately became infected. On postburn day 2, uninfected patients had a permeability index that was not different from that of controls. Although the L/M ratio subsequently increased in this population, it still was not statistically significantly different from that of the control group. Whether this represents a type II error cannot be discerned from our data, but the mean L/M ratio of the group remained statistically significantly less than that of the infected group. Our finding of increased intestinal permeability before the episode of infection suggests, but does not prove, a causal relationship. Six of the 13 infections were caused by enteric organisms (four by *Klebsiella* species and two by *Enterobacter* species). The remaining infections were caused by gram-positive organisms and other nonenteric gram-negative organisms, such as *Serratia* and *Pseudomonas* species.

The cause of the altered intestinal permeability in our patients who ultimately became infected remains unclear. Several hypotheses exist that may help explain this finding. It has been well documented in a canine model that intestinal mucosal blood flow is markedly decreased after thermal injury. At 1 hour after injury, Asch et al,¹⁰ using microsphere techniques, reported that mucosal flow was 40% of preinjury levels and returned to 70% of preinjury levels by 4 hours after injury and fluid resuscitation. Thus, the early increase in intestinal permeability documented by Deitch⁸ and this study may be explained on the basis of an ischemia-reperfusion injury. Why only some of the patients developed this abnormality may be related to their resuscitation. Eight patients, all of whom developed infections, were admitted approximately 24 hours after injury. Detailed records of the resuscitations were not available for three patients, but four had had a 4-hour delay in resuscitation. The one remaining patient who developed an infection was transferred to our unit 12 hours after injury and underwent a relatively uneventful resuscitation.

Winchur et al¹¹ noted a temporal relationship between systemic endotoxin levels and postburn day, with

peak endotoxin levels measured on postburn days 3 and 4. Although there is a positive correlation between burn size and endotoxemia, not every burned patient develops endotoxemia during the postburn course. It is possible that a periresuscitation ischemia/reperfusion injury may result in endotoxemia only in those patients who develop significant alterations in intestinal permeability. Endotoxin has been shown to alter intestinal permeability in healthy laboratory controls and laboratory animals.^{12,13} The possibility exists that the sustained elevation in intestinal permeability may be secondary to the effects of endotoxin. Navaratnam et al¹⁴ evaluated the hemodynamic effects of endotoxin on the gastrointestinal tract. Using an ovine model, they reported that endotoxin increased mesenteric vascular resistance, resulting in a more than 50% reduction in mesenteric blood flow. Sheep receiving endotoxin had a 100% incidence of bacterial translocation to mesenteric lymph nodes, while only 15% of sheep not receiving endotoxin had similar findings. The exact mechanism by which endotoxin promoted increased intestinal permeability in these animals has not been elucidated. It appears, however, that ischemia/reperfusion injury may play a central role. Endotoxin-treated mice have been shown to have increased intestinal mucosal activities of xanthine oxidase and dehydrogenase after exposure. Inhibition of xanthine oxidase with allopurinol and inactivation with a tungsten diet has been shown to inhibit endotoxin-induced bacterial translocation.^{15,16}

We have clearly demonstrated an increase in intestinal permeability to lactulose in a subset of thermally injured patients who subsequently developed an infection during their first 2 postburn weeks. It is not possible, from these data, to confirm a causal relationship, although the data suggest one. Deitch et al¹⁷ recently reported that bacterial translocation from the gut results in significant impairment of systemic immunity. This impaired systemic immunity was characterized by a decreased mitogenic response of lymphocytes isolated from mice who were monoassociated with *Escherichia coli* C25. In addition, the monoassociated mice were less able to control a local injection of *Staphylococcus aureus*, suggesting that the changes in mitogen responsiveness may be of biologic significance. Thus, the increased permeability seen in our patients early in their postburn course before an episode of infection appears to be associated with the later occurrence of infection and may even be a contributing factor to the increased susceptibility to infection in burned patients.

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Discussion

EDWIN A. DEITCH, MD, SHREVEPORT, LA: The basic hypothesis behind this study is that there is a causal association between altered intestinal permeability and the development of a hypermetabolic state, or infection. To verify this hypothesis in man, at least three conditions must be met: (1) intestinal permeability must be shown to be increased, (2) a correlation must be found between alterations in intestinal permeability and the development of a hypermetabolic response or sepsis, and (3) the hypermetabolic response should be mitigated by preventing or reducing the increase in intestinal permeability.

Condition 1 seems met, since there are increasing patient data, including the study you have presented today, documenting that permeability is increased after thermal injury. Condition 2 may be becoming met, since you showed us some data today indicating that increases in intestinal permeability are associated with an increased incidence of infection and perhaps even predict infection since the alterations in permeability were present before the onset of infection.

My first question for you is, since there was no correlation between burn size and permeability and yet permeability predicted infection, how would you explain these paradoxical findings?

My second question also relates to permeability measurement. Have you measured the hypermetabolic response of these patients to see if there is any relationship between altered permeability and metabolism?

The last step in my proposed sequence would be to try to normalize intestinal permeability and see if in fact one can prevent certain clinical problems from developing. Do you have any ideas in this regard?

I agree with most of your conclusions. However, I am not sure that I agree with your statement that permeability is not increased in uninfected patients. If you look at the ratio of these patients to controls they are 2 to 4:1, depending on where you measure them. Since only six patients were not infected, due to

the small sample size, I wonder if you might not be seeing a type II error.

Lastly, I'd like to congratulate the authors on the development of a new liquid-gas chromatographic assay for measuring these sugar probes. Since this assay seems to be much more sensitive and specific than the previous enzymatic assays, this new assay may go a long way in allowing us to more accurately investigate changes in intestinal permeability.

PALMER Q. BESSEY, MD, ST LOUIS, MO: One of the mechanisms that is touted to be able to improve the health of the gastrointestinal tract is enteral nutrition. I was wondering if by any chance you could correlate any of the permeability changes with enteral feedings and the ability to absorb gastrointestinal nutrients.

MITCHELL P. FINK, MD, WORCESTER, MASS: Lactulose has a Van der Waals radius equal to 0.62 nm. Most of the biological molecules that we're interested in that might be absorbed from the lumen of the gastrointestinal tract are substantially larger than that. I wonder if you might speculate as to what changes in permeability to a relatively small molecule, like lactulose, might have to do with changes in permeability to much larger molecules of biological significance, like endotoxin.

ORI D. ROTSTEIN, MD, TORONTO, ONTARIO: In Dr Alexander's paper last year, he demonstrated that much of the endotoxin translocating across the gastrointestinal tract actually went through the cell, directly across the enterocyte. However, you show no difference in the mannitol, which is supposed to be a measure of transit across the enterocyte. What is the relationship between intestinal permeability as you measured it here using mannitol and other measures of bacterial translocation or endotoxin egress from the gastrointestinal tract?

DR LEVOYER: In response to Dr Deitch's question, it is possible that alterations in intestinal permeability are a result of the initial injury as well as the resuscitation and subsequent treatment of the injury. In the group of patients who ultimately developed infections, eight of the patients came to our facility from outside institutions, and four of these patients had a documented delay in resuscitation.

This study was designed as a pilot study. The findings suggest a need for a larger study. In this larger follow-up study we should be able to identify other factors, such as a delay in resuscitation, that may have a role in the development of increased permeability and subsequent infection. A larger study should allow us to identify whether there is a burn size-related effect on altered intestinal permeability.

We did not routinely measure the metabolic rates of the patients. In our follow-up study, we plan to address this question. As we identify different factors that may have a role in the development of increased permeability, then we can evaluate the possibility of treatment or prophylaxis. For example, if delayed resuscitation and subsequent reperfusion injury is found to be a contributing factor, then we may be able to evaluate the use of xanthine oxidase inhibitors or inactivators.

The possibility of a type II error may exist in our noninfected patient population. A larger study will help to evaluate this possibility. Regarding Dr Bessey's question on enteral feeding, our patients were begun on enteral feedings (either oral or via a nasogastric tube) by postinjury day 3 to 5. These feedings were continued throughout the hospital course. None of the patients were started on feedings before this period during the early postburn days when permeability was also documented to be increased. During the next study this may be something to evaluate.

Concerning the question regarding the disparity between the molecular size of lactulose and clinically relevant substances, such as endotoxin or even bacteria, I believe our study affords a starting point. Winchur has shown endotoxin levels to be elevated in burn patients with peak levels occurring around postinjury days 3 and 4. Documenting an increase in intestinal permeability via the paracellular pathway will allow the evaluation of other possible routes of permeability. In our follow-up study we intend to measure endotoxin levels. We did not evaluate other pathways of permeability, such as the transcellular pathway proposed by Alexander to which Dr Rotstein is referring.

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