0031-3998/04/5506-0935
PEDIATRIC RESEARCH

Vol. 55, No. 6, 2004
Printed in U.S.A.

Vitamin A Activation of Transforming Growth Factor-β_1 Enhances Porcine Ileum Wound Healing *In Vitro*

DORIS E. YUEN AND ANDREW F. STRATFORD

Department of Pediatrics [D.E.Y.], University of Western Ontario, London, Ontario, Canada N6A 4V2; and Department of Surgery [A.F.S.], Queen's University, Kingston, Ontario, Canada K7L 3N6

ABSTRACT

Treatment with transforming growth factor-β_1 (TGF-β_1) has been shown to be effective in accelerating skin wound healing. Another approach to gain the beneficial effects of TGF-β_1 on wound healing could be the activation of tissue stores of latent TGF-β_1 with agents such as vitamin A. The aims of this study were to determine whether *1*) vitamin A is effective in enhancing intestinal wound healing *in vitro* and *2*) activation of TGF-β_1 is increased during wound healing with vitamin A treatment. We used the intraluminal chemical induction model of necrotizing enterocolitis (NEC), which was adapted to the 1-wk-old piglet. Injured (NEC) and noninjured full-thickness ileum explants harvested from the piglets were cultured for 24 and 48 h in serum-free medium supplemented with all-trans retinol (ATR; 0, 2, 5, and 10 μM). All concentrations of ATR improved recovery of normal ileal wall cytoarchitecture of NEC explants, with maximal recovery observed with 2 μM ATR after 24 h of culture. Further recovery after 48 h was observed with 5 and 10 μM ATR but did not achieve the degree of healing observed with 2 μM ATR. There were no observable adverse effects of ATR on noninjured ileal explant morphology. Active TGF-β_1 was identified only in the NEC explants incubated with ATR. The results of this study demonstrate that administration of vitamin A accelerates recovery of normal intestinal wall cytoarchitecture of injured ileum *in vitro*, without adversely affecting noninjured ileum. The increased activation of latent TGF-β_1 may, in part, be responsible for the accelerated healing of injured ileum observed with vitamin A administration. **(*Pediatr Res* 55: 935–939, 2004)**

Abbreviations

ATR, all-trans retinol
ECM, extracellular matrix
GI, gastrointestinal
NEC, necrotizing enterocolitis
TGF-β_1, transforming growth factor-beta$_1$

Irrespective of the underlying pathogenesis, a broad spectrum of gastrointestinal (GI) diseases result in disruption of the protective mucosal barrier. This breech of mucosal integrity results in further injury to underlying submucosal structures. There is a clear requirement for rapid healing of the GI wall to maximize preservation of normal structure and function. Necrotizing enterocolitis (NEC) is a disease of premature infants that is characterized pathologically by varying degrees of bowel wall inflammation and necrosis and intraluminal bacterial overgrowth (1). The incidence and case-fatality rates for infants with NEC increase with decreasing gestational age and birth weight (2). In very low birth weight infants (<1500 g), the incidence of NEC is 5–15% in most neonatal intensive care units, with a mortality of 10–44% (2, 3). There is also significant morbidity associated with NEC, including sepsis, bowel perforation, intestinal strictures, short bowel syndrome, and complications of long-term total parenteral nutrition that lead to prolonged hospitalization. Although multiple factors have been postulated to play a role in the development of the disease, the exact cause remains unclear (4). As such, preventive strategies based on our current understanding of NEC have not significantly decreased the incidence, mortality, or morbidity in premature infants who develop NEC.

In current clinical practice, the principal treatment is conservative medical management, which involves withholding enteral feeds, total parenteral nutrition, i.v. antibiotics, correction of fluid and electrolyte abnormalities, treatment of coagulopathy, and close observation for evidence of bowel perforation. Surgical intervention for intractable cases of NEC with or without evidence of bowel perforation usually involves significant bowel resection. Thus, surgery is avoided unless absolutely necessary. Even in infants who have NEC and are treated successfully with a conservative regimen, a significant number (6–33%) result in obstructive intestinal strictures (5), requiring resection and/or percutaneous bypass. The present manage-

Received February 11, 2003; accepted January 22, 2004.
Correspondence: Doris E. Yuen, M.D., Ph.D., Division of Neonatal-Perinatal Medicine, St. Joseph's Health Care, London, 268 Grosvenor St., Room E407, London, Ontario, N6A 4G5, Canada; e-mail: dyuen2@uwo.ca
Supported by the Bickell Foundation.

DOI: 10.1203/01.pdr.0000127023.22960.85

ment is not designed to modify the rate and quality of healing in the diseased parts of the bowel. If intestinal wound healing could be modulated to enhance the replacement of injured tissue with functional intestinal tissue, then it would be possible to significantly decrease the amount of bowel that is ultimately damaged.

It has been established that a substantial number of peptide growth factors play specific roles in wound healing (6). Although the role of specific growth factors has not been as clearly delineated for GI wound healing, it seems that transforming growth factor-β_1 (TGF-β_1) plays a central role. Active TGF-β_1, a 25-kD dimeric peptide (7), has been shown to modulate chemotaxis of monocytes, macrophages, and epithelial cells (6); epithelial restitution (8); deposition of extracellular matrix (ECM) proteins (9); and neovascularization (10) during wound healing. The application of TGF-β_1 to GI incisional wounds has been shown to increase wound strength (11).

Large reservoirs of latent TGF-β_1 and its receptors are present in human tissues and are ubiquitous in distribution (12). The activation of latent TGF-β_1 requires its binding to IGF-II/mannose-6-phosphate cell surface receptors (13) followed by proteolytic cleavage of the latency associated protein and latent TGF-β binding protein by cell surface proteases (14). Agents that modulate TGF-β_1 activation include vitamin A, which facilitates receptor binding of latent TGF-β_1 and increases plasmin levels (15).

Treatment of skin wounds with vitamin A results in accelerated wound healing and increased wound strength (16). Only a few studies have evaluated the effect of vitamin A on GI wound healing (17–19). The beneficial effect of vitamin A treatment on wound healing may, in part, be the result of augmented activation of tissue stores of latent TGF-β_1. The aims of this study were to determine whether vitamin A treatment is effective for *1*) enhancing intestinal wound healing *in vitro* and *2*) increasing activation of TGF-β_1 during wound healing.

METHODS

All animal studies were performed at the University of Ottawa Animal Care Facilities (Ottawa, Ontario, Canada). All animal handling and procedures were reviewed and approved by the University of Ottawa Animal Care Committee to conform to the Canadian Council on Animal Care's Guide to the Care and Use of Experimental Animals and with the Animals for Research Act.

NEC animal model. A modification of the model of NEC originally described by Clark and Miller (20) was adapted to the newborn piglet (21). This model utilizes an intraluminal chemical induction of NEC. One-week-old Yorkshire piglets (Agriculture Canada) of normal birth weight were used for these studies. Full-thickness distal ileal tissue was harvested for tissue culture from six piglets. Each animal was its own control. Acidified casein was injected into the NEC intestinal segments. Normal saline was injected into the adjacent control intestinal segments.

The animals remained sedated and intubated during the surgical procedures. Laparotomy was performed *via* a midline incision. Without causing ischemia, a ligature was placed at the proximal ileum and flushed distally with warm sterile saline using a transmural 26-gauge needle. Placing ligatures created two intestinal loops at 10-cm intervals proximal and distal to the ileocecal valve. Sterile 0.9% saline or test solution consisting of bovine casein (10 mg/mL) and calcium gluconate (50 mg/mL; Sigma Chemical Co.-Aldrich, St. Louis, MO, U.S.A.) acidified to pH 4.0 with propionic acid was injected into these distal ileum and proximal colon loops (control). A 26-gauge needle was used to inject these solutions into the lumen of the ligated bowel loops at a volume of 1 mL/2 cm length of intestine. The control loop was separated from the treated loop by an untreated "interloop" to avoid extension of the inflammatory process from the treated to the control loop. The abdominal cavity was closed, and the animals were allowed to recover. The piglets were kept nil by mouth and maintained on continuous i.v. dextrose in water (10%) and electrolyte solutions (75 mmol/L sodium chloride, 20 mmol/L potassium chloride, 22.4 mmol calcium/L calcium gluconate) at a rate of 200 mL $\cdot$ kg^{-1} $\cdot$ 24 h^{-1} $\cdot$. Demerol (1 mg/kg i.m.) was administered as needed for analgesia. The piglets were reanesthetized after 3 h, and the abdominal cavity was entered and the ligated intestinal loops were excised and inspected macroscopically for lesions. The excised ileal tissue to be used for culture was placed in ice-cold Hanks solution (GIBCO BRL, Grand Island, NY, U.S.A.) for transport. The tissue was placed in cold (4°C) 0.9% sodium hypochlorite/PBS for 30 min to decrease microorganism contamination. Additional tissue was frozen immediately in liquid nitrogen and stored at −100°C for further analyses. The animals were killed with intracardiac sodium pentobarbital (65 mg/mL).

Explant culture. Ileal tissue was cut into 25-mm^2 sections and placed villus side up on a Gelfoam platform (Pharmacia & Upjohn, Peapack, NJ, U.S.A.) in 4-mL Falcon culture plates. Four ileal sections were cultured from each of the NEC and control ileal segments for each piglet. Serum-free Dulbecco's modified Eagle's medium/F-12 culture medium (GIBCO BRL) was added to allow the explants to sit at the air–liquid interface. The culture medium was supplemented with 100 U/mL penicillin, 100 pg/mL streptomycin, 0.1 pg/mL amphotericin B, 5.0 pg/mL gentamicin, and 0.3 mg/mL ascorbic acid. Medium was changed every 24 h. The culture plates were placed on a rocker platform at six cycles per minute and incubated at 37°C with a 95% O_2 and 5% CO_2 gas mixture. These conditions were found to optimize preservation of normal gut wall morphology in porcine intestinal explants over a 48-h culture period (22). All-trans retinol (ATR; Sigma Chemical Co) was added to the culture medium to achieve 2, 5, and 10 μM.

Histology. Tissue was fixed in 10% formaldehyde and embedded in polyester wax. Sections (5 μm) were prepared by standard techniques and were stained with Carazzi's hematoxylin for microscopic evaluation. Two randomly selected sections for each ileal explant were assessed for the degree of bowel wall injury, percentage of intact mucosal epithelium, and villus height. A standardized histologic scoring system (Table 1) was used to evaluate the degree of bowel wall injury (20). Under $\times$5 magni-

Table 1. *Histologic grading system; intestinal necrosis 20*

Grade	Histologic criteria
0	Villi completely intact
1	Villous tip necrosis, preservation of basal crypts
2	Necrosis to depth of basal crypts, loss of mucosal and submucosal architecture
3	Necrosis extending into muscularis
4	Transmural necrosis

fication, measurements of the mucosal surface were made with the aid of the micron grid. The percentage of the mucosal surface covered with intact epithelium was calculated [length of mucosal surface lined with intact epithelium (μ)/total length of mucosal surface (μ) $\times$ 100]. The villus height reported for each ileal explant represents the average villus height (all villi visualized in the field of $\times$10 magnification were measured from villus tip to the junction of the lamina propria and muscularis mucosae). Two researchers who were blinded to the culture medium composition independently performed the histologic scoring and measurements.

Western blot. Tissue previously frozen at -100°C was homogenized in SDS-Stop. The protein content of the samples was measured with the Bio-Rad colorimetric method against a BSA (Bio-Rad Laboratories, Hercules, CA, U.S.A.) standard curve. Total protein was extracted from the tissue samples after paraffin fixation through SDS-PAGE. Samples of equal protein content (20 μg) were loaded and run on a 10% (wt/vol) SDS polyacrylamide mini-gel under nonreducing conditions. A standard broad-range protein ladder (Bio-Rad Laboratories) was run simultaneously on the same gel. Proteins were transferred onto a nitrocellulose membrane at 80 mV in a Bio-Rad wet transfer apparatus for 1 h and blocked with a 2% milk solution. The membrane was incubated overnight at 4°C with mouse monoclonal anti-human TGF-β_1 antibody (Serotec, Raleigh, NC, U.S.A.) followed by incubation with fluorescein-conjugated anti-mouse antibody (Oncogene Science, Cambridge, MA, U.S.A.) for 1 h. Immunofluorescence was induced with ECL reagents (Amersham Scientific, Buckinghamshire, England), and the membrane was exposed on Kodak Hyperspeed film for 10 min. The latent TGF-β_1 band is found at 200–230 kD, the propeptide at 50 kD, and active TGF-β_1 dimer at 20–25 kD .

Statistical analyses. Histologic data were analyzed by ANOVA with posttest *t* test to evaluate for differences between groups. The level of significance was $p < 0.05$.

RESULTS

There was improved recovery of normal gut wall cytoarchitecture of full-thickness ileal NEC explants cultured for 24 and 48 h with all tested concentrations of ATR (Figs. 1–3). After 24 h of culture, 2 μM ATR resulted in the most significant improvement in healing compared with 5 and 10 μM ATR (Fig. 1). There was further healing noted at 48 h with 5 and 10 μM ATR. However, the degree of healing remained less than that achieved with 2 μM ATR at either time points. Improved recovery of gut wall cytoarchitecture was also noted at 48 h for the ileal explants cultured without added ATR but was significantly less than that observed for explants cultured with supplemental ATR (Fig. 1).

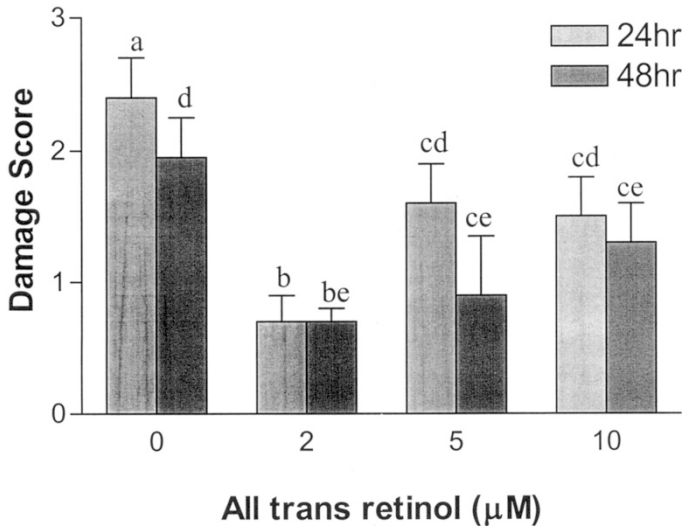

Figure 1. Effect of ATR on wound healing of injured full-thickness porcine ileum explants cultured up to 48 h. Histologic evaluation was performed on injured full-thickness porcine ileum explants cultured for 24 or 48 h in culture medium that was supplemented with or without ATR (2–10 μM). Paraffin-embedded, hematoxylin- and eosin-stained ileum sections were evaluated for the extent of ileal wall damage using a standardized scoring system (Table 1). Histologic grades range from 0 (normal gut wall cytoarchitecture) to 4 (full-thickness gut wall necrosis). Values are expressed as mean $\pm$ SD. Data sets with one common superscript are not significantly different ($p \geq 0.05$).

Recovery of mucosal surface epithelium was significantly improved with 2 μM ATR after 24 and 48 h compared with explants cultured without added ATR (Fig. 2). After 48 h, there was decreased mucosal epithelial integrity of the explants

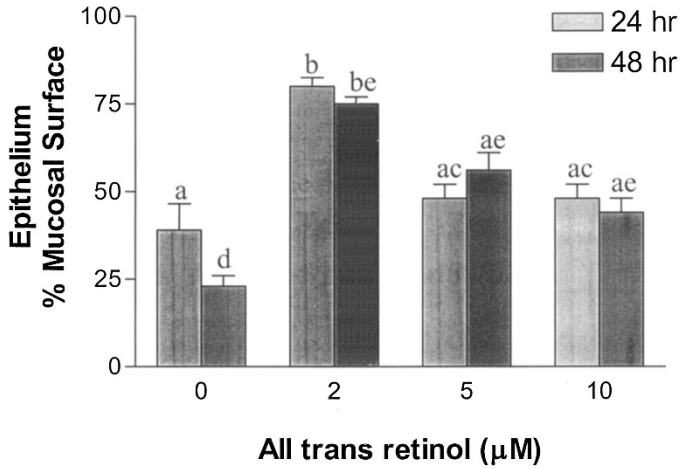

Figure 2. Effect of ATR on recovery of mucosal epithelium of injured full-thickness porcine ileum explants cultured up to 48 h. Histologic evaluation was performed on injured full-thickness porcine ileum explants cultured for 24 or 48 h in culture medium that was supplemented with or without ATR (2–10 μM). Measurements were made of the mucosal surface of paraffin-embedded, hematoxylin- and eosin-stained ileum sections under $\times$5 magnification with the aid of the micron grid. The percentage of the mucosal surface covered with intact epithelium was calculated [length of mucosal surface lined with intact epithelium (μ)/total length of mucosal surface (μ) $\times$ 100]. Values are expressed as mean $\pm$ SD. Data sets with one common superscript are not significantly different ($p \geq 0.05$).

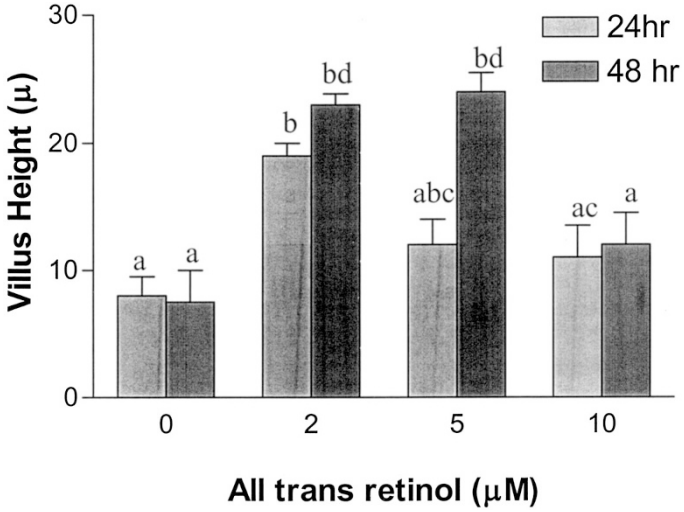

Figure 3. Effect of ATR on recovery of villi of injured full-thickness porcine ileum explants cultured up to 48 h. Histologic evaluation was performed on injured full-thickness porcine ileum explants cultured for 24 or 48 h in culture medium that was supplemented with or without ATR (2–10 μM). Measurements were made on paraffin-embedded, hematoxylin- and eosin-stained ileum sections with the aid of a micron grid. Villus height was measured from the villus tip to the junction of the lamina propria and muscularis mucosae. The reported villus height represents the average height of all villi visualized and measured in a $\times$10 magnification field. Values are expressed as mean $\pm$ SD. Data sets with one common superscript are not significantly different ($p \geq 0.05$).

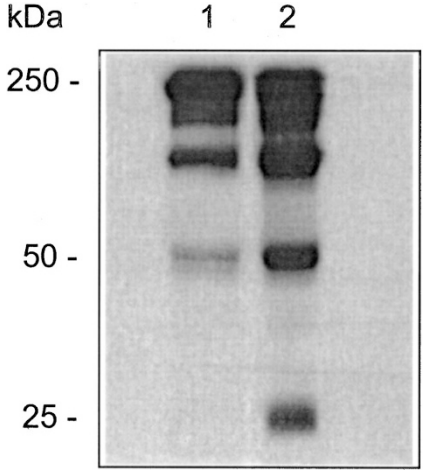

Figure 4. Identification of latent and active TGF-β_1 by Western immunoblotting of injured full-thickness porcine ileum explants cultured for 48 h with or without ATR. Twenty micrograms of total protein extracted from the ileum explants were loaded in each lane, processed on a 10% (wt/vol) SDS-PAGE under nonreducing conditions, and transferred onto nitrocellulose membranes. The membranes were incubated with mouse monoclonal anti-human TGF-β_1 antibody followed by incubation with fluorescein-conjugated anti-mouse antibody and addition of ECL immunofluorescence detection reagents. Autoradiographs were developed after 10 min of exposure. Molecular mass markers are indicated on the left. *Lane 1*, represents ileal explants cultured without all trans retinol; *lane 2*, ileal explants cultured with 2 μM ATR. Latent TGF-β_1 is identified in the 200- to 250-kD band and TGF-β_1 propeptide at 50 kD in both lanes, whereas active TGF-β_1 dimer (20- to 25-kD band) is identified only in *lane 2*.

cultured without additional ATR, whereas explants cultured with any concentration of supplemental ATR maintained the degree of epithelial integrity achieved at 24 h of culture. There was no further improvement in recovery of mucosal epithelium at 48 h compared with 24 h in the ATR-supplemented explants.

Recovery of villi height was observed for explants cultured with all concentrations of supplemental ATR and reached significance with 2 μM ATR after 24 h and 2 and 5 μM after 48 h (Fig. 3). There was no further significant improvement in recovery of villi at 48 h compared with 24 h of culture with all concentrations of ATR except for 5 μM.

The addition of ATR to the culture medium of noninjured control porcine ileal explants did not result in any histologic changes over a 48-h culture period compared with explants cultured without supplemental ATR (Table 2). NEC explants cultured for 48 h without supplemental ATR or with the optimal dose of ATR (2 μM) were used for Western blot identification of TGF-β_1. Under nonreducing conditions, the active TGF-β_1 dimer was identified only from the explants cultured with supplemental ATR (Fig. 4).

DISCUSSION

The results of this study demonstrate that vitamin A enhances wound healing of injured full-thickness porcine ileal explants *in vitro* without causing any observable adverse effects on normal ileal cytoarchitecture. The increased activation of tissue stores of TGF-β_1 with vitamin A may, in part, be responsible for the improved ileal wound healing. These findings suggest that vitamin A holds the potential as an effective adjuvant medical therapy for accelerating GI wound healing.

Over the 48-h study period, addition of vitamin A to the culture medium resulted in the accelerated recovery of the overlying mucosal epithelium and villus structures. Rapid recovery of an intact mucosal barrier after GI injury would provide greater protection of the underlying gut wall structures from damage, as a result of exposure to noxious intraluminal contents once the mucosal barrier is breached. As such, treatment with vitamin A after GI injury may decrease the extent of gut wall damage, which would have an impact on decreasing

Table 2. *Histologic evaluation of cultured noninjured ileum explants*

	Damage score*		Epithelium (% mucosal surface)		Villus height (μ)	
ATR (μM)	24 h	48 h	24 h	48 h	24 h	48 h
0	1.0 $\pm$ 0.8	1.2 $\pm$ 1.0	63.3 $\pm$ 35.1	46.7 $\pm$ 47.3	13.7 $\pm$ 2.3	20.0 $\pm$ 9.9
2	1.8 $\pm$ 0.3	1.3 $\pm$ 0.3	25.0 $\pm$ 20.0	64.0 $\pm$ 26.9	14.5 $\pm$ 7.0	21.3 $\pm$ 9.3
5	0.7 $\pm$ 0.7	0.8 $\pm$ 0.3	71.0 $\pm$ 23.5	78.3 $\pm$ 24.7	22.0 $\pm$ 4.0	18.0 $\pm$ 2.6
10	1.5 $\pm$ 0.6	1.5 $\pm$ 0.9	36.7 $\pm$ 28.9	65.0 $\pm$ 13.2	9.0 $\pm$ 5.7	15.7 $\pm$ 6.8

Values are expressed as mean $\pm$ SD. Values for all concentrations of retinol and duration of culture for damage score, epithelium, and villus height were not significantly different ($p > 0.05$).

* See Table 1 for explanation of damage score.

the mortality rate and the risk of developing associated morbidities such as need for surgical bowel resection and development of intestinal strictures, short gut syndrome, and septicemia.

The mechanism by which vitamin A accelerates intestinal epithelial restitution after mucosal injury is not known. The limited number of studies on the cellular and molecular mechanisms of GI wound healing have shown that intestinal mucosal healing occurs in two phases: *1*) restitution, involving the migration of sheets of enterocytes across the mucosal defect, which starts within minutes from the time of injury, and *2*) proliferation of mucosal epithelial cells, which starts in the order of hours from the time of injury (23, 24). Growth factors that promote intestinal epithelial migration seem to achieve this effect through activation of TGF-β_1, which may be the final common signal in this cytokine-mediated cascade (8). As such, the increased activation of TGF-β_1 with vitamin A observed in our study may, in part, account for the improved recovery of mucosal epithelium after injury. Various ECM proteins of the basal lamina and epithelial cell integrin expression have been shown to influence epithelial cell migration and differentiation (25, 26). Because vitamin A and TGF-β_1 modulate ECM protein synthesis and integrin expression (9, 27, 28), this may be another mechanism by which these compounds modulate wound healing.

The addition of vitamin A to the culture medium of injured porcine ileal explants improved recovery of villi. However, these histologic observations do not provide information on the extent of recovery of the normal functional capacity of the villi. Growth factors, ECM proteins, and integrins, as discussed previously in relation to epithelial cell migration, have also been shown to play important roles during GI adaptation after injury (29, 30).

Current management of NEC does not include specific therapies for enhancing GI wound healing. On the basis of our findings, vitamin A treatment of premature infants who develop NEC holds the potential of conferring the following benefits: *1*) accelerate recovery of the intestinal mucosal epithelial barrier, *2*) minimize the severity and extent of intestinal injury, and *3*) enhance recovery of normal mucosal function. Follow-up studies will be conducted to test the *in vivo* efficacy of vitamin A treatment on enhancing GI wound healing in the newborn piglet model for NEC.

Acknowledgments. We thank Li Dong for technical assistance and Haroon Sheikh for expertise in imaging. We are grateful to Dr. Victor Han and Dr. Gregor Reid for invaluable input in the preparation of this manuscript.

REFERENCES

1. Ballance WA, Dahms BB, Shenker N, Kliegman RM 1990 Pathology of neonatal necrotizing enterocolitis: a ten-year experience. J Pediatr 117(suppl):S6–S13
2. Stoll BJ 1994 Epidemiology of necrotizing enterocolitis. Clin Perinatol 21:205–218
3. Chan K, Ohlsson A, Synnes A, Lee DS, Chien LY, Lee SK, Canadian NN 2001 Survival, morbidity, and resource use of infants of 25 weeks' gestational age or less. Am J Obstet Gynecol 185:220–226
4. Caplan MS, Jilling T 2001 New concepts in necrotizing enterocolitis. Curr Opin Pediatr 13:111–115
5. Kanto WP Jr, Hunter JE, Stoll BJ 1994 Recognition and medical management of necrotizing enterocolitis. Clin Perinatol 21:335–346
6. Moulin V 1995 Growth factors in skin wound healing. Eur J Cell Biol 68:1–7
7. Sporn MB, Roberts AB, Wakefield LM, Assoian RK 1986 Transforming growth factor-beta: biological function and chemical structure. Science 233:532–534
8. Dignass AU, Podolsky DK 1993 Cytokine modulation of intestinal epithelial cell restitution: central role of transforming growth factor beta. Gastroenterology 105:1323–1332
9. Kimura Y, Torimura T, Ueno T, Inuzuka S, Tanikawa K 1995 Transforming growth factor beta 1, extracellular matrix, and inflammatory cells in wound repair using a closed duodenal loop pancreatitis model rat. Immunohistochemical study. Scand J Gastroenterol 30:707–714
10. Mahida YR, Ciacci C, Podolsky DK 1992 Peptide growth factors: role in epithelial-lamina propria cell interactions. Ann N Y Acad Sci 664:148–156
11. Slavin J, Nash JR, Kingsnorth AN 1992 Effect of transforming growth factor beta and basic fibroblast growth factor on steroid-impaired healing intestinal wounds. Br J Surg 79:69–72
12. Massague J, Cheifetz S, Laiho M, Ralph DA, Weis FM, Zentella A 1992 Transforming growth factor-beta. Cancer Surv 12:81–103
13. de Bleser PJ, Jannes P, Buul-Offers SC, Hoogerbrugge CM, van Schravendijk CF, Niki T, Rogiers V, van den Brande JL, Wisse E, Geerts A 1995 Insulin like growth factor-II/mannose 6-phosphate receptor is expressed on CCl4-exposed rat fat-storing cells and facilitates activation of latent transforming growth factor-beta in cocultures with sinusoidal endothelial cells. Hepatology 21:1429–1437
14. Dallas SL, Miyazono K, Skerry TM, Mundy GR, Bonewald LF 1995 Dual role for the latent transforming growth factor-beta binding protein in storage of latent TGF-beta in the extracellular matrix and as a structural matrix protein. J Cell Biol 131:539–549
15. Glick AB, McCune BK, Abdulkarem N, Flanders KC, Lumadue JA, Smith JM, Sporn MB 1991 Complex regulation of TGF beta expression by retinoic acid in the vitamin A-deficient rat. Development 111:1081–1086
16. Elson ML 1998 The role of retinoids in wound healing. J Am Acad Dermatol 39:S79–S81
17. de Waard JW, Wobbes T, van der Linden CJ, Hendriks T 1995 Retinol may promote fluorouracil-suppressed healing of experimental intestinal anastomoses. Arch Surg 130:959–965
18. Nagai Y, Horie T, Awazu S 1993 Vitamin A, a useful biochemical modulator capable of preventing intestinal damage during methotrexate treatment. Pharmacol Toxicol 73:69–74
19. Phillips JD, Kim CS, Fonkalsrud EW, Zeng H, Dindar H 1992 Effects of chronic corticosteroids and vitamin A on the healing of intestinal anastomoses. Am J Surg 163:71–77
20. Clark DA, Fornabaio DM, McNeill H, Mullane KM, Caravella SJ, Miller MJ 1988 Contribution of oxygen-derived free radicals to experimental necrotizing enterocolitis. Am J Pathol 130:537–542
21. Di Lorenzo M, Bass J, Krantis A 1995 An intraluminal model of necrotizing enterocolitis in the developing neonatal piglet. J Pediatr Surg 30:1138–1142
22. Nietfeld JC, Tyler DE, Harrison LR, Cole JR, Latimer KS, Crowell WA 1991 Culture and morphologic features of small intestinal mucosal explants from weaned pigs. Am J Vet Res 52:1142–1146
23. Ciacci C, Lind SE, Podolsky DK 1993 Transforming growth factor beta regulation of migration in wounded rat intestinal epithelial monolayers. Gastroenterology 105:93–101
24. Yanaka A, Muto H, Fukutomi H, Ito S, Silen W 1996 Role of transforming growth factor-beta in the restitution of injured guinea pig gastric mucosa in vitro. Am J Physiol 271:G75–G85
25. Lotz MM, Nusrat A, Madara JL, Ezzell R, Wewer UM, Mercurio AM 1997 Intestinal epithelial restitution. Involvement of specific laminin isoforms and integrin laminin receptors in wound closure of a transformed model epithelium. Am J Pathol 150:747–760
26. Lotz MM, Rabinovitz I, Mercurio AM 2000 Intestinal restitution: progression of actin cytoskeleton rearrangements and integrin function in a model of epithelial wound healing. Am J Pathol 156:985–996
27. Tamariz-Dominguez E, Castro-Munozledo F, Kuri-Harcuch W 2002 Growth factors and extracellular matrix proteins during wound healing promoted with frozen cultured sheets of human epidermal keratinocytes. Cell Tissue Res 307:79–89
28. de Waard JW, Wobbes T, van der Linden CJ, Hendriks T 1995 Retinol may promote fluorouracil-suppressed healing of experimental intestinal anastomoses. Arch Surg 130:959–965
29. Dieckgraefe BK, Santoro SA, Alpers DH 1996 Immunolocalization of alpha-integrin subunits and extracellular matrix components during human colonic organogenesis. Gastroenterology 110:58–71
30. Howarth GS, Shoubridge CA 2001 Enhancement of intestinal growth and repair by growth factors. Curr Opin Pharmacol 1:568–574