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Folate Stimulation of Wound DNA Synthesis

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Background. **Whereas nutrition supply is known to be beneficial to wound healing, the role of folate in wound healing is not clear.**

Materials and methods. **The experiment consisted of a surgery on day 0 and a tracer infusion on day 5 after surgery. During the surgery a donor wound was created on the back and indwelling catheters were inserted in the carotid artery and jugular vein in rabbits under general anesthesia. A pharyngostomy feeding tube was also inserted in a group receiving enteral feeding after injury (TEN group, $n = 6$), but not in other two groups receiving rabbit chow after injury and intravenous amino acids with or without folate during tracer infusion (IVAA and IVAA-folate groups, $n = 6$ each). On day 5 stable isotope tracers were infused in conscious rabbits to determine the fractional synthetic rate (FSR) of wound DNA, and FSR and fractional breakdown rate (FBR) of wound protein. In the IVAA-folate group 5-methyltetrahydrofolate (the active form of folate) was infused at 0.1 mg/h during the tracer infusion.**

Results. **Serum folate concentration in the IVAA-folate group (380 ± 78 ng/mL) was greater ($P < 0.001$) than those in the TEN (20 ± 6 ng/mL) and IVAA (33 ± 22 ng/mL) groups. DNA FSRs in the IVAA-folate group (5.02 ± 1.26%/d) and in the TEN group (4.51 ± 0.98%/d) were greater ($P < 0.01$–0.05) than that in the IVAA group (2.87 ± 0.61%/d). In the IVAA and IVAA-folate groups net protein deposition (FSR–FBR) was correlated with chow intake ($P < 0.001$–0.002).**

Conclusions. **Folate supplementation has a stimulatory effect on wound DNA synthesis, which would be expected to accelerate wound healing. Nutrition intake is essential for tissue repair by a mechanism that increases net protein deposition in the wound.**

Key Words: **skin wound; rabbit; stable isotopes; metabolism.**

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INTRODUCTION

It is well known that nutrition supply is necessary for wound healing. Among various forms of trauma, severe burn injury gives rise to the most hypermetabolic state and requires aggressive nutritional support [1–4]. Early enteral feeding is believed to be beneficial to burned patients [5–9]. However, it is not known what nutrients are rate-limiting to the anabolic response in local wound under the current nutritional regime. Whereas folate is necessary for the de novo biosynthesis of purines and thymidylate and thereby plays a crucial role in DNA synthesis, its role in wound healing has not been reported. Following a burn injury, active skin regeneration and increased cell proliferation in some tissues (e.g., liver and gut mucosa) all require folate as a building block to synthesize DNA of new cells. Thus, a relative lack of folate could become a limiting factor if sufficient replacement is not provided.

We found in our previous experiment that protein and DNA metabolism in the skin wound responds differently [10]. The turnover rate of wound protein increased several folds of the basal rate during the flow phase, whereas DNA synthesis rate was not greater than the normal skin rate. Because DNA synthesis is a reliable measure of cell proliferation, a stimulation of wound DNA synthesis is expected to accelerate wound angiogenesis and re-epithelialization, thereby accelerating wound healing. On the other hand, as the majority of tissue loss from a burn injury is derived from protein loss, net protein deposition is necessary to repair the tissue defect. Thus, from the metabolic perspective the nutrition supply in burns should target at increasing cell proliferation and net protein deposition [11–14].

To investigate wound metabolism in relation to wound healing, we have developed stable isotope methods to measure the rate of DNA synthesis as well as protein synthesis and breakdown in the skin

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donor site wound [10–15]. The skin donor site wound is suitable for measuring DNA and protein kinetics, as it is free of dead tissues and bacterial colonization. Our previous study [10] demonstrated that the infiltration of inflammatory cells in the wound was minimal. Thus, the measured DNA and protein kinetics predominately reflect metabolic events in skin cells (mainly keratinocytes and fibroblasts). The present experiment was conducted to compare wound protein and DNA kinetics in rabbits receiving amino acid infusion with or without concomitant 5-methyltetrahydrofolate (5-MTHF, the active form of folate) infusion. An additional group of rabbits receiving total enteral nutrition (TEN) via an indwelling feeding tube was also studied to provide reference values that showed the metabolic responses with the optimal nutrition supply.

MATERIALS AND METHODS

Animals

Male New Zealand white rabbits (Myrtle's Rabbitry, Thompson Station, TN) weighing 4 to 5 kg, were used for this study. The rabbits were housed in individual cages and were given 150 g/d of Lab Rabbit Chow HF 5326 (Purina Mills, St. Louis, MO) for weight maintenance; daily intake of 150 g chow contains crude protein 4.7 g/kg and calories 38 kcal/kg. This protocol complied with NIH guidelines and was approved by the Animal Care and Use Committee of The University of Texas Medical Branch at Galveston.

Isotopes

D-[U-$^{13}C_6$]glucose (99% enriched), L-[ring-$^{13}C_6$]phenylalanine (Phe, 99% enriched), L-[1-^{13}C]leucine (Leu, 99% enriched), were purchased from Cambridge Isotope Laboratories (Woburn, MA). L-[1,2-$^{13}C_2$]Leu (99% enriched) was purchased from MASSTRACE (Woburn, MA).

Surgical Procedures

The experiment included a surgical procedure on day 0 and a stable isotope tracer infusion on day 5. The surgical procedures were performed using aseptic techniques under ketamine and xylazine anesthesia. Hair on the back and neck was removed by a clipper and hair remover lotion. Two partial thickness skin donor wounds (~40 cm^2 each) were created on both sides of the back using an electric dermatome set at 0.015 inch. Sterile catheters were placed in the left carotid artery and jugular vein through an incision on the left side of the neck [10, 14]. In the TEN group, a pharyngostomy feeding tube was placed as is described below. An extension tube (length 89 cm, volume 5.0 mL; Baxter Healthcare Corporation, Deerfield, IL), cut into 50 cm length, was passed p.o. through to the stomach. The surgeon's finger was then placed in the mouth of the rabbit to palpate the thin tissue area caudal to the point of the mandible. The surgeon's finger then elevated the tissue facilitating a skin incision over the tissue. The forceps were used to penetrate the mucosa and advanced into the mouth cavity. The "oral" end of the feeding tube was pulled out through the incision with the forceps so that 15 cm of the tube was visible and 35 cm of the tube is located in the esophagus and stomach. The visible portion of the tube was attached to the skin with suturing.

The wound was covered with Aquaphor gauze (Smith & Nephew, Largo, FL), OpSite membrane (Smith and Nephew, Hull, England) and surgical gauze, and was protected by a rabbit jacket (Harvard Apparatus, Boston, MA). The removed split skin was measured for area (cm^2) and weight (mg) and the wound depth was indicated by the ratio of weight/area (mg/cm^2). The vascular catheters were filled with heparin solution (1000 U/mL) and were attached to Jelco intermittent injection caps (Johnson and Johnson, Medical Division of Ethicon, Arlington, TX). A skin sample was taken from the incision during surgery, and frozen in liquid nitrogen. This skin sample was stored at −80°C for later analysis of background enrichment.

Immediately after the surgery, a single dose (50,000 U/kg) of antibiotic (Bicillin; Wyeth Laboratories, Philadelphia, PA) was injected intramuscularly. When the animals had awakened from anesthesia, buprenorphine (0.015 mg/kg) was injected intramuscularly twice a day for 2 d as an analgesic. Heparin solution (0.45 mL at 1000 U/mL) was injected via the intermittent injection caps every morning to flush the vascular lines. The wound dressings were changed on day 3 and day 5 (before tracer infusion), when the wound was inspected to ascertain there was no infection.

Experimental Design

There were 18 rabbits with partial thickness wounds on their backs, which were divided in 3 groups. In two groups the rabbits received the same rabbit chow as before surgery. The amount of chow intake was recorded every day. During the tracer infusion period, the rabbits were given either intravenous amino acid infusion (10% Travasol; Baxter Healthcare Corporation, Deerfield, IL) alone (IVAA group) or the amino acids plus 5-MTHF (IVAA-folate group).

In the third group, the rabbits received TEN via the feeding tube (TEN group). The TEN recipe included 30 mL/kg/d of Promote (ROSS Nutrition, Columbus, OH) and 3 g/kg/d of additional caseinate (NOW Sports, Bloomingdale, IL). The TEN recipe provided protein at 4.875 g/kg/d and calories at 42 Kcal/kg/d, which was comparable to daily 150 g chow intake in normal rabbits. On days 1 and 2 after injury, 50% of the daily dose was given. On days 3 and 4, the full dose was given. The daily TEN was evenly divided into two aliquots and given at 8:30 and 17:00 through the feeding tube. During the stable isotope infusion period on day 5, 70% of the full dose was constantly infused into the feeding tube.

Tracer Infusion Protocol

At 17:00 on day 4, the rabbit chow was removed and water was available all of the time. The stable isotope tracers were infused on day 5 after injury (Fig. 1). At 08:00 the nutrition supply was started. The daily dose of TEN (30 mL/kg/d of Promote plus 3 g/kg/d of additional caseinate) was mixed with water to a final volume of 225 mL and infused at 17.5 mL/h (prime: 17.5 mL) into the feeding tube. The total volume infused (8 h constant infusion plus 1 h prime) was 157.5 mL. In the IVAA and IVAA-folate groups, 10% Travasol was infused into the catheter in the jugular vein at 1.5 mL·kg^{-1}·h^{-1} after a prime of 1 mL/kg. This infusion rate doubled plasma amino acid concentrations in our previous experiment [13]. In the IVAA-folate group 5-MTHF was also infused at 0.1 mg/h (prime: 80 μg). One hour later the infusion of stable isotope tracers was started. The infusion rate (IR) and priming dose (PD) of stable isotopes were as follows: D-[U-$^{13}C_6$]glucose (IR = 1.5 to 2.0 μmol/kg/min; PD = 120–160 μmol/kg), L-[ring-$^2H_5$]Phe (IR = 0.2 μmol/kg/min; PD = 8 μmol/kg), L-[1-^{13}C]Leu and L-[1,2 -$^{13}C_2$]Leu (IR = 0.45 μmol/kg/min; PD = 27 μmol/kg). The glucose tracer was used to determine the fractional synthetic rate (FSR) of wound DNA; the Phe tracer was used to determine FSR of wound protein; the two Leu tracers were to determine the fractional breakdown rate (FBR) of wound protein. L-[1-^{13}C]Leu was stopped at hour 6 for measurement of enrichment decay in the arterial blood, while L-[1,2-$^{13}C_2$]Leu was infused continuously until the end. The use of two Leu tracers was to obtain both plateau and decay enrichment from one wound sample taken at the end of infusion [10, 14].