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Intraperitoneally administered, hydrogen-rich physiologic solution protects against postoperative ileus and is associated with reduced nitric oxide production

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Background. *Postoperative ileus, a transient impairment of bowel motility initiated by intestinal inflammation, is common after an abdominal operation and leads to increased hospital stays and costs. Hydrogen has potent anti-inflammatory and antioxidant properties and potential therapeutic value. Solubilized hydrogen may be a portable and practical means of administering therapeutic hydrogen gas. We hypothesized that intraperitoneal administration of hydrogen-rich saline would ameliorate postoperative ileus.*

Methods. *Ileus was induced via surgical manipulation in mice and rats. The peritoneal cavity was filled with 1.0 mL saline or hydrogen-rich saline (≥1.5–2.0 ppm) before closure of the abdominal incision. Intestinal transit was assessed 24 hours postoperatively. Inflammation was examined by quantitation of neutrophil extravasation and expression of proinflammatory markers. Nitric oxide production was assessed in cultured muscularis propria.*

Results. *Surgical manipulation resulted in a marked delay in intestinal transit and was associated with upregulation of proinflammatory cytokines and increased neutrophil extravasation. Bowel dysmotility, induced by surgical manipulation and inflammatory events, was significantly attenuated by intra-abdominal administration of hydrogen-rich saline. Nitric oxide production in the muscle layers of the bowel was inhibited by hydrogen treatment.*

Conclusion. *A single intraperitoneal dose of hydrogen-rich saline ameliorates postoperative ileus by inhibiting the inflammatory response and suppressing nitric oxide production. (Surgery 2016;160:623-31.)*

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Postoperative ileus (POI) is characterized by a transient impairment of bowel motility and is a common complication of abdominal operations and several other operative procedures.[1] POI causes a change in gastrointestinal function and is an important factor preventing the early hospital discharge of patients after an abdominal operation, even after a laparoscopic operation.[2,3] Additionally, POI contributes to many comorbid postoperative complications, including delayed enteral nutrition, poor wound healing, poor ambulation, atelectasis, and pneumonia, which then lead to longer hospital stays and increased health care costs. It is critical, therefore, to establish both preventative and therapeutic strategies for POI.

Once believed to be an inert and nonfunctional molecule in our body, hydrogen exerts anti-inflammatory and antioxidant properties.[4,5] The safe medical use of inhaled hydrogen gas under strict monitoring is certainly possible, as hydrogen is not explosive at concentrations <4% (vol/vol) and is not flammable at temperatures <527°C.[6] Treating patients with inhaled hydrogen for prolonged periods, however, might pose some logistic problems for hospitals due to the potential flammability of hydrogen, which decreases the attractiveness of therapeutic strategies using inhaled hydrogen. Solubilizing hydrogen in water may be a more practical and controllable way of delivering hydrogen to biologic systems and could promote hydrogen use in clinical settings.

Previous studies have shown that intestinal manipulation of the bowel during an operation initiates a local inflammatory reaction within the muscularis propria, and this surgical manipulation (SM) is the intrinsic origin of POI.[7,8] We demonstrated that hydrogen inhalation could prevent POI caused by intestinal ischemia/reperfusion injury.[9] We hypothesized that intraperitoneal administration of hydrogen-saturated saline would exert protective effects against POI caused by SM, and we tested our hypothesis using mouse and rat models.

METHODS

Preparation of hydrogen-rich saline. Hydrogen-saturated, physiologic saline was produced by mixing saline with hydrogen gas in a pressure-resistant container (Fig 1, *A*). A hydrogen-generating agent (0.65 g) was prepared by mixing aluminum powder and calcium hydroxide at a ratio of 76 to 24 by weight. The agent was reacted with water (0.5 mL) in an acrylic resin tube to generate H_2 as follows: $2Al + Ca(OH)_2 + 6H_2O \rightarrow Ca[Al(OH)_4]_2 + 3H_2$. The tube was closed tightly with a secure cap attached to a check valve and immersed in a 500-mL, pressure-resistant, polyethylene terephthalate bottle filled with saline. H_2 generated in the tube was transferred to the saline through the check valve.

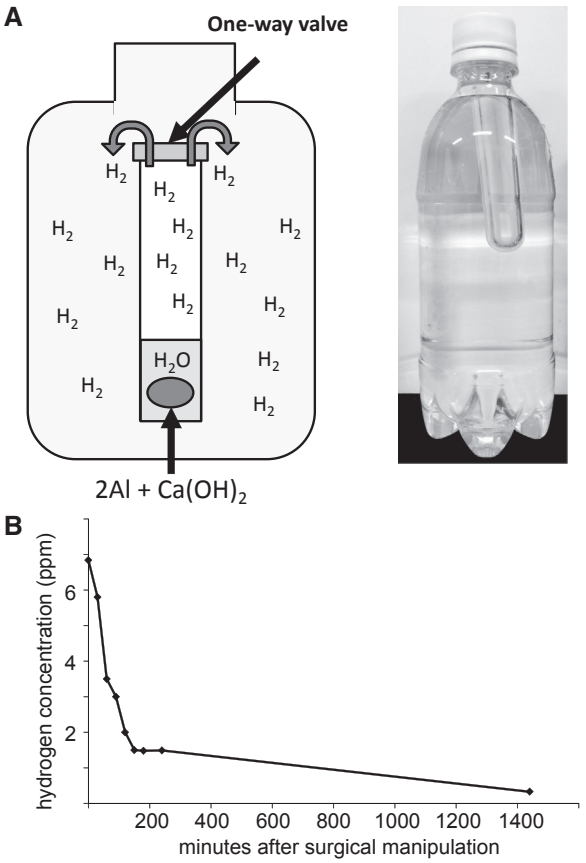

Fig 1. Preparation of hydrogen-rich saline. (*A*) Schematic representation of protocol for generating hydrogen-saturated saline. A tube containing an H_2-generating agent and water was closed tightly with a secure cap, attached to a check valve, and immersed into a pressure-resistant bottle filled with saline. (*B*) Concentration of H_2 as a function of time after opening the bottle.

The concentration of H_2 was measured using a methylene blue, platinum colloid, reagent-based titration method, as described previously, and was verified using an electrochemical gas sensor (model DHD1-1; DKK-TOA Co, Tokyo, Japan).[10] The concentration of hydrogen in the saline was measured every hour after opening the container. The initial concentration of hydrogen was 6.84 ppm, and within 24 hours, it was decreased gradually to 0.26 ppm. Because the concentration of hydrogen decreased gradually after the bottle was opened, we used the hydrogen-rich saline 3–5 hours after generation (hydrogen concentration was 1.5–2.0 ppm; Fig 2, *B*).

Animals. Twenty-week old, male C57BL/6J (wild-type) mice (Clea Japan, Tokyo, Japan) and Spraque Dawley rats (Clea Japan) were used in all