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Opinion/Position paper

Acute exposure to space flight results in evidence of reduced lymph Transport, tissue fluid Shifts, and immune alterations in the rat gastrointestinal system

W.E. Cromer*, D.C. Zawieja

Department of Medical Physiology, Texas A&M University Health Science Center, United States

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ABSTRACT

Space flight causes a number of alterations in physiological systems, changes in the immunological status of subjects, and altered interactions of the host to environmental stimuli. We studied the effect of space flight on the lymphatic system of the gastrointestinal tract which is responsible for lipid transport and immune surveillance which includes the host interaction with the gut microbiome.

We found that there were signs of tissue damage present in the space flown animals that was lacking in ground controls (epithelial damage, crypt morphological changes, etc.). Additionally, morphology of the lymphatic vessels in the tissue suggested a collapsed state at time of harvest and there was a profound change in the retention of lipid in the villi of the ileum. Contrary to our assumptions there was a reduction in tissue fluid volume likely associated with other fluid shifts described. The reduction of tissue fluid volume in the colon and ileum is a likely contributing factor to the state of the lymphatic vessels and lipid transport issues observed. There were also associated changes in the number of MHC-II$^+$ immune cells in the colon tissue, which along with reduced lymphatic competence would favor immune dysfunction in the tissue.

These findings help expand our understanding of the effects of space flight on various organ systems. It also points out potential issues that have not been closely examined and have to potential for the need of countermeasure development.

1. Introduction

Spaceflight is associated with physiological changes in various organs and tissue types from muscle to bone the immune system and the cardiovascular system (Arbeille et al., 2016; Hughson et al., 2016; Smith et al., 2014a; Smith et al., 2014b; Petersen et al., 2016; Petersen et al., 2017; Kulkarni et al., 2005; Shearer et al., 2005; Sonnenfeld et al., 2003; Sonnenfeld and Shearer, 2002). The changes in these organ systems can have a major impact on immediate astronaut health and are primary focuses for researchers in the space biology field. All of these factors just like those mentioned before are critical to immediate astronaut health and mission readiness. Disturbances in the gastrointestinal tract (GI tract) have also been noted and range from acute space sickness to altered gastric emptying and disrupted peristaltic motion of the intestines (Chen et al., 2016; Thornton and Bonato, 2013; Harm et al., 2002). This was accompanied by evidence of changes in nutritional status in early space flight studies as well as current studies and the development of improved food stuffs and dietary supplements to compensate for these changes (Stephens et al., 1968; Lane et al., 2013; Smith and Zwart, 2008; Smith et al., 2005). There still are many unanswered questions as to what the base causes of these deficiencies were, however some hypothesized that altered intestinal microbiota, poor diet and impaired intestinal absorption may play a role in these.

Data from the human studies have shown that there is a delay in gastric emptying and evidence for acute changes in gastric motility (Harm et al., 2002; Amidon et al., 1991). This is also accompanied by alterations in the pharmokinetics of orally administered drugs (Amidon et al., 1991). There were also indications of reduced levels of nutrients in the serum of early astronauts that included gamma-tocopherol, vitamin D and vitamin K though the data on vitamin K has been challenged recently (Vermeer et al., 1998; Zwart et al., 2011). This would suggest that there are underling issues with digestion or absorption of nutrients during space flight.

Two studies performed on young Mongolian gerbils launched on a Bion satellite showed that there were aberrations in the development of the GI tract and the authors included in those finding a histological assessment of what they deemed lymphostasis in the tissue (Atiashkin and Bykov, 2012; Atiashkin et al., 2012). While this is hard to

* Corresponding author.
E-mail address: cromer@tamhsc.edu (W.E. Cromer).

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determine if this truly was loss of lymph transport in the tissue or another form of tissue swelling using the methods used was impossible. The findings of those papers do suggest that there may be further issues with absorbing lipids, protein loss and inflammation seen in other forms of lymphatic insufficiency in the bowel such as lymphangiectasia (Hoshina et al., 2009; Loreti et al., 2003). Our own studies have shown that simulated microgravity impacts the activity of the lymphatic transport and another group has shown that simulated space flight can cause rearrangement of immune tissues associated with the lymphatic system (Gashev et al., 2006; Grigorenko et al., 2015). Another Study more recently has shown that acute space flight (2 weeks) was sufficient to change the makeup of the microbiome of mice which may suggest that the regulation of the host-microbiome interaction is disturbed (Ritchie et al., 2015). This regulation is in part controlled by the ability of the host to sample antigen from the gut lumen and efficiently transport that sample to the lymph node for screening.

Given these findings and that lymphatic impairment is either preceded by or followed by gross inflammation of the tissue we hypothesize that there is lymphatic insufficiency in the GI tract of space flown animals that is associated with inflammation or damage of the tissue (Cromer et al., 2015; Mathias and von der Weid, 2013). We will use surrogate measures of lymphatic function the analyze tissue lymphatic function including lipid deposition in tissue and lymphatic vessel number, size and shape. We will also examine the tissue using histopathological assessment methods used in models of murine inflammatory bowel disease as well as tissue cytokine analysis.

2. Methods

2.1. Tissue acquisition and preparation

Tissues were obtained from the NASA Biospecimen Sharing program after approval of the proposed use. Male rat colon and ileum from the PARE0.3 mission (9 day shuttle flight) were shipped as collected (cleared of intestinal content and snap frozen) on dry ice and were stored immediately at −80 °C until use. Tissues were removed one at a time from storage and placed into liquid nitrogen and forceps were used to gently break the tissue along the longitudinal axis. Approximately half the resulting tissue pieces were placed and oriented in OCT media while still frozen to provide cross sectional sections to be cut. The OCT and tissue were immediately snap frozen in liquid nitrogen before being placed in −80 °C storage. The remaining tissue was placed in cryovials for storage (−80 °C) and protein analysis. Tissue blocks were removed from storage one at a time and placed at −20 °C for 2 hours before transferring them to a cryotome for sectioning. The temperature of the cryotome was set at −22 °C and sections were cut at 10μm thickness and 3 sections were adheared to each Superfrost Plus® glass slide. The slides were allowed to air dry for 30 min each and were then stored at −80 °C until use.

2.2. Histopathology

Tissue slides (1 each per tissue per animal, colon and ileum) were retrieved from storage and fixed in 10% paraformaldehyde (PFA) in phosphate buffered saline (PBS) for 20 min. The slides were then washed 3 times in PBS for 5 min per wash prior to staining with hematoxylin and eosin (H & E). Slides were stained for 30 s in hematoxylin followed by 3 1 min washes in water then stained in eosin for 2 min followed by 3 1 min washes in water. Slides were scored as previously described using an established system for measuring tissue damage in inflammatory bowel disease. Epithelial cell layer: 0 = no damage and a contiguous cells layer with sharp defined edges,1 = ill-defined cell border and cell separations, 2 = occasional breaks in the cell layer amounting to no more than 10% of the surface area (pulling away from one another, 3 = patchy epithelial cell loss of no more than 25% of the surface area 4 = complete ablation of the epithelial cell layer in greater than 50% of the section. Immune cell infiltration (cellularity): 0 = no cell infiltration, 1 = 10% involvement of the tissue, 2 = 25% involvement, 3 = 50% involvement, 4 = 100% involvement. Crypt damage: 0 = normal crypts, 1 = crypts showing signs of mild cell loss, 2 = crypts showing minor cell loss and displacement, 3 = major cell loss and major displacement, 4 = ablation of crypts. Edema: 0 = normal tissue, 1 = mild signs (irregular staining of structures such as muscle), 2 = minor separations in the tissue within the muscle and between the muscle and mucosa, 3 = major separations in the muscle layer that resemble intracellular tears and large gaps between muscle and mucosa, 4 = massive tearing and holes I the muscle layer and near complete disassociation between the muscle and mucosa. Vascularity: 0 = normal number and shape of vessels in the tissue, 1 = slight increase in the abundance of 3rd order vessels, 2 slight increase in 3rd order vessels and mild dysplasia in the 2nd order vessels, 3 = large increase in the number of 3rd order vessels and major dysplasia in the 2nd order vessels, 4 = evidence of granulosing disease around the vessels

2.3. Oil Red-O staining of tissue

Tissue slides (1 each per tissue per animal, colon and ileum) were retrieved from storage and fixed in 10% paraformaldehyde (PFA) in phosphate buffered saline (PBS) for 20 min. Slides were washed in PBS 3 times for 5 min each prior to immersion in 100% propylene glycol for 2 min. Slides were then immersed in pre-warmed (60 °C) Oil Red-O staining solution. The solution with slides were placed at room temperature and allowed to incubate for 15 min. Slides were then placed in 85% propylene glycol for 1 min to differentiate and then rinsed in running water for 1 min. Colon slides were counter stained with Gill's hematoxylin for 30 s followed by 3 1 min washes in water, while ileal slides were not counter stained. Slides were imaged and post processed in Image J (NIH) as listed in Table 1.

2.4. Immunofluorescent tissue staining

Colon slides as prepared above were fixed in 4% PFA for 20 min at room temperature followed by a wash in PBS at room temperature for 5 min. The slides were then blocked in 5% normal goat serum, 0.01% triton X-100 in PBS for 2 hours followed by a wash in PBS for 5 min. An additional Fc blocking step was used for MHC-II antibody staining to reduce cell background staining. Lyve-1 and Prox-1 antibodies were diluted in antibody incubation buffer (0.5% normal goat serum, 0.01% trition X-100, PBS) at 1:200 and applied to the slide and allowed to incubate overnight at 4*C as was the anti-MHC-II antibody (SantaCruz, clone# 10.3-6). Slides were then washed 3 times in PBS for 5 min each wash and secondary antibody (Goat anti-rabbit IgG Alexa488, [Lyve-1, Prox-1] or Goat anti-Mouse IgG H + L Alexa488 [MHC-II]) was diluted 1:200 in antibody dilution buffer and applied to the slides for 2 hours followed by 3 washes in PBS for 15 min each. Slides were imaged on an Olympus wide field upright microscope at 4, 10 and 20× magnification

Table 1
Steps taken to analyze Oil-Red-O staining images using Image J software. All images were acquired and processed identically.

Step	Action in Image J
1	Raw images were separated into individual color channels (RGB)
2	Red channel image was despeckled
3	Red channel image was sharpened
4	Red channel image was converted to 8-bit
5	Threshold was adjusted (set to 40–140 low/high) the same on all images
6	Threshold was applied to images
7	Intestinal area was outlined using the freehand tool (ileum) or epithelial layer (colon)
8	Particle analysis was performed with the following settings; particle size 80–500 μm, roundness 0.5–1, fill in holes, do not count on edges