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Case Series

Unexpected Cardiomyopathy and Cardiac Dysfunction after Administration of Sulfadiazine-trimethoprim Medicated Diet to ICR mice (*Mus musculus*)

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For many years, the University of Chicago administered sulfamethoxazole-trimethoprim sulfate (SMZ-TMP) oral suspension to select immunocompromised mouse colonies via the drinking water. In 2014, SMZ-TMP oral suspension was placed on back-order and medicated diet with a different sulfonamide, sulfadiazine-trimethoprim (SDZ-TMP) was used as a replacement. Months after this transition, sentinel mice from the same room as one of the remaining immunocompromised colonies on this diet were found dead or appeared sick. Necropsies revealed cardiomegaly, and histology confirmed myocardial fibrosis in the first 4 sentinel mice examined, consistent with cardiomyopathy. Subsequent sequential monitoring of 2 sentinel mice via echocardiography showed their progression toward decreased cardiac function. Investigation of the housing room revealed that the sentinel mice had been accidently placed on SDZ-TMP diet upon entering the colony housing room. This case report describes cardiomyopathy in 6 ICR mice after long term consumption of SDZ-TMP medicated feed.

Abbreviations: SDZ-TMP, Sulfadiazine-trimethoprim; SMZ-TMP, Sulfamethoxazole-trimethoprim

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Potentiated sulfonamides, such as sulfadiazine-trimethoprim (SDZ-TMP) and sulfamethoxazole-trimethoprim (SMZ-TMP) have a broad spectrum of activity and are used commonly in both veterinary and human medicine. The combination of the 2 drugs are bacteriostatic and bactericidal, and act synergistically by inhibiting folic acid metabolism in bacteria and some protozoa and fungi.[7] They are commonly administered via water or feed to immunocompromised mice, to prevent or treat opportunistic infections such as *Pneumocystis murina*.[3,6,8,15,22,27,28]

Adverse side effects associated with potentiated sulfonamides include keratitis sicca, hypothyroidism, anemia, leukopenia, and hypersensitivity reactions.[23] Even though sulfonamides have been associated with these adverse side effects, a 2005 toxicology study on SMZ determined it was not a carcinogen after it was administered in the feed at 400 mg/kg/day to mice for 26 wk.[26] Another study found that hypothyroidism in mice was dose-dependent and only induced after administration of a high dose of SMZ-TMP (2400 mg/kg/day) medicated diet for 4 wk.[1] To avoid the onset of hypothyroidism, medicated commercial diets containing sulfonamides should not exceed 240 mg/kg/day.[1] Despite the knowledge we have gained from these studies, other potential adverse side effects have not yet been described in association with long term oral administration of potentiated sulfonamides in mice. Identification of other adverse side effects would provide veterinarians and investigators with more information on safe prophylactic and therapeutic use as an adjunct measure in the maintenance of immunocompromised rodent colonies. This case report identifies an adverse side effect identified in ICR sentinel mice that received SDZ-TMP medicated diet for 3 to 6 mo.

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Case Series

In the summer of 2014, several companies manufacturing sulfamethoxazole-trimethoprim (SMZ-TMP) oral suspension had discontinued production, and the price of the product had risen dramatically. Many investigators at our university with immunocompromised or irradiated mice in their colonies had maintained their animals on this prophylactic antibiotic via the drinking water to prevent infections caused by opportunistic pathogens. We discussed using more stringent barrier procedures instead of using prophylactic antibiotics to avoid infection. However, many investigators requested an alternative to SMZ-TMP oral suspension. Irradiated sulfadiazine-trimethoprim (SDZ-TMP) medicated diet (Teklad TD. 06596, Envigo, Indianapolis, IN) was used as a replacement because SDZ-TMP is similar in structure and function to SMZ-TMP and an 18-wk controlled study performed at our institution revealed it was relatively safe and had minimal side effects.[12] In addition, recent research had shown that commonly used antibiotics, including enrofloxacin, doxycycline, amoxicillin, and SMZ-TMP, did not reach efficacious levels in mice when administered via the drinking water, prompting the move toward medicated diets.[21]

Approximately 6 mo after this transition, the veterinarians started receiving reports of sick (hunched, ruffled and lethargic) or dead sentinel mice that were all housed in the same room. This housing room was located in a facility that was transitioning veterinary oversight, and 3 different veterinarians independently necropsied 4 sentinel mice within a 2-wk period. All 3 veterinarians subjectively noted enlarged hearts in these mice. Organs including the heart and lungs were submitted for histopathology, which later confirmed cardiomyopathy. Affected mice were approximately 4 to 5 mo of age.

Upon further investigation and review of the housing room, we concluded that sentinel mice, including the mice that were recently necropsied, were being fed SDZ-TMP medicated diet. These mice had been placed on SDZ-TMP medicated diet as soon as they entered the housing room and remained on the diet for at least 3 mo. Maintaining sentinel mice on SDZ-TMP medicated diet is not our institution's standard practice. The diet error was likely due to a misunderstanding by the husbandry staff, as the room only housed an immunocompromised colony that was exclusively being fed SDZ-TMP medicated diet. The combination of acute to peracute compromise or death caused by cardiomyopathy, and the coincidental finding of all affected animals fed SDZ-TMP medicated diet over a protracted-time period, led to the consideration that SDZ-TMP diet might have been a contributing factor. Therefore, we pursued additional diagnostics on the remaining 3 clinically unaffected sentinels.

Another important factor in this case report is the identification of a control animal. Approximately 2 mo prior to all of these findings, a veterinary technician who was replacing one of the sentinel mice for this housing room serendipitously noticed the sentinel cage was being fed SDZ-TMP diet. That cage was switched to a standard rodent diet at the time, but the veterinary technician did not further evaluate the diet status of the other sentinel cages in the room. The new sentinel mouse had never been fed SDZ-TMP diet and was only fed standard rodent diet; therefore, it was considered a control animal.

The 2 remaining nonclinical sentinel mice on SDZ-TMP diet plus the control mouse were moved to a nonbarrier room where they continued to be maintained on their respective diets. Nonbarrier rooms are tested quarterly for all excluded pathogens listed below; however, these rooms were established to allow investigators to transport mice into and out of central facilities for various procedures such as echocardiography, which was conducted on these 3 mice. At this time, all sentinel cages in the reference housing room were assessed and confirmed to be on standard rodent diet.

Materials and Methods

Animals and Colony Health Monitoring. Female ICRTac:ICR mice (age 4 to 5 wk) were ordered from Taconic Biosciences (Rensselaer, NY) for use as sentinel mice. This case report summarizes the findings from 7 sentinel mice housed in 5 cages from the same housing room. Six mice from 4 cages were fed SDZ-TMP medicated diet for approximately 3 to 6 mo, while one received standard rodent diet. These animals represent all of the sentinel mice for the housing room during this time period.

Health monitoring was performed on soiled bedding sentinels quarterly. Every 2 wk during cage change, approximately 5 grams of soiled bedding from a maximum of 69 cages was transferred by hand to a clean sentinel cage. The sentinel mice were pair-housed on corncob bedding (Teklad 7097, Envigo, Indianapolis, IN) and maintained in cages on positively pressurized individually ventilated cage racks (Allentown 75 Jag Micro-Barrier, Allentown, NJ). Sentinel mice typically received a standard irradiated rodent diet (Teklad 2918 Envigo, Indianapolis, IN), and a cotton square (Ancare, Bellmore, NY) as enrichment. Animal rooms were maintained on a 12:12 h light:dark cycle with humidity ranging from 30% to 70% and temperatures ranging from 68 to 76 °F. Mice were checked daily by the animal care staff to assure that they were in good health and had adequate food, water, and cage conditions.

Colony mice were considered free of the following viral, bacterial, and parasitic agents: mouse hepatitis virus, Sendai virus, pneumonia virus of mice, mouse parvovirus, minute virus of mice, Theiler murine encephalomyelitis virus, reovirus 3, mouse rotavirus, ectromelia virus, lymphocytic choriomeningitis virus, polyoma virus, mouse cytomegalovirus, mouse adenovirus, K virus, mouse thymic virus, hantavirus, lactate-dehydrogenase elevating virus, *Mycoplasma pulmonis*, *Salmonella* spp., *Citrobacter rodentium*, *Clostridium piliforme*, *Streptobacillus moniliformis*, *Filobacterium rodentium, Corynebacterium kutscheri*, *Syphacia obvelata*, *Aspicularis tetraptera*, *Myobia musculi*, *Myocoptes musculinus*, *Radfordia affinis*, *Psoregates simplex*, *Giardia muris*, *Encephalitozoon cuniculi*, and *Hymenolepis* sp. Organisms known to be endemic at the University of Chicago are *Helicobacter* spp., *Rodentibacter* spp, and Mouse Norovirus with the exception of a few designated rooms. All animal care and use were conducted in accordance with federal policies and guidelines[14] and were approved by the University of Chicago's IACUC. The University of Chicago has a PHS assurance with OLAW, is a USDA registered research facility, and the animal care program has been accredited by AAALAC International since 2002.

Histopathology. All 7 mice assessed in this case report were necropsied by a board-certified laboratory animal veterinarian. Organs were fixed in 10% formalin (Thermo Fischer Scientific, Pittsburg, PA). Heart and lungs (from all mice), and kidneys, liver, and spleen from select mice were submitted to IDEXX BioAnalytics (Colombia, MO). After fixation, trimmed tissues were processed for paraffin embedding, sectioned, stained with hematoxylin and eosin or Masson's trichrome, and evaluated. Hearts were further scored for severity of change(s) and interpreted as to the significance of the findings in the context of epidemiologic information provided. The same board-certified pathologist evaluated all organs.

Echocardiography. The necropsy and histopathology findings from the 4 initial mice prompted an assessment of cardiac function in the remaining 3 live mice. For echocardiogram assessment, mice were anesthetized with isoflurane (Henry Schein Animal Health, Dublin, OH). Temperature was monitored and maintained at 37 °C using a heat pad and heat lamp. Heart rate was monitored and maintained at or above 400 to 450 bpm. The same echocardiographer captured and interpreted all M-mode and pulse Doppler images (Vevo 770 Fujifilm Visual Sonics Toronto, On, Canada). Two-dimensional images were recorded in parasternal long- and short-axis projections, with guided M-mode recordings at the midventricular level in both views. Left ventricular cavity size and percent fractional shortening were measured in at least 3 beats from each projection and averaged.

Serology and serum chemistry. Serology for the adventitious pathogens listed above was only performed for the initial mouse that presented with clinical signs of illness. After euthanasia by CO_2 asphyxiation using a gas displacement rate of 10% to 30% of the chamber volume/minute, cardiac blood was collected onto an Opti-Spot strip (IDEXX BioAnalytics, Columbia, MO). At the time of their euthanasia, cardiac blood collection was also performed on the remaining 3 mice, including the age-matched control, and serum was submitted for blood chemistries. Frozen

Table 1. Histopathologic findings.

Animal ID	Degree of cardiomyocyte degeneration	Fibrosis severity and location	Luminal dilation severity and location
Mouse 1	Chronic degeneration	Marked at base of ventricles; Mild multifocal in LVFW and IVS	None seen
Mouse 2	Chronic degeneration	Marked at base of ventricles; Moderate multifocal in LVFW, IVS; mild RVFW	None seen
Mouse 3	Chronic degeneration	Marked at subepicardial region of ventricle (most likely LVFW); Moderate multifocal in RVFW	Moderate RV
Mouse 4	Chronic degeneration	Marked at base of ventricles, subepicardial region of LVFW, subendocardial region of IVS, multifocally in a ventricle wall (LVFW or IVS); mild multifocal in RVFW	None seen
Mouse 5	Chronic degeneration	Marked at base of ventricles, subepicardial region of LVFW (with extension into rest of LVFW); moderate multifocal in RVFW	Moderate RV
Mouse 6	Chronic degeneration	Moderate at base of ventricles; mild multifocal in IVS	None seen
Control	Mild degeneration	None seen	None seen

LVFW = left ventricular free wall; IVS = interventricular septum; RVFW = right ventricular free wall; RV = right ventricle.

serum was sent to Comparative Clinical Pathology Services, (Columbia, MO). The chemistries were performed on a Beckman Coulter AU680 Automated Chemistry Analyzer (Beckman-Coulter, Brea, CA). Blood from the other mice was not submitted for serology or serum chemistry, as they were either found dead or initially euthanized for nonspecific clinical signs.

Results

Histopathology. All 6 mice maintained on SDZ-TMP diet showed changes associated with moderate to marked chronic cardiomyopathy (Table 1). Histologic changes associated with cardiomyopathy included cardiomyocyte degeneration characterized by vacuolation and fragmentation with fibrosis. For the age-matched control, only a mild amount of cardiomyocyte degeneration was noted (Figure 1 A and B). However, the pathologist noted moderate amounts of cardiomyocyte degeneration in mice fed SDZ-TMP (Figure 1 C and D). Chronicity was denoted by moderate amounts of fibrosis within the ventricles and interventricular septum (Figure 2). No evidence of fibrosis (Figure 2) or dilation was seen in the age-matched control mouse.

Echocardiograms. Echocardiograms were performed on 3 of the 7 mice; 2 had been on SDZ-TMP medicated diet (mice 5 and 6), and one (the control) had been on a standard diet. As listed in Table 2, at the time of euthanasia, mice 5 and 6 had decreased fractional shortenings and ejection fractions compared with the age-matched control mouse (Table 2). After the first echocardiogram assessment, mouse 5 was euthanized due to a severe decrease in its ejection fraction and fractional shortening. Mouse 6 underwent weekly echocardiograms for 3 wk. During the first week, this mouse had a normal ejection fraction (57.3%) and fractional shortening (30%). By the third week, both its ejection fraction and fractional shortening had decreased by almost half, and the mouse was euthanized (Table 2).

Serology and serum chemistry. Serology for adventitious pathogens excluded at our facility was negative in the first sentinel mouse that presented with clinical signs of illness. Most of the values from the serum chemistry (including calcium) for mouse 5 (SDZ-TMP) and the age-matched control were either within the reference range or within acceptable levels relative to the reference range (data not shown). Mouse 6 had an insufficient quantity of serum to run all analytes.

Discussion

Within a 4-wk period, 4 sentinel mice from the same housing room at The University of Chicago were reported as acutely sick or were found dead. Subjective cardiac enlargement was noted on gross necropsy, and cardiomyopathy was confirmed via histopathology. Assessment of the animal housing room revealed that these sentinel mice had been inadvertently receiving SDZ-TMP diet for a few months. The remaining 3 sentinel mice, including 2 on SDZ-TMP medicated diet, and one that had only received standard diet, were monitored by sequential echocardiography and serum chemistry. Echocardiograms were performed in the hope of identifying the development of cardiomyopathy, as the age of these mice and time on SDZ-TMP mirrored that of the previously affected sentinel mice. Cardiomyopathies are associated with decreased ejection fractions and fractional shortenings because the heart muscle is weakened and can no longer pump efficiently. The 2 sentinel mice that had received SDZ-TMP diet for 4 to 6 mo had decreased ejection fractions and fractional shortenings, compared with the age-matched sentinel mouse on standard diet. Furthermore, histopathology confirmed cardiomyopathy in the mice with decreased ejection fractions and fractional shortenings, but was not evident in the age-matched control animal.

At the time of this case report, all 3 rodent barriers at this institution used exposure of sentinel mice to soiled bedding for pathogen testing, resulting in a total census of over 400 sentinel cages. Approximately 100 sentinel mice were ordered quarterly to replace previously used animals. The clinical signs and lesions described in this case report could be due to a spontaneous mutation such as rodent progressive cardiomyopathy.[2,10,16,17] However, none of the sentinel mice from the same shipment that had been fed standard rodent diet rather than feed containing SDZ-TMP showed any clinical signs of cardiac disease. In addition, the vendor was contacted, and reported no occasions of unexpected cardiomyopathy in their outbred stock of ICR mice. Furthermore, unexpected death and clinical illness were not reported in the immunocompromised mice housed in the same room, despite being maintained on the SDZ-TMP diet. Personal communication with the investigator whose mice were in the same housing room as these sentinel mice indicated that the majority of mice housed in that room were inbred strains

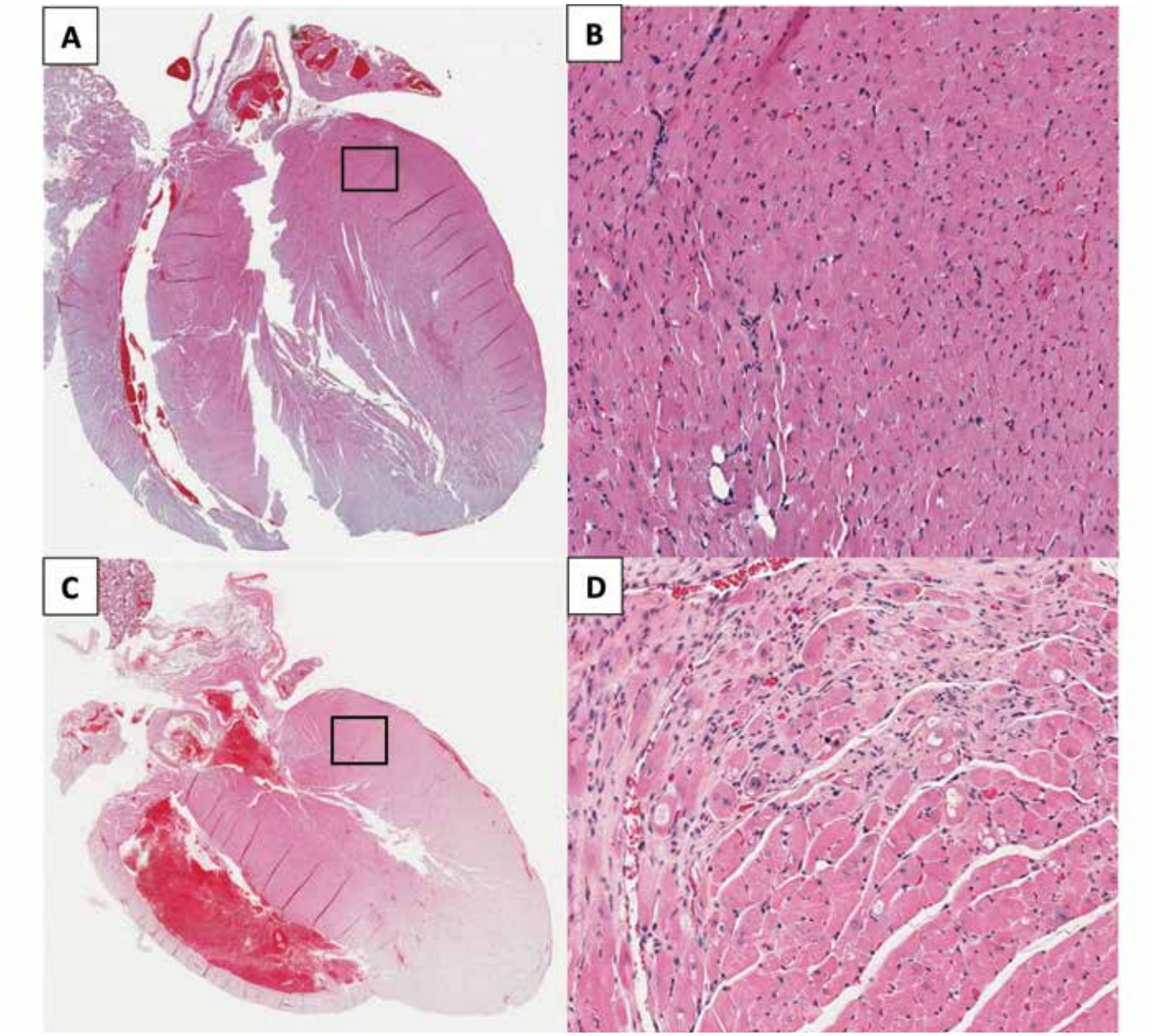

Figure 1. Representative pictomicrograph of the heart from age-matched control (A) and mouse on SDZ-TMP diet (C). Hematoxylin and eosin stain; magnification 2×. Region indicated by square in both A and C are shown in panels B and D under higher magnification. Note the mild amount of cardiomyocyte degeneration (vacuolation and fragmentation) in the age-matched control mouse (B) compared to the moderate degree of degeneration with mice fed SDZ-TMP (D). Hematoxylin and eosin stain; magnification, 5×.

ordered from commercial suppliers for short term studies. Therefore, these mice had been on the SDZ-TMP diet for a much shorter period than the sentinel mice. Some strains of mice were maintained by inhouse breeding; however, the investigator and colony manager had not noted any breeding problems under their standard breeder replacement strategy. For these reasons, the veterinary staff did not have an indication to examine or perform diagnostics on the colony mice.

Immunocompromised mice are susceptible to opportunistic pathogens, many of which are commonly found in vivaria. To avoid opportunistic infections, investigators may therefore elect to place their mice on oral antibiotics. Prophylactic and long-term use of antibiotics may have confounding effects not only on the animal's wellbeing but also on research results. Depending on the duration of the study, adverse side effects of prophylactic antibiotic administration may not always be detected. Although SDZ-TMP medicated diet has been used in numerous rodent colonies at our institution, this case report represents the first time we have observed the development of cardiac dysfunction after 3 to 6 mo of administration of SDZ-TMP medicated diet in ICR mice. We hypothesize that if SDZ-TMP contributed to the cardiomyopathy seen in these 6 sentinel mice, disease development may have been dependent on the duration of exposure and warrants further investigation.

To our knowledge, the literature contains no reports of mice developing cardiomyopathy in association with long term use of SDZ-TMP. If the observation of cardiomyopathy in ICR mice in this case report developed due to long term exposure to SDZ-TMP, 3 mechanisms are possible: direct cardiac toxicity, anemia, or nutrient deficiency due to the disruption of the gut microbiota. Direct cardiac toxicity is associated with damage to the cardiomyocytes, weakening the heart muscle and alterating its contractility. Even though the data in this case report show a decrease in cardiac function and myocardial fibrosis, further research is needed to assess the potential direct impact of sulfonamides on cardiomyocytes. Although CBCs were not

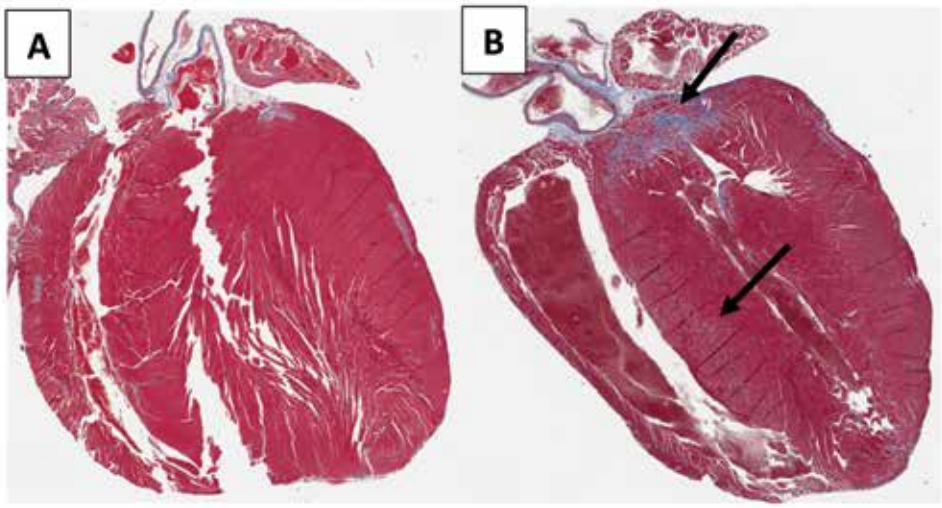

Figure 2. Representative pictomicrograph of the heart from age-matched control (A) and mouse on SDZ-TMP diet (B) with Masson's trichome staining. Note the marked fibrosis (arrows) at the base of the left ventricle and within the interventricular septum in B compared with A. Magnification, 2×.

Table 2. Ejection fraction and fractional shortening prior to euthanasia.

Animal ID	Fractional shortening (%)	Ejection fraction (%)
Mouse 5 (SDZ-TMP)	16	35
Mouse 6 (SDZ-TMP)	19	34
Control	36	66

performed in our mice, anemia has been reported in dogs and cats after receiving SDZ-TMP.[5,23] This can be due to either bone marrow suppression, a hypersensitivity reaction, or hypothyroidism.[23] The thyroid hormone has many functions, which include stimulating the production of erythrocyte precursors and erythropoietin. The pathophysiology of how anemia leads to cardiomyopathy is still unknown. One hypothesis is that cardiomyocytes die from inadequate oxygenation, and to compensate, the heart increases its output to maintain adequate oxygenation. This ultimately causes the ventricles to become worn down and thin. Calcium is an essential nutrient for myocardial contraction, and one study found low serum calcium and vitamin D levels in patients with dilated cardiomyopathy.[24] To determine whether potential dysbiosis due to prolonged antibiotic use was creating a nutrient deficiency and contributing to the development of cardiomyopathy, calcium levels from mouse 5 were evaluated and found to be normal.

This case report has inherent limitations. These limitations include lack of male subjects, limited serum chemistry samples, no inclusion of CBCs, and the availability of only one control animal. The use of sentinel female mice is a common practice in the laboratory animal community, as female mice adjust better to social housing, even as they age. Therefore, we did not use male ICR mice for soiled bedding sentinel monitoring, nor were any male ICR mice exposed to long term SDZ-TMP medicated diet. Veterinarians were not aware that the first 4 mice were being fed SDZ-TMP diet, therefore, blood was not collected for serum chemistry. Despite having a full chemistry panel only from mouse 5 and the single control, nutrient deficiency was considered unlikely, as calcium values were normal in both animals, and no other serum chemistry values indicated a cause for disease or illness in mouse 5. Anemia associated with the administration of sulfonamides has not been reported in mice; therefore, the authors elected not to perform CBCs at the time of the case report. Even though hypothyroidism has been associated with anemia and the administration of sulfonamides, we do not believe these animals were hypothyroid because the development of that condition is reported to be dose-dependent[1] and the commercial medicated diet did not exceed 240 mg/kg/day of SDZ. As described above, the control mouse was available serendipitously and was the only age-matched sentinel on a standard diet in the housing room under investigation.

Potentiated sulfonamides have been known to cause adverse side effects in humans and animals.[1,4,5,9,11,13,18,19,20,23,25] This case report identified a correlation between the long-term delivery of potentiated sulfonamide SDZ-TMP diet to ICR mice and the development of cardiomyopathy. Based on the limitations of this case report, it is premature to conclude causality of cardiomyopathy in ICR mice due to long term use of potentiated sulfonamides. Further studies are needed to determine if observations made in this case report can be recapitulated and, if so, what causal mechanisms might be elucidated.

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