Published in final edited form as:

Radiat Res. 2017 February ; 187(2): 241–250. doi:10.1667/RR14557.1.

Development of A Novel Murine Model of Combined Radiation and Peripheral Tissue Trauma Injuries

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Abstract

Detonation of a 10-kiloton nuclear bomb in an urban setting would result in >1 million casualties, the majority of which would present with combined injuries. Combined injuries, such as peripheral tissue trauma and radiation exposure, trigger inflammatory events that lead to multiple organ dysfunction (MOD) and death, with gastrointestinal (GI) and pulmonary involvement playing crucial roles. The objective of this study was to develop an animal model of combined injuries, peripheral tissue trauma (TBX animal model) combined with total body irradiation with 5% bone marrow shielding (TBI/BM5) to investigate if peripheral tissue trauma contributes to reduced survival. Male C57BL/6J mice were exposed to TBX10%, irradiation (TBI/BM5), or combined injuries (TBX10% + TBI/BM5). Experiments were conducted to evaluate mortality at day 7 after TBI/BM5. Serial euthanasia was performed at day 1, 3 and 6 or 7 after TBI/BM5 to evaluate the time course of pathophysiologic processes in combined injuries. Functional tests were performed to assess pulmonary function and GI motility. Postmortem samples of lungs and jejunum were collected to assess tissue damage. Results indicated higher lethality and shorter survival in the TBX10% + TBI/BM5 group than in the TBX10% or TBI/BM5 groups (day 1 vs. day 7 and 6, respectively). TBI/BM5 alone had no effects on the lungs but significantly impaired GI function at day 6. As expected, in the animals that received severe trauma (TBX10%), we observed impairment in lung function and delay in GI transit in the first 3 days, effects that decreased at later time points. Trauma combined with radiation (TBX10% + TBI/BM5) significantly augmented impairment of the lung and GI function in comparison to TBX10% and TBI/BM5 groups at 24 h. Histologic evaluation indicated that combined injuries caused greater tissue damage in the intestines in TBX10% + TBI/BM5 group when compared to other groups. We describe here the first combined tissue trauma/radiation injury model that will allow conduction of mechanistic studies to identify new therapeutic targets and serve as a platform for testing novel therapeutic interventions.

INTRODUCTION

Since the terrorist attacks on September 11, 2001, the U.S. Government has recognized the need to prepare for potential attacks using weapons of mass destruction involving nuclear

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weapons, radiological dispersal devices or attacks on nuclear plants (1). The government is working actively on the development of medical countermeasures against radiation syndromes. Modeling scenarios, as well as previous experience (detonation of atomic bombs in Hiroshima and Nagasaki in 1945), have predicted that a majority (65–70%) of victims would present with combined radiation injuries (2). Only a small percentage of individuals would be exposed to uniform total-body irradiation (TBI), with most individuals experiencing exposure with variable amounts of bone marrow sparing (3). This would potentially reduce the lethality of the gastrointestinal acute radiation syndrome (GI-ARS) and allow development of other pathologies with longer prodromal phases, such as hematopoietic acute radiation syndrome (H-ARS) (4). GI-ARS plays significant role in the mortality and morbidity in the first several weeks after high-dose irradiation.

Experience has shown that survival after irradiation is affected adversely by concomitant trauma (1, 2, 5). Although it is anticipated that many patients will also suffer burn injuries, significant numbers are also likely to experience other trauma, including blast injuries and crush injuries severe enough to affect skin, underlying muscle, and bones (2, 6, 7). The incidence of severe traumas is important to consider in developing and testing medical countermeasures against acute radiation syndromes (ARS) for two reasons: (1) they have additive effects in combination with radiation and could increase the lethality of otherwise survivable doses of radiation or trauma (1, 2, 4, 8); (2) they will confound the ability to establish reliable biodosimetry because they augment the complexity of the radiation response by triggering cellular, molecular and systemic pathways not affected by radiation alone. In a mass casualty event, it may be necessary to allocate limited resources based on the likelihood for survival (8), therefore, understanding the effects of combined trauma and radiation exposure on survival outcomes is critical for optimizing medical management and triage of the injured.

In preclinical studies radiation has been combined with a number of different injuries (1, 4) including burns (9–11), sepsis (12), incisional wounds (13) and hemorrhage (14). These studies show the increased lethality in combined injuries, more severe tissue damage and the importance of timing of the wounding for survival. Aforementioned combined injuries models, however, have not included sterile trauma with severe tissue damage to the muscle and bones as part of the combined injuries (15). The body's response to different types of harmful impacts varies (16), and therefore, there is unmet need for an animal model of severe crush injury combined with radiation. We previously developed an animal model of peripheral tissue trauma-induced systemic inflammation and GI stasis that reliably and dose-dependently [dose = amount of implanted tissue–bone matrix (TBX) expressed as a percent of total-body weight of the recipient mouse]. This model is particularly useful for studying GI motility because it does not require the use of narcotics, which are known to have immunomodulatory effects. The TBX trauma model is easy to execute and allows dose-dependent control of the degree of tissue injury. In our previous work, we showed that TBX induced systemic inflammation, vascular leakage and dose-dependent impairment of GI motility (15, 17). Heating and decellularisation of the TBX tissue with paracetic acid abrogated the effects on GI motility and elevation of serum cytokines and chemokines, demonstrating the presence and importance of living cells within the implant that drive these pathophysiological processes. Use of TBX model allowed us to precisely determine the dose

of the peripheral tissue damage that will cause significant and transient impairment of the lung and gut function and in the same time being 100% survivable.

The aim of the current study is to describe the combination of the crush injury and radiation in an animal model to investigate the effects of radiation alone, TBX alone or combined injuries on survival and GI and lung function up to day 7 postirradiation. The main objective was to determine contributory role of crush injury to the lethality and pathophysiology of the combined injuries. To the best of our knowledge, this is the first study to simultaneously evaluate changes in function of the gut and lung function after combined injuries.

MATERIALS AND METHODS

Animals and Experimental Design

All experiments in this study were approved by the University of Maryland School of Medicine Institutional Animal Care and Use Committee. We used age-matched, 12-week-old male C57Bl/6J mice purchased from Jackson Laboratories (Bar Harbor, ME). Animals were assigned to one of the following four groups: naïve controls (Sham, $n = 4$); sterile trauma group receiving TBX equal to 10% of body weight (TBX10%, $n = 6$); radiation-only group receiving 12.6 Gy TBI with 5% bone marrow shielding (TBI/BM5, $n = 6$); and animals receiving combined sterile trauma and radiation injury (TBX10% + TBI/BM5, $n = 4$). Animals receiving combined injuries were exposed first to radiation and followed immediately by subcutaneous implantation of TBX10%. After irradiation/TBX implantation, mice were evaluated every 12 h for up to 7 days.

Combined Injury Model

The peripheral tissue trauma model was described previously (15). Briefly, long bones, including muscles and rib cage with intercostal muscles, were isolated and minced (yielding TBX), and then implanted in the subcutaneous pouch of a healthy, syngenic, recipient mouse through a small (1 cm) incision. All irradiated animals were exposed to radiation prior to TBX implantation. Unanesthetized mice were placed in a custom-made jig that allowed for TBI with lead-shielding positioned to cover the tibia, fibula and foot to protect 5% of bone marrow cells and avoid the acute hematopoietic syndrome. The 5% bone marrow shielding was previously calculated and reported by Boggs *et al.* (18). Animals were irradiated using a Precision X-ray 320 irradiator (North Branford, CT) at dose of 12.6 Gy (320 kV, 12.5 mA; 2 mm aluminum filter; HVL ~ 1 mm copper) and a dose rate of 1.58 Gy/min. Immediately after irradiation, mice were anesthetized with Isoflurane (Primal Healthcare Limited, Bethlehem, PA) and the surgical procedure for TBX implantation was performed (15). Kaplan-Meier survival curves were constructed for 7 days, at which time all surviving animals were euthanized.

Unrestrained Whole-Body Plethysmography

Pulmonary function was assessed using unrestrained whole-body plethysmography (Buxco-Data Sciences International, St. Paul, MI) according to a previously reported protocol (19). Baseline measurements were taken for each animal before treatment and lung function was assessed every 12 h after treatment for the 7-day study. We have evaluated (1) tidal volume

(total volume of air that was inspired during a breath), (2) respiratory rate, (3) total minute volume (product of the tidal volume and the number of breaths-per-minute) and (4) Penh (Enhanced Pause, dimensionless index which is a marker of airway hyperactivity or airflow) to determine the functional changes in lungs in Sham, TBX10%, TBI/BM5 and TBX10% + TBI/BM5 groups.

Gastrointestinal Transit Assay

GI transit was assessed by oral gavage of 15 μ L FITC-labeled dextran [fluorescein isothiocyanate (FITC)–dextran, mw 70 kD; Sigma-Aldrich, St. Louis, MO] (15, 20–22). The size of the FITC-dextran molecules ensures that there will be no loss of fluorescence due to passage across the mucosal barrier. After 75 min, mice were euthanized and the GI tract excised en bloc from the stomach to anus and sectioned into stomach, small intestine (10 equal segments), cecum and colon (3 equal segments). Each segment was opened longitudinally along the mesenteric border and placed in 800 μ L phosphate-buffered saline. Tubes were vigorously shaken for 1 min to release all FITC-dextran and then centrifuged for 10 min at 5000g. Fluorescence in the supernatant was measured in duplicate samples (2 $\times$ 100 μ L) using a plate reader (Synergy HT; BioTek Instruments, Inc.; Winooski, VT).

Histology

At the time of euthanasia, a section of middle small intestine was excised for histological evaluation of tissue damage. The small intestine was opened along the mesenteric aspect, pinned and fixed in 4% paraformaldehyde (Sigma-Aldrich). Tissues were embedded in paraffin, sectioned (5 μ m) and stained with hematoxylin and eosin (H&E). Brightfield images of H&E-stained jejunal sections were acquired with a ZEN 2012 (Carl Zeiss Microscopy GmbH; Jena, Germany) and used to measure of villus height (according to a previously established method) measured starting at the crypt neck and ending at the villus tip (9).

Statistical Analysis

Plethysmography data were analyzed according to the Drorbaugh and Fenn method and statistical analyses were performed using Fine Point software (Data Sciences International St. Paul, MN). All other data were analyzed using GraphPad Prism 6 software (San Diego, CA). For nonparametric variables, Kruskal-Wallis and Mann-Whitney U tests were performed (GI transit), and ANOVA with Tukey or Dunnett post hoc tests (villus length) was used for parametric variables. Differences were considered significant when $P < 0.05$.

RESULTS

Survival

All animals receiving TBX10% alone survived the entire length of the follow-up period (7 days), while mice subjected to 12.6 Gy TBI/BM5 died at day 6 postirradiation (Fig. 1). The combination of TBX and radiation dramatically reduced survival and all mice in TBX10% + TBI/BM5 group died within 24 h of combined injuries.

Combined Injury Induces Structural Changes in the Gut

The GI-ARS is a major driver of early lethality in individuals exposed to total-body irradiation doses greater than 8 Gy, although the onset of GI-injury may occur at doses as low as 5 Gy. The extent of tissue damage caused by radiation has been reported previously (23, 24), but the effects of combined radiation and severe peripheral crush injury remain unclear. Histologic evaluation of the small intestine showed that initial damage to the gut at day 3 worsened at day 7 after TBX implantation (Fig. 2). GI damage after TBI/BM5 was observed on day 3 postirradiation and was the most severe on day 6. Animals receiving combined injuries had the worst microscopic damage at 24 h, with extensive areas denuded of villi, submucosal edema and collagen deposition, and thickening of the muscularis externa. Villus length was significantly decreased at day 1 after combined injuries (Fig. 3). Trauma alone induced tissue damage starting at day 3 and worsened at day 7 postirradiation. Loss of villi starting at day 3 and shortening of villi progressed to day 7. In the TBI/BM5 group there was no difference in microscopic appearance and villus length at day 3 postirradiation when compared to the sham group. However, a significant decrease in villus length was seen at later time points in both the TBX10% group (day 7) and TBI/BM5 group (day 6). Interestingly, there was a significant reduction in villus length on day 3 vs. day 7 in the TBI/BM5 group (Fig. 3).

Combined Injury Augments Gastrointestinal Ileus in Mice

GI dysmotility contributes to development of multiple organ failure and is a recognized driver of systemic inflammatory response syndrome in patients suffering severe trauma (25). GI ileus increases the risk of bacterial translocation from the gut lumen and can contribute to development of sepsis (27). Here, we confirmed our previous findings (15) that 24 h after trauma-induced ileus maximal fluorescence is seen in segment 2 of the small intestine, consistent with a slowing of gut motility (Fig. 4A). At this same early time point after irradiation, GI motility in the TBI/BM5 group was comparable to that in the sham group, with the highest concentrations of marker in the more distal segments of the small intestine (segments 9 and 10). The combined trauma group (TBX + TBI/BM5) had significantly delayed transit with the majority of fluorescence in segment 1 of the small intestine. At 72 h, GI transit was recovered in mice that received TBX, whereas irradiated mice showed an onset of ileus, with fluorescence highest in segment 4 of the small intestine (Fig. 4B). At day 7 after treatment (Fig. 4C), transit in TBX mice remained comparable to that in sham-treated mice, whereas at day 6 the TBI/BM5 group continued to exhibit severe GI ileus. All animals receiving combined injuries died by day 3 postirradiation, due to combined injuries, so this group is not represented in Fig. 4B and C.

Combined Injury Impairs Lung Function

Histologic evaluation of the lungs showed no significant morphological differences among the groups (data not shown). The dose of 10% implanted TBX was identified in the preliminary studies as most suitable, because it was nonlethal, but impaired lung function (data not shown). The reduction in lung tidal volume (Fig. 5A) was transient in mice receiving TBX10%, with a maximum effect at 12 h and resolution by 72 h after implantation. In the animals receiving radiation alone (TBI/BM5) change in tidal volume

was first observed at 72 h postirradiation and continued to decline throughout the 6-day study period. Combined injuries caused rapid decrease in tidal volume, starting at 12 h after combined injuries.

Changes in breathing frequency followed the same pattern, early changes in TBX10% group that resolved in 72 h after the TBX implantation and later changes in TBI/BM5 starting at 72 h and continued to decline until day 6 (Fig. 5B). Minute volume, the product of number of breaths in one min and tidal volume, and represents the total amount of air inhaled in one min. In the TBX10% group, minute volume decreased early after the TBX implantation and recovered to baseline at 72 h later. In the TBI/BM5 group, these changes started to occur later (72 h postirradiation) and continued until day 6. The increase in Penh is usually seen in the animals with severe respiratory distress and precedes lethality (27). This process resolved in the animals that received TBX10%, there was increase in the Penh that resolved at later time points similarly to other evaluated parameters; while in the animals that received radiation alone (TBI/BM5 group) these changes started to occur later, and continued to be higher ~2-fold higher at 144 h postirradiation when compared to TBX10% and sham group at same time point. Penh dramatically increased in the first 24 h after combined injuries.

DISCUSSION

A nuclear detonation in a densely populated area will result in nonhomogeneous total body irradiation exposures of a large number of individuals. There is a high probability in this scenario that irradiation would be accompanied with other types of injuries which would further complicate the triage and treatment of affected individuals. Development of medical countermeasures is currently hampered by the lack of animal models of combined radiation injury and severe peripheral tissue trauma that will expand understanding of the pathophysiologic mechanisms that contribute to multiple organ failure. Here we used TBX model in combination with TBI/BM5. Data presented in this study suggest that trauma is major contributor to the early lethality of the combined injuries. We confirmed that combined injuries worsen survival by affecting, both, the GI function and structures, as well as significant impairment in lung function.

Several models of combined radiation injuries exist in which radiation is administered in combination with burns, sepsis, skin incisions and hemorrhage (13, 14). In the model of combined burn (15% total-body surface) and TBI, combined injuries significantly increased lethality (9–11, 24). In an animal model of combined incisional wound inflicted 1 h prior to 9.6 Gy TBI, Kiang *et al.* showed an increase in lethality of combined injuries. However, in separate study, skin wounds inflicted 24 h prior to TBI increased survival (28, 29), which emphasizes the importance of the timing of the secondary impact for the survival. In the current study, we combined radiation with TBX implantation immediately after the radiation exposure to mimic the scenario in which radiation and trauma occur simultaneously or within a short time interval. Our study results are in alignment with other models in which exposure is followed by the secondary impact, different types of trauma or sepsis and contradict the results that incisional wounds followed by irradiation 24 h later increase survival.

The gut is recognized as a major contributor to the development of systemic inflammatory response syndrome (SIRS) and multiple organ failure (MOF) and is of utmost importance to the early survival after irradiation. In the current animal model of combined injuries, we successfully evaluated functional and structural changes in gut and lungs. In the animal model of combined (TBI + burn) injuries, researchers reported increase in gut damage when compared to TBI or burn injuries alone (9–11, 24). They observed the characteristic shortening of villi in the small intestine, with an increase in apoptosis and loss of crypts that was exaggerated by combined injuries (24). Additionally, there is an increase in neutrophil infiltration and edema in the gut. In an animal model of combined TBI with incisional wounding, Kiang *et al.* showed an increase in lethality of combined injuries and increases in systemic levels of pro-inflammatory cytokines. These effects were correlated with an increase in structural damage to the gut caused by combined injuries (13). In our model of combined injuries, we have confirmed the results of other researchers that radiation and combined injuries induce shortening of the villi and gastrointestinal edema. These occurred in the absence of infiltration of the immune cells in the small intestine. Here we showed that radiation and trauma synergistically contribute to functional and structural impairment in gut. Gut stasis is known to promote bacterial overgrowth and translocation and, therefore, our results provide a mechanism to explain the observed increase in bacterial translocation after combined TBI and burn injury (24). Using our novel model of combine injuries, we will be able to investigate factors that contribute to the development of ileus after combined injuries.

Limited data is available on the effects of combined injuries on the lung function. Available data is on the effects of combined burn and radiation injury on the lung structure showing increased neutrophil infiltration and alveolar edema in mice (10). Our results differ from these because in our model we did not observe neutrophil infiltration in lungs (data not shown). The reason for this may lie in different time points for tissue collection and/or differences in trauma. It is well known that lungs respond very differently to different injuries (30). Therefore, potential differences in the DAMP molecules released during burn or sterile crush injury might be the cause of the observed differences. To the best of our knowledge, this is the first study to investigate functional impairment in the lungs after combined injuries. Evaluation of the lung function suggests that, similarly to the dynamic of gut changes, initially the 72 h trauma has more significant impact on the lungs and that radiation contributes later time point. Our results additionally suggest that functional impairment of both lungs and gut precedes damage and that it is important to determine functional impairment of organs during screening and triage of the patients. Lung function needs further investigation to determine the true cause of the observed changes and determine if it is due to the combined injuries or it is because of animals being moribund. Nevertheless, in the animals with combined injuries this impairment in lung function was exacerbated. Palmer *et al.*, showed lung edema in animas after combined radiation and burn injury (10). We have observed significant decrease in tidal volume in mice receiving combine injury when comparing to trauma alone and radiation alone which could be explained by pulmonary congestion after combined injuries showed by Palmer (10). Combined with decrease in breathing frequency, there is a significant decrease in pulmonary function in animals exposed to combined injuries. Penh is often used as a screening

measurement of respiratory distress following irradiation. Here we have observed an increase in Penh which resolved in the animals exposed to TBX alone and increased after combined injuries. Physiological processes involved in the observed functional changes in the lungs are currently investigated.

Predictions of catastrophic scenarios (31, 32) suggest that the majority of treatments in the first 24 h should be focused on trauma for two reasons: (1) it presents more acute contributor to lethality and (2) due to development of H-ARS at later time points and increased risk of infection due to neutropenia. Our data confirm that trauma plays a more critical role in the lethality and impairment of gut and lung function in the first 24 h, whereas radiation has a more prominent role in lethality and gut dysfunction after 6–7 days. We observed transient changes in lung and gut function in the first 72 h in animals receiving TBX alone. These changes resolved at day 7. Similarly, histologic evaluation of the gut showed structural damage to the villi after TBX at early time points, with signs of recovery at day 7. In contrast, radiation injury began to develop at later time points with significant damage observed on day 6 postirradiation. Animals receiving combined injuries had the worst histologic presentation 24 h postirradiation and developed structural damage to tissues very rapidly. Functional impairment of the lungs followed the same pattern. Changes in lung function in the radiation-only group were most likely indirect and induced remotely by GI damage. Combined injuries promotes bacterial translocation and, therefore, ileus seen in our model, at least partially, supports the hypothesis that gut damage drives lung injury. Indeed, suppression of inflammation in the gut by administration of ghrelin in the model of combined radiation and sepsis injury suppressed neutrophil infiltration in lungs, also supporting our hypothesis (12).

Future studies are needed to evaluate the time course of inflammation and systemic response to combined injuries as well as to evaluate the expression of relevant genes and proteins in affected tissues and sera. Completion of these experiments will contribute to a better understanding of the mechanistic role of the gut–lung axis after trauma in general and specifically in combined injuries.

In conclusion, we have described a novel model of combined injuries that does not require the use of narcotics, as in other models, and therefore eliminates the confounding pharmacologic effects of narcotics on immune, lung and GI functions. Our novel model of combined injuries will allow us to search for serum biomarkers for the prediction of lethality and for therapeutic targets as medical countermeasures against radiation.

Acknowledgments

This work has been supported by the NIH grant number U19AI067898.

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FIG. 1. Results of Kaplan-Meier survival curves analysis. There is a significant difference in survival between Sham and TBX10% group when compared to TBI/BM5 and combined injuries group (TBX10% + TBI/BM5) ($P < 0.05$). Additionally, there is a significant difference between TBI/BM5 group when compared to TBX10% + TBI/BM5 group ($P < 0.05$).

FIG. 2.

Sections of jejuna taken from control mice showed characteristic tall villi and associated crypts, goblet cells, compact submucosal space, and a muscularis externa composed of an outer longitudinal and inner circular muscle layer (sham). At day 3 (D3) after TBX10% alone, there was a loss of villi, increase in the number of goblet cells and submucosal edema. The muscularis externa was unchanged (TBX10%, day 3). These changes were exacerbated at day 7 (D7) after TBX10%, with a greater loss of intact villi and reduced numbers of crypts (TBX10%, day 7). At day 3 postirradiation alone, there was an increase in crypt depth, reduced numbers of goblet cells and a slight thickening of the muscularis externa (TBI/BM5, day 3). The injury was more severe at day 6 (D6) after TBI/BM5, with blunted villi, exaggerated crypt loss and greater thickening of the muscularis externa (TBI/BM5, day 6). The combination of TBX10% and 12.6 Gy total-body irradiation with 5% bone marrow sparing worsened the injury when compared to either intervention alone at 24 h (D1) with reduced numbers of intact villi, crypt drop out (areas with no crypts), submucosal edema and increased thickening of the muscularis externa (TBX10% +TBI/BM5 day 1). (10× magnification)

FIG. 3. Results of the measurements of the jejunal villi length. Results were presented as mean $\pm$ SEM. ANOVA with Dunnett's post hoc analysis. * $P < 0.05$ vs sham. § $P < 0.05$ day 3 (D3) vs. day 7 (D7) for each group.

FIG. 4.

Effects of radiation (TBI/BM5), trauma (TBX10%) and combined injuries (TBX + TBI/BM5) on gastrointestinal motility in mice on day 1 (panel A), 3 (panel B) and 6 (TBI/BM5) or 7 (TBX20%) (panel C) postirradiation. Our results indicate that in early time points trauma and combined injuries have more significant influence on gastrointestinal motility. The segment of the small intestine where highest percentages of the FITC-Dextrane 70kD are 1 and 2 in TBX10% + TBI/BM5 and TBX10% group, respectively. There was no difference between the sham and TBI/BM5 groups. At 72 h postirradiation time point, there

is a significant change in the gastrointestinal transit. Animals that received only trauma started to recover from the injury and their motility is similar to sham group where most of the fluorescent marker was concentrated in segments 9 and 10. As expected, animals that received radiation (TBI/BM5) had most of the dye in the segment 4 and 5, showing a decrease in gastrointestinal motility after irradiation ($P < 0.05$ vs. sham and TBX10%). On day 6 postirradiation, GI injury caused by radiation is fully developed and its effects are seen by severely impaired motility, most of the fluorescent dye is in segment 1 of small intestine. Gastrointestinal motility of the animals receiving only trauma (TBX10%) is comparable to controls segments 9 and 10 were the place of highest concentration of FITC-Dextrane 70 kD ($P < 0.05$). [Stomach (st); 1–10 segment of small intestine; cecum (cm); c-1, c-2 and c-3 - segments of colon 1–3].

FIG. 5.

Results of the assessment of lung function using unrestrained whole-body plethysmography every 12 h after the trauma, irradiation or combined injuries. We have evaluated tidal volume (panel A), respiratory frequency (panel B), total minute volume (panel C), and Penh (panel D). In the first 72 h, there was no significant change between TBI/BM5 and sham. After 72 h, animals started to develop functional impairment in breathing that progressed until day 6 when all TBI/BM5 animals died. In the trauma group (TBX10) animals started to developed functional impairment in lung function 12 h after the TBX implantation and it lasted 24–48

h after injury. Breathing parameters improved at 60 h and continued to improve over the course of follow-up period. The animals receiving combined injuries (TBX10 + TBI/BM5) developed the most severe impairment of the lung function 12 h postirradiation, which continued to progress until death 24 h after the combined injuries. Statistically significant values are presented in the tables below the graphs. $P < 0.05$ was considered statistically significant.