

Ghrelin as a Novel Therapy for Radiation Combined Injury

Asha Jacob, Kavin G. Shah, Rongqian Wu and Ping Wang

Laboratory of Surgical Research
The Feinstein Institute for Medical Research, Manhasset, NY 11030

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Department of Surgery
North Shore University Hospital and Long Island Jewish Medical Center

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Please address correspondence, proofs and reprint requests to:

Ping Wang, MD
Laboratory of Surgical Research
The Feinstein Institute for Medical Research
350 Community Drive
Manhasset, NY 11030
Tel: (516) 562-3411
Fax: (516) 562-1022
Email: pwang@nshs.edu

Abstract

There is a growing concern in the world about the exposure to radiation due to the threat of nuclear terrorism. Acute radiation syndrome or radiation sickness develops after a whole body or a partial body irradiation with a high dose of radiation. In the terrorist radiation exposure scenario, however, radiation victims likely suffer from additional injuries such as trauma, burn, wound, or sepsis. This concern has resulted in the need to study whole-body radiation related injuries to intervene therapeutically if such need arises. Despite advances of our understanding of the pathophysiology of radiation injury, very little information is available on the therapeutic approaches of radiation combined injury. In this review, we describe briefly the pathological consequences of ionizing radiation and provide an overview of the animal models of radiation combined injury. We highlight the combined radiation and sepsis model we recently established and suggest the use of ghrelin, a novel gastrointestinal hormone, as a potential therapy for radiation combined injury.

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Introduction

The detonation of nuclear devices in Hiroshima and Nagasaki caused the death of >100,000 people (1). A mathematical model derived by the US Department of Defense using a 12.5 Kiloton nuclear device, which can be delivered in a large suitcase, predicts tens of thousands of casualties with isolated trauma, radiation and combined injury syndrome. Furthermore, it is also estimated that detonation of a 20-megaton warhead would result in 2 million deaths and a large number of casualties (2). Acute radiation syndrome (ARS) or radiation sickness develops after a whole-body or partial-body irradiation with a high dose of radiation (3-6). Radiation injury is most often accompanied by trauma, burn, infection and sepsis and hence termed radiation combined injury.

Radiation Injury

The nuclear attacks in Hiroshima and Nagasaki provide the only direct information regarding the nature of expected injuries following a nuclear attack (1). Even though medical records of about 170 autopsies and clinical observations from 14,000 patients are available, clinical applicability of these reports are limited. In addition, since >100,000 people are believed to have perished in the attack, the limited number of autopsies are unlikely to reflect the pathological consequences of the affected population. Nonetheless, those people who were dying shortly after the attack were presented with injuries to nearly every organ system which included brain, cardiovascular, gastrointestinal tract, respiratory, and renal as well as hematologic/infectious complications (1). In addition to the nuclear attack at Hiroshima and Nagasaki, there were a number of criticality accidents including the accident at Chernobyl on April 1986 which provided important information on the severity of injury caused by ionizing

radiation (3,7-13). Though limited, these observations pinpoint the need for understanding radiation induced injuries to develop therapeutic measures to overcome these defects.

Radiation Sickness or Acute Radiation Syndrome (ARS):

Acute radiation syndrome (ARS) also known as radiation sickness is defined as the signs and symptoms following an exposure to radiation (3). These symptoms develop after either a total or partial body irradiation with a high dose of radiation. These changes are more sensitive in those cells with a high turnover rate which include the skin, hematopoietic system, gut and cerebrovascular system (4,5). The levels of ARS are dependent on the area of irradiation, dose, dose rate, particle type (alpha, beta, gamma, or neutrons) and whether there are concomitant injuries such as trauma, burn and infection. The concomitant injuries results in higher mortality even at lower doses than those with radiation alone.

ARS and the gastrointestinal (GI) tract

The earliest presentation of ARS is GI toxicity (3). The GI tract is among the most radiosensitive organ systems in the body due to the high rate of turnover of mucosal and intestinal cells. In addition to the intestinal epithelium crypt, radiation exposure damages supporting structures such as endocrine glands of the GI tract (14). The GI tract has been considered as the largest endocrine organ because of its complement of endocrine cells which produce a variety of peptides that are involved in GI motility, secretion, absorption, growth, and development (15). Alterations in either hormone secretion or action can have deleterious effects on the GI function.

To test whether GI hormone levels were altered due to radiation in an experimental animal model, we exposed rats to whole-body irradiation using a

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Gammacell 1000 irradiator (Atomic Energy of Canada Ltd). The radioactive source used was Cesium-137 (Cs-137). The irradiator was set to deliver gamma-irradiation at a dose rate of approximately 360 rad/min for 1.4 min, a total of 5 Gy per animal. Our study showed that whole body irradiation significantly decreased plasma cholecystokinin (CCK) and secretin levels at 1-2 days after irradiation. The levels returned to normal at either 4 or 8 days after irradiation presumably due to the differences in radiosensitivity even in different regions of the GI tract (16).

ARS and the systemic inflammatory response syndrome (SIRS)

While individual organ score is useful, a more suitable view for ARS is the concept of multiple organ failure or multiple organ dysfunction syndrome (MODS) caused by a systemic inflammatory response. This notion of MODS became evident from the victims of the recent accidents of Neshvitzh and Tokai-mura who showed a mixed pathology involving the liver and kidney rather than the classical target organs such as the hematopoietic tissues and the gut (17,18). Experimental data from a primate model supported the existence of radiation induced systemic inflammatory response. Pro-inflammatory cytokines such as IL-8 and IL-6 peaked in the blood of these primates during the initial 24 h with whole body radiation of 6-8 Gy and returned to normal by Day 2. Interestingly, the animals irradiated with sublethal dose of 6 Gy showed a second elevation of IL-8 and IL-6 after several days following the irradiation (19). Those animals irradiated with 8 Gy showed significant elevation of these cytokines several days prior to their demise. Marked stimulation of IL-12 and IL-18 presumably by the activation of the Toll signaling pathway has been shown in macrophages of animals which undergone whole body irradiation with as low as 2 Gy (20). Different target organs are affected due to the activation of the innate immune system resulting in the significant release of the pro-inflammatory cytokines (19). The excessive release of these cytokines, may lead to

uncontrolled inflammatory reaction with adverse metabolic and hemodynamic responses (21-23) and eventually multiple organ dysfunction.

In the classical view of the ARS, the role of the central nervous system has been underestimated. It was generally thought that the brain is highly radioresistant and it is only affected at radiation doses as high as 20-30 Gy where a transient or permanent incapacitation of the CNS occurs, leading to coma and death (24,25). This idea is based on the knowledge that the CNS is consisted of a great extent of non-proliferating cells. In fact, the CNS is highly radiosensitive in terms of its electrical activity and neurochemical metabolism with doses as low as 1-2 Gy (26,27). Studies also show early acute dose-dependent overexpression of TNF- α , IL-1 α , IL-1 β 4 to 8 h following mid-brain X-ray irradiation in mice with 7-25 Gy. Increases in IL-1 α and IL-1 β following 5 Gy whole body irradiation were also seen in the cortex of mice at 10 h after irradiation. These data, though limited, supports the hypotheses that CNS is highly responsive to ionizing radiation, induction of cytokines is crucial in radiation-induced responses, and radiation causes local neuroimmune and inflammatory responses.

These studies raise a critical question whether the inflammatory responses produced in the CNS is due to the consequences of systemic inflammatory responses after whole body irradiation or is it due to a direct effect on the CNS. Peripheral pro-inflammatory cytokines are capable of affecting the CNS by either direct entry or by indirectly by the vagal nerve stimulation (28-30). Recent studies demonstrated that electrical stimulation of the vagus nerve subsequent to LPS administration in rats prevented the release of TNF- α from macrophages (31-33). These studies further indicate that action potentials from the vagus nerve traverse the subdiaphragmatic vagus nerve and reach macrophages present in the spleen and suppress TNF- α through molecular mechanism that requires signaling through the nicotinic acetylcholine receptor

subunit $\alpha 7$ (34). Therefore, it is conceivable that the CNS inflammatory response after whole body irradiation could be a consequence of the systemic inflammatory response.

Animal Models of Radiation Combined Injury

Only a limited amount of research has been done to study the consequences of ARS and due to the nature of the studies, they are limited solely to experiments in animals. Most of these studies are performed to circumvent the pathological complications associated with radiation therapy. A number of reports show that radiation-induced production of pro-inflammatory cytokines contribute to the disorders associated with radiotherapy in the blood, peripheral lymphoid tissues and lungs (35-37). In most cases, the inflammatory response is resolved by the release of endogenous anti-inflammatory cytokines. The persistent accumulation and activation of immune cells however lead to inflammation and later adverse effects. The current clinical approach for treatment is to inhibit the production of inflammatory mediators and suppress the initiation of the inflammatory response (38).

Radiation and thermal injury (burn) or wounds

Current animal models of radiation combined injury are limited to radiation exposure and thermal injury (burn) or wounds (39-43). Key features of radiation and burn are [1] shock, which occurs early and often becomes the main cause of death at early times following injury, [2] the dramatic suppression of hematopoiesis and the immune system, [3] the extensive and severe gastrointestinal damages which lead to dysfunction in absorption and secretion and increased risk of infection and [4] delayed wound healing (44). Studies on radiation and burn injuries propose imbalances in the cytokine feedback as the underlying mechanism for radiation combined injury (45).

Radiation induced increases in IL-1 and TGF β 1 expressions are predictive of

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fibrovascular changes after high doses of radiation in several mouse strains and in human patients (46-48). These data suggest that therapies that reduce IL-1, IL-6 and/or TGF β expression might change the fibrovascular effects of radiation exposure and thus, enhance survival. Ran *et al.* showed that serum levels of TNF- α and IL-6 are significantly increased after 12-Gy gamma radiation followed by 30% body surface burn in the rat (49). Similarly, Budagov *et al.* demonstrated that serum levels of IL-6 increase at 6-24 h after radiation and burn injury (50). Peritoneal macrophages from these animals exhibit increased production capacity of IL-6 and IL-1 β in the radiation combined injury group (51). The authors suggest that endogenous infection and bacterial toxins are important to the increased mortality after radiation and burn (52). Several studies using animal models of radiation injury and wounds have shown that radiation exposure delays wound healing (53-58). Thermal injury alone can lead to delayed wound healing, increased susceptibility to sepsis and multi-organ failure (59,60) and additional injuries can exacerbate these immunological complications. Logically, a significant factor involved in the delay is the severity of irradiation one receives at the time of a nuclear attack.

Radiation and sepsis

Regarding infection and sepsis after radiation injury, a study was reported for radiation injury and endotoxemia (61). In that study, mice were exposed to 3-Gy radiation and 10 days later they were injected with 1 mg/kg BW lipopolysaccharide (LPS, endotoxin). LPS-induced thymic atrophy is worsened by radiation. Mice that received both radiation and LPS had lower red blood cells and platelet counts than those received either insult alone. They suggest that radiation has significant influence on LPS-induced changes in several organs and cell populations such as leukocytes, red blood cells, and

platelets, which may increase severity of infection due to Gram-negative bacteria (61). In another study, mice irradiated with 7 Gy and infected with *Klebsiella pneumoniae* exhibited 0% survival while radiation or infection alone produced 100% or 95% survival, respectively (45).

Recently, we have developed an animal model of radiation exposure followed by sepsis induced by cecal ligation and puncture (CLP) [i.e., radiation combined injury (RCI)]. Male Sprague-Dawley rats were exposed to whole-body irradiation and forty-eight hours later, polymicrobial sepsis was induced by CLP (62). These studies show that compared to sham operated rats, serum levels of liver enzymes, AST and ALT were increased by 3 fold and 5 fold in RCI, respectively. Likewise, lactate, creatinine and LDH were increased by 15 fold, 6 fold and 4.8 fold respectively. Similarly, serum levels of pro-inflammatory cytokines, TNF- α and IL-6 were also markedly increased following RCI. Myeloperoxidase activities, used as a measure for neutrophil infiltration to the injured sites, were also markedly elevated in the lungs, small intestine and kidneys in RCI rats (62). In a 10-day survival study, CLP subsequent to radiation exhibited survival rates of 69% at day 1, 50% at days 2 and 3 and decreased to 38% at days 4-10 (62). These studies clearly provided a suitable animal model for RCI to further pursue studies on potential therapies for such injuries.

Therapeutic Approaches to Radiation Combined Injury

Despite our advances in the pathophysiology of radiation combined injury, very little information is available for treatment strategies. Nevertheless, a few studies have been reported as treatment for such injuries. Studies have shown that the administration of anti-IL-6 monoclonal antibodies 1 hour prior and at 1,2, and 3 days following radiation combined burn injury improved survival rate from 0% to 60% in 30 days. Prolonged antibody treatment up to 8 days increased the survival rate to 90% (63,64).

Intraperitoneal application of nerve growth factor (NGF) in radiation and wound model in rats increased the survival rate, peripheral white blood cell counts and bone-marrow nucleated cells suggesting NGF could be therapeutic for wound injuries caused by ionizing radiation (58). Pretreatment with curcumin in radiation and wound model in mice significantly increased the rate of wound healing, increased collagen synthesis, and improved fibroblast and vascular densities in mice (65). In addition, ascorbic acid treatment improved survival and caused dose dependent elevation of wound contraction in mice exposed to combined radiation and wound. COX2 inhibitors, pentoxifylline, esculetoside A are proposed to be beneficial in controlling inflammatory cytokines and protect against radiation (66,67).

With such limited information in treatment strategies, we tested whether ghrelin, which has been shown to be beneficial in sepsis (68-70), could be a potential therapy for RCI. Ghrelin is a novel gastrointestinal hormone, first discovered as an endogenous ligand for the secretagogue receptor type 1a (GHSR-1a) (71). Ghrelin was originally reported to induce growth hormone release through pituitary GHSR-1a stimulation (72-74). However, a large body of evidence has indicated other physiological functions of ghrelin mediated by the central and peripheral ghrelin receptors (75). It has been linked to the regulation of pituitary hormone secretion, feeding, energy homeostasis, gastrointestinal function, and cardiovascular and immune system (76-78). Our recent studies have shown that circulating levels of ghrelin decreased significantly in a rat model of CLP, and ghrelin administration decreases inflammatory responses, improves organ blood flow, attenuates tissue injury, and reduces mortality under such conditions (69,70,78).

To test the effect of ghrelin on RCI, we subjected rats to RCI and treated with human ghrelin (62). Our studies show that serum levels of ghrelin and its gene expression in the stomach were markedly decreased at 20 h after RCI. Administration of

human ghrelin significantly reduced serum levels of AST and ALT by 54% and 41% from the vehicle group, respectively. Similarly, serum LDH, lactate and creatinine were decreased by 57%, 32% and 41% in the treatment group, respectively. Treatment with human ghrelin reduced serum TNF- α and TNF- α gene expression in the small intestine by 50% and 48% as compared to the vehicle group, respectively. Likewise, serum IL-6 and IL-6 gene expression in the gut were decreased by 42% and 29% as compared to the vehicle group, respectively. Ghrelin treatment significantly attenuated myeloperoxidase activities in the lungs, gut and kidneys as well. Finally, treatment with ghrelin improved the survival rate to 69% which was significantly higher than that in the vehicle treated RCI rats. These results suggest that ghrelin is beneficial in preventing RCI in rats (62).

Mechanism of Action of Ghrelin in RCI

Studies from our lab (79) and Kovarik et al. (80) have shown that systemic levels of norepinephrine (NE) are increased significantly during polymicrobial sepsis or CLP. Enterectomy prior to CLP markedly reduce the circulating levels of NE (81). Interestingly, about 50% of the NE formed in the body is produced by the sympathetic nerve fibers in the gut (82,83). These studies demonstrated that gut is the major source of the increased circulating NE in sepsis. We have also shown that NE released from the gut during CLP is crucial in causing the upregulation of pro-inflammatory cytokines and that NE induced hepatocellular dysfunction in early sepsis is mediated by the activation of α_{2A} -adrenoceptors (84,85). In this regard, we examined if circulating levels of NE is altered during RCI and determine whether ghrelin has any effect on NE release. These studies showed that plasma levels of NE were significantly increased in RCI rats as

compared to sham operated rats. Interestingly, intravenous administration of ghrelin decreased these levels by 35% (62).

Previously we have demonstrated that ghrelin's beneficial effect in sepsis was mediated by the activation of the vagus nerve (68). To further determine the mechanism by which ghrelin exerts its effect on RCI, sham and RCI rats subjected to vagotomy immediately prior to ghrelin treatment were examined for inflammatory responses and organ injury markers. These studies show that at 20 h after CLP, serum levels of AST, ALT, LDH, lactate and creatinine were significantly increased in both sham vagotomized and vagotomized RCI rats and were comparable to vagus nerve intact RCI rats. Similarly, serum TNF- α , IL-6 and MPO activities in the intestine, lungs and kidneys were increased in sham vagotomized and vagotomized RCI rats. Interestingly, when sham vagotomized rats were treated with ghrelin, the various organ injury parameters were markedly reduced. In contrast, ghrelin treatment in vagotomized RCI rats exhibited increased levels of these markers as similar to vehicle treated sham vagotomized and vagotomized RCI rats (62). These data strongly supported the notion that ghrelin's beneficial effect on RCI is mediated by the activation of the vagus nerve.

From these studies, we postulate the following mechanism of ghrelin's action in RCI (Figure 1). During RCI, the activation of the sympathetic nervous system causes the release of NE from the gut. The NE released from the gut travels through the portal vein, reaches the liver and binds to α_{2A} -adrenoceptors on the surface of the Kupffer cells and activates them to release pro-inflammatory cytokines such as TNF- α , IL-1 β , IL-6 and HMGB-1. Ghrelin treatment activates the vagus nerve causing the inhibition of the sympathetic fibers and leads to the decrease in NE release from the gut and thereby inhibits the pro-inflammatory cytokines.

Future Studies and Perspectives

We provide herein a comprehensive review of the literature on radiation injury and the pathophysiological consequences of those injuries. We also provided a detailed review of the animal models of radiation combined injury and, though limited, the preclinical studies on the therapeutic approaches available for such injuries. It is important to recognize that no absolute therapies are in use for patients suffering from radiation sickness or in the case of a nuclear attack. It is well accepted that radiation injury combined with trauma, burn and sepsis would be a most likely scenario following a nuclear attack. In such cases, a more suitable view of ARS is multi organ dysfunction due to exacerbated innate immune responses.

The role of the CNS in ARS has been implicated and several studies support the notion that the CNS is involved in the systemic inflammatory response observed after radiation injury. Recently it has been shown that blocking the cervical sympathetic ganglion with diazepam or ketamine following radiation and burn produced significant decrease in mortality rates (from 90% to 55%) and depressed the increases in serum inflammatory factors, TNF- α , IL-1 and IL-6, generally observed after radiation and burn injuries (44). In addition, these studies indicated that overactivation of the hypothalamic-pituitary-adrenal (HPA) axis could be rebalanced to near normal level after sympathetic ganglion block. Since one of the key features of radiation and burn is shock which occurs early and becomes the main cause of death at early times following such injuries, it is plausible that the sympathetic ganglion block could also be beneficial in RCI. Future studies are warranted for such conclusions.

Our studies show that ghrelin treatment in RCI attenuates the inflammatory responses and organ injury, and improves survival. These studies further indicated that the ghrelin's effect on RCI is mediated by the activation of the vagus nerve and

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subsequent inhibition of the sympathetic nervous system leading to a rebalance of the dysregulated sympathetic/parasympathetic nervous system caused by RCI. These studies further support the role of the CNS in the systemic inflammatory response observed in RCI.

It is well recognized that ghrelin is predominantly secreted by the stomach and that it enters the circulation and also crosses the blood brain barrier. We have previously shown that co-administration of ghrelin and LPS, a potent activator of cytokine release, did not decrease LPS-induced TNF- α from Kupffer cells suggesting that the ghrelin's downregulatory effect on cytokine release during sepsis may not be mediated by ghrelin receptors on Kupffer cells (68). Others have shown that ghrelin and ghrelin receptor (GHS-R) are expressed on immune cells (86). Ghrelin inhibits the production of pro-inflammatory cytokines by activated T cells, monocytes and endothelial cells (86,87). This suggests ghrelin functions in the immune cells to regulate local inflammatory response. Therefore, the targets of the exogenous ghrelin could also be immune cells. However, it is still unknown that ghrelin released from the GI tract, which is sensitive to RCI, can regulate inflammatory responses locally. Further studies are required for such findings.

It has been postulated that radiation followed by trauma, burn or wound would lead to multi-organ dysfunction due to uncontrolled systemic inflammatory responses. Thus, ghrelin could also be beneficial in radiation injuries associated with such complications. However, future studies are warranted for such conclusions. Nevertheless, our studies on ghrelin effects on RCI suggest that ghrelin can be developed as a potential therapy for RCI.

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Figure Legend

Figure 1: Whole body radiation (Radiation) combined with polymicrobial sepsis induced by cecal ligation and puncture (CLP) [RCI] activates the sympathetic nervous system (SNS) and causes the release of norepinephrine (NE) from the sympathetic fibers in the gut. The NE then travels through the portal vein into the liver. While in the liver, NE binds to the α_{2A} -adrenoceptors (α_{2A} -AR) and activates the signaling pathway(s) responsible for the production and release of pro-inflammatory cytokines, TNF- α , IL-6, IL-1 β and HMGB-1, from Kupffer cells (KC). Ghrelin, a stomach-derived peptide, reaches the dorsal vagal complex (DVC) in the brain by crossing the blood brain barrier, stimulates GHSR-1a receptors (ghrelin receptors), activates the vagus nerve and in turn, through the cholinergic pathways, downregulates TNF- α and other pro-inflammatory cytokines. While activating the cholinergic pathway, ghrelin can inhibit the SNS, decrease the release of the sympathetic neurotransmitter, NE, and cause the downregulation of the pro-inflammatory cytokines as well. Therefore, ghrelin's beneficial effect in RCI is caused by the rebalance of the dysregulated sympathetic/parasympathetic nervous systems.

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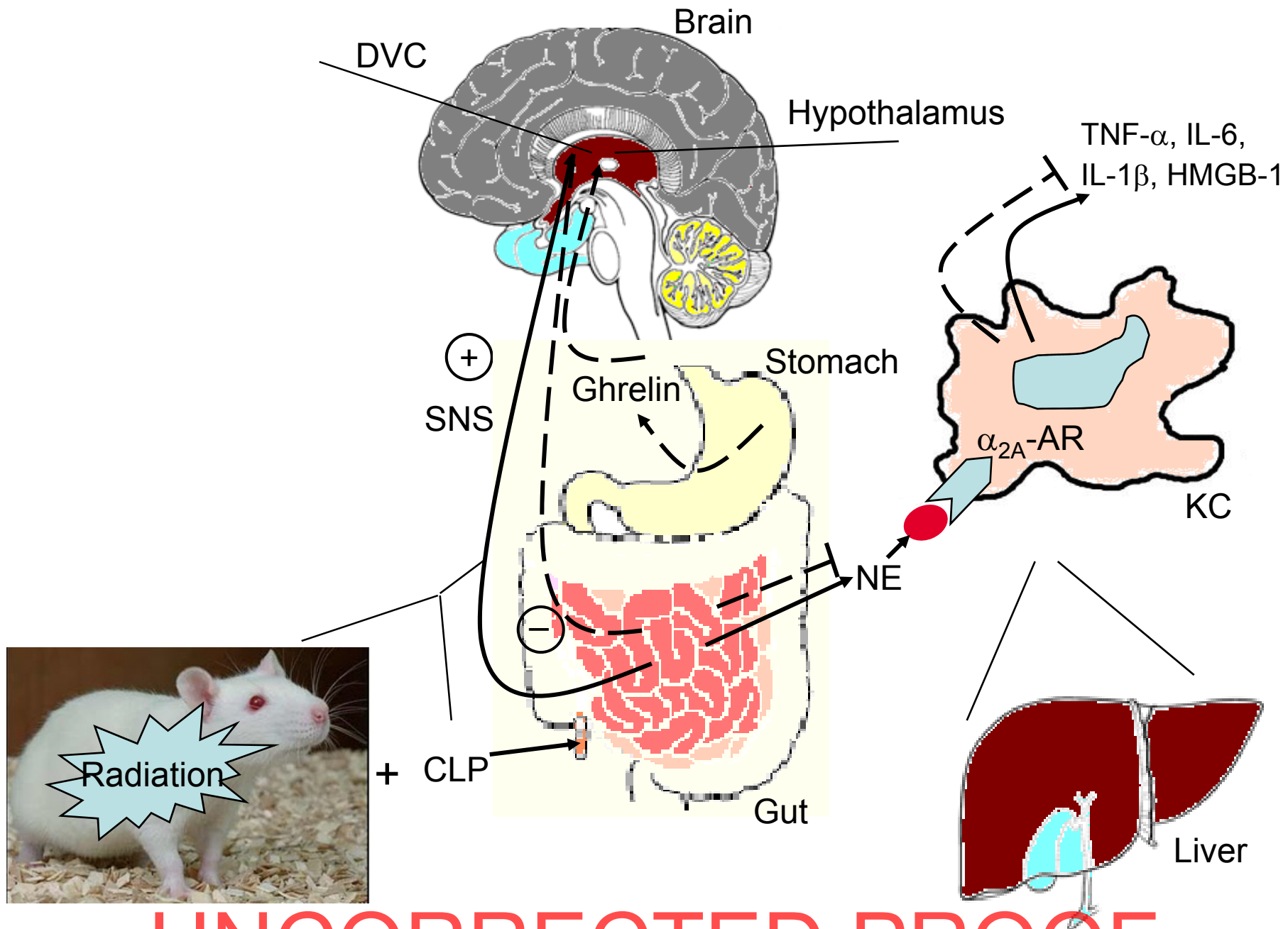
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