Arch. Bas. App. Med. 10 (2022):100– 106
www.archivesbamui.com
www.ojshostng.com/index.php/abam

Research Article

Evaluation of the Effect of Immunocal on Radiation-Induced Mucositis in Wistar Rats.

***Aladelusi T.O.[1], Ogun G.O.[2], Adenipekun A.A.[3], Ladipo J.K.[4]**

[1]Department of Oral and Maxillofacial Surgery, [2]Department of Pathology, [3]Department of Radiation Oncology, [4]Department of Surgery, College of Medicine, University of Ibadan, Ibadan.

Accepted: October 25, 2022

Abstract

Mucositis is a common complication of head and neck radiotherapy. The alleviation of a severe form of this complication is important as it might lead to treatment interruptions which may impact local tumour control. Immunocal, a natural food protein concentrate helps in maintaining glutathione concentration required for building a strong immune system. This study was designed to evaluate the effect of Immunocal on radiation-induced mucositis in buccal and ileal tissues of Wistar rats.

Forty-five Wistar rats divided into five groups were used for this study. Group A served as normal control, Group B did not receive immunocal, Group C and D were treated with Immunocal (oral, 300 mg/kg body weight) from day 1 to 14 and from day 8 to14 respectively while Group E received Immunocal (oral, 300 mg/kg body weight) for 3 days post-irradiation. However, Groups B, C, D and E had a single dose of 4 Gy gamma irradiation at 100.87cGy per minute on day 15. All rats were sacrificed on the third-day post-irradiation, while the buccal mucosa and ileal tissues were harvested for histomorphological assessment.

Comparison of the histological features of harvested buccal and ileal mucosa showed no significant difference in the modified radiation injury scores of the groups which had pre and post irradiation Immunocal supplement compared to the group that had only the irradiation. Gross histomorphology showed significant alteration in the histology of the mucosal epithelium with an early attempt at repair evident in post-irradiation Immunocal-supplemented group. There was a significant reduction ($p < 0.05$) in the weight of all experimental groups on the third day post-irradiation. These findings suggest that post-irradiation supplementation might aid epithelial mucosal healing.

Key Words: Radiation-induced mucosal injury, Immunocal, radiotherapy, histomorphology

INTRODUCTION

Radiotherapy, the medical use of ionizing radiation as part of treatment to control malignant cells, is a major cancer treatment modality and may be used for cancer treatment with palliative or therapeutic intent (Hall and Giaccia 2019) (The precise treatment intent depends on the tumour type, location, stage, as well as the general health status of the patient (Bidram *et al*. 2019). It is also common to combine radiotherapy with surgery, chemotherapy, hormone therapy or other treatment modalities (Mokhtari *et al*. 2017).

Radiation therapy is painless with minimal or no side effects at lower dose palliative treatments, although short-term pain flare-up may be experienced in the days following treatment due to oedema compressing the nerves in the treated area (Wang and Tepper 2021). However, treatment at higher doses which is often required for curative intent, causes varying side effects during treatment (acute side effects), in the months or years following treatment (long-term side effects), or after re-treatment (cumulative side effects) (Brook 2020; Brook 2021). The nature, severity, and longevity of side effects depends on the organs that receive the radiation, the treatment regimen and the patient (Symonds, Mills, and Duxbury 2019). The side effects from radiation are usually limited to the area of the patient's body that is under treatment and most side effects are predictable and expected (Brook 2021).

Common unavoidable acute side effects include damage to the epithelial surfaces of the skin, oral, pharyngeal, bowel mucosa, urothelium, the oral, pharyngeal, oesophageal and intestinal mucosa (Symonds, Mills, and Duxbury 2019). Almost all patients receiving radiation therapy to the head and neck will develop some degree of oral mucositis with the severity influenced by both treatment- and patient-related factors (Pulito *et al*. 2020). It is estimated that approximately 15% of patients treated with radical radiotherapy to the oral cavity and oropharynx will require hospitalization for treatment-related complications (Dragun 2018). Dysphagia secondary to mucositis, loss of taste, loss of appetite and thickened secretions may lead to weight loss in patients with head and neck cancer (Sroussi *et al*. 2017; Cristofaro *et al*. 2021). Furthermore, severe oral mucositis may interfere with the ability to deliver the intended course of therapy, leading to significant interruptions in treatment, possibly impacting on local tumour control and overall patient survival (Anderson and Lalla 2020). Other acute side effects are oedema (as part of the general inflammation) (Turcotte *et al*. 2018) infertility,

**Author for Correspondence:* Tel: +2348033662155

E-mail: *drtimmylee@gmail.com*

if the gonads are involved (De Felice *et al*. 2019) and generalized fatigue (Hsiao *et al*., Saligan 2016). Medium and long-term side effects include fibrosis (Brook 2020), hair loss (Phillips *et al*. 2020), dryness (oral and skin) (Frowen, Hughes, and Skeat 2020), radiation-induced malignancy (Khanna *et al*. 2021) and death (Liu *et al*. 2022).

The use of ionizing radiation in cancer management is based on tissue interaction. Ionizing radiation interacts with biologic matter in several ways. Overall, high-energy photons produce electrons that directly ionize atoms and break chemical bonds (Wakeford and Hauptmann 2022). Subsequently, free radical and other reactive species such as hydroxyls, singlet oxygen, superoxide, and hydrogen peroxide are generated mostly from ionization of water (Çalişkan and Çalişkan 2016) These oxygen-containing molecules are often collectively referred to as reactive oxygen species (ROS) and they cause oxidative damage by virtue of their unpaired valence shell electrons. The role of ROS in radiation-induced tissue injury has been confirmed by the finding that superoxide dismutase (SOD) overexpression and the use of SOD mimetics can mitigate tissue injury following ionizing radiation exposure (McBride, Withers, and Schaue 2019)

Glutathione (GSH) is an endogenous tri-peptide antioxidant synthesised from cysteine. It plays a critical role in preventing oxidative stress, thereby preserving mitochondrial function and averting cellular apoptosis (Gaucher *et al*. 2018) Immunocal® (Immunotec Inc., Vaudreuil-Dorion, Quebec, Canada) is a whey protein supplement that contains abundant amounts of cystine, a cysteine precursor, due to its unique non-denaturing preparation (Bounous 2000) Immunocal has previously been shown to substantially increase blood or lymphocyte GSH levels in patients with HIV infection and cystic fibrosis respectively, owing to its high concentration of non-denatured whey proteins containing the cysteine precursor, cysteine (Micke *et al*., 2002; Grey *et al*., 2003). Immunocal has also been reported to be protective against neurodegenerative diseases and effective in the management of autism (Winter *et al*. 2017; Castejon *et al*. 2021). The aim of this study was to evaluate the effect of Immunocal on alleviation of the histomorphological changes observed at the earliest stage of radiation-induced mucosal injury in the digestive tract of the male Wistar rats. The outcome of the study will be helpful in the exploration of the options available for reduction of the severity of mucositis in patients undergoing radiotherapy for head and neck cancer.

MATERIALS AND METHODS

Animals and treatment group: Forty-five male Wistar rats weighing 100 - 240 g were obtained from Central Animal house, Department of Physiology, College of Medicine, University of Ibadan, Ibadan. All animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NRC 1996). They were acclimatized in the postgraduate animal room of the Department of Anatomy, University of Ibadan, for one week and were assigned to the experimental and control groups by applying the random sampling technique. The rats were divided into five groups:

Group A: Ten (10) rats that received no treatment, and therefore served as normal control.

Group B: Ten (10) rats that were treated with gamma irradiation only, and therefore served as experimental control.

Group C: Ten (10) rats that received oral, 300 mg/kg body weight, Immunocal supplement for 14 days before irradiation. (Day 1 to 14)

Group D: Ten (10) rats that received oral, 300 mg/kg body weight Immunocal supplement for 7 days before irradiation. (Day 8 to 14)

Group E: Five (5) rats that received oral, 300 mg/kg body weight Immunocal supplement for 3 days post irradiation. (Day 15 to 17)

All the rats were kept according to the experimental group to which they belong in Makrolon cages in the same room in a well-ventilated animal house facility at room temperature. The animals were weighed on days 1, 8, 14 and 18 (3 days post-irradiation) using a perforated transparent bowl with a lid placed on a digital weighing scale. They were fed with rat feed (Bendel Feeds, Edo State, Nigeria) and were provided unrestricted access to water. Rats in the treatment groups were given appropriate dosage of Immunocal through the oral route for the proposed period before/after exposure to irradiation at the Radiotherapy Department, University College Hospital, Ibadan.

Exposure of experimental rats to irradiation: Irradiation of the rats was done on day fifteen of the study. The experimental rats were injected with 2.5 mg/kg of diazepam intraperitoneally. Each rat was stationed in a supine position in the radiation chamber within 2 minutes of the administration of diazepam and was exposed to four Gray of gamma rays obtained from a Cobalt 60 source by an Atomic Energy of Canada Limited (AECL) medical Theratron 780C machine. The duration of exposure to the gamma rays delivered to the axial structures of the rat was 13.3 minutes at a dose rate of 100.87/cGy per minute and was delivered in a single dose. The field size used was 25 cm while the source-to-skin distance was 80 cm.

Assessment of the outcome of irradiation: The study of Paris and co-worker on high turnover rate of rat's mucosa and post-irradiation attrition guided this study (Paris *et al*. 2001). Rats in both control and experimental groups were subjected to chloroform anaesthesia and sacrificed on day three post-irradiation. Each rat, after sacrifice, was dissected to harvest the gut. A midline incision was used to access the abdomen. Thereafter, the entire gut from the stomach to the caecum was resected. A 1 cm section of the ileum was taken at 15 cm from the ileo-caecal junction for study. A full thickness resection of the cheek including the buccal mucosa was also harvested for assessment of the buccal mucosa. The specimens thus harvested were placed in 10% formalin. The specimen was processed for Hematoxylin and Eosin staining. Microscopy was done using Axioskop 40 Carl-Zeiss hydra headed light microscope at varying powers (x 10, x 40, x 100). The buccal mucosa and ileal mucosa were microscopically examined for edema, acute inflammation and/or villi atrophy.

The radiation injury score, as described previously by Langberg *et al*. (1992), was modified to highlight the acute nature of the characteristics being measured and the characteristics native to the tissue under observation as seen on light microscopy. A cumulative radiation injury score (as

modified), which is the sum of all the characteristics observed, was allotted.

Data analysis: The obtained data was analysed using SPSS 20.0 software package. Quantitative parameters were expressed as mean ± standard error of mean (SEM). Statistical comparisons among the groups were performed using one-way analysis of variance (ANOVA). The Chi-square test assessed the relationship between the modified radiation injury score of the different groups. The level of significance was set at less than 5%.

RESULTS

General observations

During the investigation, forty-five rats were studied, and the following general observations were made. Firstly, there was progressive weight gain in all the rats from their recruitment into the study to the day of irradiation. However, only the control group remained active (agile and aggressive) throughout the experimental period while all the irradiated rats became weak, slow, and inactive following irradiation. Secondly, the experimental rats passed watery faeces, had diarrhoea post irradiation and did not feed well, whilst those in the control group continued to feed well. There was a significant reduction in the average body weight of rats within each experimental group following irradiation compared with the control group (Table 1).

Table 1:
Weight gain/loss within each group of rats

	Mean weight (mg)	SEM	P – value
Group A: Normal Control			
X	213	67.41	0.008*
Y	227	71.84	
Group B: Experimental control (irradiation only)	229	72.47	0.001*
X	196	62.03	
Y			
Group C: Pre irradiation Immunocal for 14 days	209	66.14	
X	185	58.54	0.001*
Y			
Group D: Pre irradiation Immunocal for 7 days	173	54.75	0.001*
X	147	46.51	
Y			
Group E: Post irradiation Immunocal for 3 days	154	68.75	
X	140	62.5	0.025*
Y			

X – Mean pre-irradiation weight, *Y* – Mean post-irradiation weight, * - P value significant at < 0.05, SEM – standard error of mean, N = 5

Histological findings: Buccal mucosa

Following irradiation, the changes in the buccal mucosa included edema and inflammation. These are reflected in the varying modified radiation injury scores. The comparison of the modified radiation injury score of the buccal mucosa of the experimental rats is as shown in Table 2. There was a statistically significant difference in cumulative modified radiation injury score of the experimental groups when compared with the control group (Table 2 and 3). The histological findings in Group A included presence of minimal inflammation (black arrows), no edematous changes and prominent rete pegs (Figure 1A). The histological findings in Group B were minimal inflammation (black arrows) and moderate edema (white arrows) (Figure 1B). When the cumulative radiation scores were compared with that of Group A, there was a statistically significant difference with p = 0.025. Furthermore, the histological findings in Group C in rats given Immunocal for 14 days and exposed to irradiation on day 15 were moderate inflammation only (black arrows) (Figure 1C), while Group D and E were moderate inflammation and minimal/ moderate edema (white arrows) respectively (Figure 1D-E). When the cumulative radiation scores of Group C, D and E were compared with that of Group A, there was a statistically significant difference with p-value of 0.046, 0,025 and 0.025 respectively (Table 3). There was, however, no statistically significant difference when the cumulative modified radiation injury score for Group B which had irradiation only was compared with that of other irradiated groups that had Immunocal for varying periods of time as the general histomorphological trend was a minimal to moderate response in all the experimental groups (Group B to C, D and E; 0.157, 1.000 and 0.564 respectively). There was also no statistically significant difference when the groups with varying periods of Immunocal administration were compared with one another; Group C to E (p = 0.314), Group D to E (p = 1.000), and Group C to D (p = 0.083).

Table 2:
Modified radiation injury scores for the histological sections of the buccal mucosa of the rats

Rats	Mean ± S. E	
	Edema	Inflammation
Group A	0.40 ± 0.25	1.00 ± 0.00
Group B	2.00 ± 0.32	1.40 ± 0.25
Group C	1.20± 0.20	1.40± 0.25
Group D	1.60± 0.25	1.60 ± 0.25
Group E	1.80 ± 0.20	1.40 ± 0.25

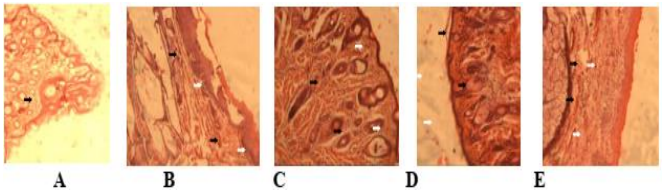

Figure 1A-E: Histological examination of rat buccal mucosa. Photomicrographs of buccal mucosa sections (H & E) rat in Group A (Normal – Control group), Group B (experimental control), Group C (pre irradiation Immunocal for 14 days), Group D (pre irradiation Immunocal supplement for 7 days) and Group E (Post irradiation Immunocal for 3 days) at x100 Magnification.

Table 3:
Analysis of the comparison of the cumulative modified radiation injury scores (mRIS) for rat buccal mucosa in all rat groups

Groups	Statistical Value	Group A	Group B	Group C	Group D	Group E
Group A	χ^2		5.0	4.0	5.0	5.0
	n	5	5	5	5	5
	P-value		0.025*	0.046*	0.025*	0.025*
Group B	χ^2	5.0		2.0	0.001	0.333
	n	5	5	5	5	5
	P-value	0.025*		0.157	1.00	0. 564
Group C	χ^2	4.0	2.0		3.0	1.0
	n	5	5	5	5	5
	P-value	0.046*	0.157		0.083	0.314
Group D	χ^2	5.0	0.001	3.0		0.001
	n	5	5	5	5	5
	P-value	0.025*	1.00	0.083		1.00
Group E	χ^2	5.0	0.333	1.0	0.001	
	n	5	5	5	5	5
	P-value	0.025*	0.564	0.314	1.00	

χ^2 - Chi-square value, n - Number of rats, *P-value significant at <0.05. Group A: Normal control Group B: Experimental control (irradiation only). Group C: Pre irradiation Immunocal for 14 days Group D: Pre irradiation Immunocal for 7 days. Group E: Post irradiation Immunocal for 3 days.

Histological findings: Ileal mucosa

Following irradiation, the changes in the ileal mucosa included varying degrees of villi atrophy, edema and inflammation. These are reflected in the modified radiation injury scores (Table 4 and 5). The histological findings in Group A (normal control) were normal villi (red arrows), minimal inflammation (black arrows) and no edematous changes (Figure 2A). The histological findings in Group B (experimental control) were severe villi atrophy (red arrows), moderate inflammation (black arrows) and moderate edema (white arrows) (Figure 2B). The histological findings in Group C (14 days Immunocal plus irradiation) were moderate villi atrophy (red arrows), mild inflammation (black arrows) and moderate edema (white arrows) (Figure 2C). The histological findings in Group D (7 days Immunocal plus irradiation) were moderate villi atrophy (red arrows), minimal inflammation (black arrows) and moderate edema (white arrows) (Figure 2D). The histological findings in Group E (3 days post-irradiation Immunocal) were mild villi atrophy (red arrows), mild inflammation (black arrows) and moderate edema (white arrows) with increased regenerative attempt seen in the basal layer (Figure 2E).

Table 4:
Modified radiation injury scores for the histological sections of the ileal mucosa of the rats

Rat	Mean ± S. E		
	Villi atrophy	Edema	Inflammation
Group A	0.00 ± 0.0	0.00 ± 0.00	1.00 ± 0.00
Group B	2.40 ± 0.25	1.60 ± 0.25	1.60 ± 0.25
Group C	1.00 ± 0.32	1.00 ± 0.00	1.2 ± 0.25
Group D	1.40 ± 0.25	1.60 ± 0.40	1.00 ± 0.00
Group E	1.60 ± 0.40	2.40 ± 0.25	1.60 ± 0.40

Table 5:
Analysis of the comparison of the cumulative modified radiation injury scores (mRIS) for rat intestinal mucosa in all rat groups

Groups	Statistical Value	Group A	Group B	Group C	Group D	Group E
	χ^2		5.0	5.0	5.0	5.0
Group A	n	5	5	5	5	5
	P-value		0.025*	0.025*	0.025*	0.025*
Group B	χ^2	5.0		1.8	1.8	0.2
	n	5	5	5	5	5
	P-value	0.025*		0.180	0.180	0.655
Group C	χ^2	5.0	1.8		1.0	4.0
	n	5	5	5	5	5
	P-value	0.025*	0.180		0.317	0.046*
Group D	χ^2	5.0	1.8	1.0		3.0
	n	5	5	5	5	5
	P-value	0.025*	0.180	0.317		0.083
Group E	χ^2	5.0	0.2	4.0	3.0	
	n	5	5	5	5	5
	P-value	0.025*	0.655	0.046*	0.083	

χ^2 Chi-square value. n - Number of rats = 5. *P-value significant at < 0.05, Group A: Normal control, Group B: Experimental control (irradiation only), Group C: Pre irradiation Immunocal for 14 days, Group D: Pre irradiation Immunocal for 7 days, Group E: Post irradiation Immunocal for 3 days

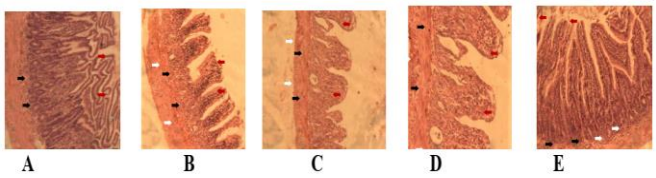

Figure 2A - E: Histological examination of rat small intestine taken from the segments of ileum. Photomicrographs of ileum sections (H & E) of rat in Group A (Normal – Control group), Group B (experimental control), Group C (pre irradiation Immunocal for 14 days), Group D (pre irradiation Immunocal supplement for 7 days) and Group E (Post irradiation Immunocal for 3 days). Magnification x100.

The comparison of the cumulative radiation injury score of the ileal tissue of the groups of experimental rats is shown in Table 4. There was a statistically significant difference in the score of the experimental groups when compared with the control group (group A) with p value of 0.025 for groups B, C, D and E (Table 5). There was no statistically significant difference when group B which had irradiation only was compared with other irradiated groups that had Immunocal for varying period. However, the comparison of group C which had Immunocal for fourteen days before irradiation with group E which had Immunocal for 3 days post irradiation showed a significant difference ($p = 0.046$) in the cumulative modified radiation injury score. From the microscopic findings, there were severely atrophic villi seen in rats that belong to group C while rats in Group E showed moderately formed villi with increased mitotic activity at the basal layer.

DISCUSSION

The study evaluated the effect of Immunocal, a natural food protein concentrate that assists in maintaining concentration of glutathione radiation-induced mucosal injury in the digestive tract of male Wistar rats. The gross observation indicates that the acute dose generated radiation sickness, which led to a significant weight loss similar to previous reports (Malomo *et al*. 2005; Najafi *et al*. 2019). The reason for the weight loss recorded in the study may be attributed to reduced appetite which made the animals less active. Increased catabolism due to inflammatory response to irradiation may have also contributed to the weight loss (Lee *et al*. 2013). The sensitivity of tissues to radiation exposure varies according to the tissue type but is proportional to the rate of cellular division with rapidly regenerating tissues such as intestinal mucosa being the most radiosensitive (Zhao *et al*. 2017; McBride and Schaue 2020; Kosmin and Rees 2022) and whole abdominal irradiation causes its inflammation with submucosal edema, hyperemia, and infiltration of lamina propria with activated inflammatory cells, such as macrophages and neutrophils (Lu *et al*. 2019; Gu *et al*. 2022). The development of copious diarrhea by all rats that had irradiation was due to radiation injury and subsequent inflammatory responses of the continuously cycling cells of the gastrointestinal epithelium (Lee *et al*. 2013; Lu *et al*. 2019; Gu *et al*. 2022). This leads to loss of water absorptive capacity of the simple columnar epithelial cells the decreased absorption and consequent loss of water and nutrients may have worsened the weight loss (McBride and Schaue 2020; Wang and Tepper 2021)

The present study suggests that pre-irradiation administration of Immunocal has no significant protective effect on the buccal mucosa and ileal mucosa when exposed to acute radiation injury. This finding is similar to the report of Ribeiro and Co-workers in 2004. In their investigation on rats that had intestinal resection, they found out that the adaptation response in the group receiving the glutamine-enriched diet was not improved over that of the group fed with regular chow (Ribeiro *et al*. 2004). Also, McGough *et al*., (2004) reviewed the efficacy of nutritional intervention on bowel symptoms during pelvic radiotherapy in data from 2646 patients and found no evidence base for nutritional interventions identified to mitigate bowel symptoms following radiotherapy (McGough *et al*. 2004). This finding is not unexpected as tissue response to acute injury is stereotypical and the mucosa tissues are especially vulnerable to inflammatory process because of their high turnover rate (McBride and Schaue 2020; Wang and Tepper 2021)

In the buccal mucosa sections, there was no statistically significant difference in the cumulative modified radiation injury scores between the groups as radiation injury elicited a mild to moderate response in all the groups. This may be due to the presence of keratin in the buccal mucosa of the rats, which might have conferred some protection on the underlying mucosa. This protective effect is absent in the human buccal mucosa which is usually non-keratinised therefore response to radiation injury is more severe (Mescher, 2018). Radiation-induced oral mucositis is a major dose-limiting toxicity in patients receiving treatment for head and neck cancer. It is a normal tissue injury caused by radiotherapy and it has marked adverse effects on patients' quality of life and cancer therapy continuity (Pulito *et al*. 2020). Also, the finding of severe ileal mucosal injury in the experimental control group while the other groups that had varying administration of Immunocal presented mild to moderate irradiation injury, suggests a positive effect of Immunocal administration. This finding was however not statistically significant, this may also be due to the labile characteristics of the ileal mucosa and its vulnerability to acute inflammation which was however only tempered but not eliminated by the antioxidative effect of glutathione(Winter *et al*. 2017; Teskey *et al*. 2018; Gaucher *et al*. 2018).

The study also reports the non-significant effect of the pre-irradiation duration of Immunocal administration if prolonged from 7 days to 14 days. It was, however, noted that the morphological distortion was less marked in the 14 days administration of Immunocal as compared to the 7 days administration on gross comparison of the slides from each of the groups. This finding further emphasises the vulnerability of the mucosa to acute injury, the pre injury status of the mucosa only attenuates but does not eradicate the acute inflammatory response. The post-irradiation group showed an early attempt at recovery (as at the 3rd day post-irradiation) as shown by the regeneration at the basal layer of the mucosa. This suggests that continuous administration of the agent both pre and post irradiation may have a more potent effect than pre-irradiation administration only. This agrees with previous findings by Bounous in his several works on whey protein concentrate which demonstrated improved clinical outcome and recovery in patients on cancer therapy and in HIV seropositive patients (Bounous *et al*. 1989; Bounous 2000) as well as similar findings by Wischmeyer and Co-workers on glutamine protective effect in intestinal epithelial cells (Wischmeyer *et al*. 1997; Teskey *et al*. 2018)

Glutamine has been demonstrated to have a protective effect against disruption of the mucosal epithelial lining by hindering

the production of proinflammatory cytokine and its metabolite glutathione regulates intracellular oxidative process, thus, buffering the action of reactive oxygen species that play a critical role in the initiation of oral mucositis (Dantas *et al*. 2020). While some authors reported a delay in development and decrease in the frequency of oral mucositis occurrence in patients with glutathione supplements contrary to the findings in the present study (Chattopadhyay *et al*. 2014; Widjaja *et al*. 2020), other authors reported a finding of no significant difference in the occurrence and severity of oral mucositis which is similar to our findings (Sornsuvit *et al*. 2008; Tanaka *et al*. 2016). While the difference in findings may be related to the dosage and mode of administration of the supplements and regimen of the intervention, our study also suggests that post exposure administration of the supplement is more effective in mucosa repair.

In conclusion, the pre-irradiation administration of Immunocal did not significantly protect the buccal or ileal mucosa of male Wistar rats against radiation-induced mucositis. However, the post-irradiation administration of Immunocal showed improved mucosal recovery in the early post-injury phase, suggesting that clinical transfer is feasible. More research is necessary to evaluate the overall role of Immunocal supplementation in the care of patients receiving radiotherapy.

Acknowledgement

The authors would like to acknowledge the efforts of the members of staff of the Department of Anatomy (animal house) and the Department of Radiation Oncology for their support during the execution of this study.

REFERENCES

Anderson, P.M., and R. V Lalla. 2020. "Glutamine for Amelioration of Radiation and Chemotherapy Associated Mucositis during Cancer Therapy*." Nutrients* 12: 1675.

Bidram, E., Y. Esmaeili., H. Ranji-Burachaloo., N. Al-Zaubai., A. Zarrabi., A. Stewart., and D.E. Dunstan. 2019. "A Concise Review on Cancer Treatment Methods and Delivery Systems." *J Drug Deliv Sci Tech* 54 : 101350.

Bounous, G. 2000. "Whey Protein Concentrate (WPC) and Glutathione Modulation in Cancer Treatment." *Anticancer Res* 20 : 4785–92.

Bounous, G., F. Gervais., V. Amer., G. Batist., and P. Gold. 1989. "The Influence of Dietary Whey Protein on Tissue Glutathione and the Diseases of Aging." *Clin Investigat Med 12: 343-49.*

Brook, I. 2020. "Late Side Effects of Radiation Treatment for Head and Neck Cancer." *Radiat Oncol J* 38 : 84–92.

Brook, I. 2021. "Early Side Effects of Radiation Treatment for Head and Neck Cancer." *Radiat Oncol J* 25: 507–13.

Çalişkan, B., and A.C. Çalişkan. 2016. "Interaction of Ionizing Radiation and Radiation Damages (Radicals)." *Intech* 11: 13.

Castejon, A.M., J.A. Spaw., I. Rozenfeld., N. Sheinberg., S. Kabot., A. Shaw., P. Hardigan., R. Faillace., and E.E. Packer. 2021. "Improving Antioxidant Capacity in Children With Autism: A Randomized, Double-Blind Controlled Study With Cysteine-Rich Whey Protein." *Frontiers Psychiat* 12: 1548.

Chattopadhyay, S., A. Saha., M. Azam., A. Mukherjee., and P. Sur. 2014. "Role of Oral Glutamine in Alleviation and Prevention of Radiation-Induced Oral Mucositis: A Prospective Randomized Study." *South Asian J Cancer* 3: 8–12.

Cristofaro, M.G., I. Barca., F. Ferragina., D. Novembre., Y. Ferro., R. Pujia., and T. Montalcini. 2021. "The Health Risks of Dysphagia for Patients with Head and Neck Cancer: A Multicentre Prospective Observational Study - PMC." *J Translat Med* 19: 472–80.

Dantas, J.B. de L., G.B. Martins., H.R. Lima., M. Carrera., S.R. de A. Reis., and A.R.A.P. Medrado. 2020. "Evaluation of Preventive Laser Photobiomodulation in Patients with Head and Neck Cancer Undergoing Radiochemotherapy: Laser in Patients with Head and Neck Cancer." *Special Care Dent* 40: 364–73.

Dragun, A. 2018. "Altered Fractionation Schedules." In Perez and Brady's Principles and Practice of Radiation Oncology, edited by Edward C Halperin, David E. Wazer, Carlos A. Perez, and Luther W. Brady, 7th ed., 1000. Philadelphia: Wolters Kluwer.

Felice, F. De., C. Marchetti., F. Marampon., G. Cascialli., L. Muzii., and V. Tombolini. 2019. "Radiation Effects on Male Fertility." *Androl* 7: 2–7.

Frowen, J., R. Hughes., and J. Skeat. 2020. "The Prevalence of Patient-Reported Dysphagia and Oral Complications in Cancer Patients." *Support Care Cancer* 28: 1141–50.

Gaucher, C., A. Boudier., J. Bonetti., I. Clarot., P. Leroy., and M. Parent. 2018. "Glutathione: Antioxidant Properties Dedicated to Nanotechnologies." *Antioxidants* 7: 62–83.

Gu, J., L. Zhao., Y.Z. Chen., Y.X. Guo., Y. Sun., Q. Guo., G.X. Duan., C. Li., Z. B. Tang., Z. X. Zhang., L. Q. Qin., and J. Y. Xu.2022. "Preventive Effect of Sanguinarine on Intestinal Injury in Mice Exposed to Whole Abdominal Irradiation." *Biomed Pharmacother* 146: 112496.

Hall, E.J., and A.J. Giaccia. 2019. Radiobiology for the Radiologist. 8th ed. Philadelphia: Wolters Kluwer.

Hsiao, C.-P., B. Daly., and L.N. Saligan. 2016. "The Etiology and Management of Radiotherapy-Induced Fatigue." *Expert Rev Qual Life Cancer Care* 1: 323–28.

Khanna, L., S.R. Prasad., S. Yedururi., A.M. Parameswaran., L.P. Marcal., K. Sandrasegaran., S.H. Tirumani., C.O. Menias., and V.S. Katabathina. 2021. "Second Malignancies after Radiation Therapy: Update on Pathogenesis and Cross-Sectional Imaging Findings." *Radiographics* 41: 876–94.

Kosmin, M., and J. Rees. 2022. "Radiation and the Nervous System." *Pract Neurol* 22: 450–60.

Langberg, C.W., T. Sauer., J.B. Reitan., and M. Hauer-Jensen. 1992. "Tolerance of Rat Small Intestine to Localized Single Dose and Fractionated Irradiation." *Acta Oncologica* 31: 781–87.

Lee, B., D. Kim., W. Kim., J. Lee., Y. Lim., D. Shin., J. Nam., and Y. Ki. 2013. "Changes in the Gastric Ghrelin Concentration after Whole-Abdominal Irradiation in Rats: Is This Related to the Radiation-Induced Anorexia and Weight Loss?" *Int J Radiat Res* 11: 131–36.

Liu, E., X. Guan., R. Wei., Z. Jiang., Z. Liu., G. Wang., Y. Chen., and X. Wang. 2022. "Association Between Radiotherapy and Death from Cardiovascular Disease Among Patients With Cancer: A Large Population-Based Cohort Study." *J Am Heart Assoc* 11: e023802.

Lu, L., M. Jiang., C. Zhu., J. He., and S. Fan. 2019. "Amelioration of Whole Abdominal Irradiation-Induced Intestinal Injury in Mice with 3,3′-Diindolylmethane (DIM)." *Free Radical Biol Med* 130: 244–55.

Malomo, A.O., O. Owoeye., T.N. Elumule., E.U.U. Akang., A. Adenipekun., O.B. Campbell., and M.T. Shokunbi. 2005. "The Effect of Dexamethasone, Metronidazole and Ascorbic Acid on the Morphological Changes Induced by Gamma Rays on the Spinal Cord of Wistar Rats." *Afr J Med Med Sci* 34: 161–65.

McBride, William H., and D. Schaue. 2020. "Radiation-Induced Tissue Damage and Response." *J Pathol* 250 : 647–55.

McBride, Williams H., H.R. Withers., and D. Schaue. 2019. "Biologic Basis of Radiation Therapy." In Perez and Brady's Principles and Practice of Radiation Oncology, edited by Edward C Halperin, David E Wazer, Carlos A Perez, and Luther W Brady, 7th ed., 333–412. Philadelphia: Wolters Kluwer.

McGough, C., C. Baldwin., G. Frost., and H.J.N. Andreyev. 2004. "Role of Nutritional Intervention in Patients Treated with Radiotherapy for Pelvic Malignancy." *Br J Cancer* 90: 2287.

Mokhtari, R.B., T.S. Homayouni., N. Baluch., E. Morgatskaya., S. Kumar., B. Das., and H. Yeger. 2017. "Combination Therapy in Combating Cancer." *Oncotarget* 8: 38022–43.

Najafi, M., M. Cheki., G. Hassanzadeh., P. Amini., D. Shabeeb., and A.E. Musa. 2019. "Protection from Radiation-Induced Damage in Rat's Ileum and Colon by Combined Regimens of Melatonin and Metformin: A Histopathological Study." *Anti-Inflammat Anti-Allergy Ag Med Chem* 19: 180–89.

Paris, F., Z. Fuks., A. Kang., P. Capodieci., G. Juan., D. Ehleiter., A. Haimovitz-Friedman., C. Cordon-Cardo., and R. Kolesnick. 2001. "Endothelial Apoptosis as the Primary Lesion Initiating Intestinal Radiation Damage in Mice." *Science (N.Y.)* 293: 293–97.

Phillips, G.S., M.E. Freret., D.N. Friedman., S. Trelles., O. Kukoyi., A. Freites-Martinez., R.H. Unger., J.J, Disa., L.H. Wexler., C.L. Tinkle., J.G. Mechalakos., S.W. Dusza., K. Beal., S.L. Wolden., and M.E Lacouture.. 2020. "Assessment and Treatment Outcomes of Persistent Radiation-Induced Alopecia in Patients with Cancer." *JAMA Dermatology* 156: 963–72.

Pulito, C., A. Cristaudo., C. La Porta., S. Zapperi., G. Blandino., A. Morrone., and S. Strano. 2020. "Oral Mucositis: The Hidden Side of Cancer Therapy." *J Experiment Clin Cancer Res* 39: 1–15.

Ribeiro, S.R., P.E. Pinto Jr., A.C. da Miranda., S.H. Bromberg., F.P. Lopasso., and K. Irya. 2004. "Weight Loss and Morphometric Study of Intestinal Mucosa in Rats after Massive Intestinal Resection: Influence of a Glutamine Enriched Diet." *Rev Hosp Clin Fac Med Sao Paulo* 59: 349–56.

Sornsuvit, C., S. Komindr., S. Chuncharunee., P. Wanikiat., N. Archararit., and P. Santanirand. 2008. "Pilot Study: Effects of Parenteral Glutamine Dipeptide Supplementation on Neutrophil Functions and Prevention of Chemotherapy-Induced Side-Effects in Acute Myeloid Leukaemia Patients." *J Int Med Res* 36: 1383–91.

Sroussi, H.Y., J.B. Epstein., R.J. Bensadoun., D.P. Saunders., R. V. Lalla., C.A. Migliorati., N. Heaivilin., and Z.S. Zumsteg. 2017. "Common Oral Complications of Head and Neck Cancer Radiation Therapy: Mucositis, Infections, Saliva Change, Fibrosis, Sensory Dysfunctions, Dental Caries, Periodontal Disease, and Osteoradionecrosis." *Cancer Med* 6: 2918–31.

Symonds, R., J. Mills., and A. Duxbury. 2019. Walter and Miller's Textbook of Radiotherapy: Radiation Physics, Therapy and Oncology. 8th ed. Amsterdam: *Elsevier*.

Tanaka, Y., T. Takahashi., K. Yamaguchi., S. Osada., T. Shimokawa., and K. Yoshida. 2016. "Elemental Diet plus Glutamine for the Prevention of Mucositis in Esophageal Cancer Patients Receiving Chemotherapy: A Feasibility Study." *Support Care Cancer* 24: 941.

Teskey, G., R. Abrahem., R. Cao., K. Gyurjian., H. Islamoglu., M. Lucero., A. Martinez., *et al*. 2018. "Glutathione as a Marker for Human Disease." *Adv Clin Chem* 87: 141–59.

Turcotte, M.C., E.G. Herzberg., M. Balou., and S.M. Molfenter. 2018. "Analysis of Pharyngeal Edema Post-Chemoradiation for Head and Neck Cancer: Impact on Swallow Function." *Laryngoscope Invest Otolaryngol* 3: 377–83.

Wakeford, R., and M. Hauptmann. 2022. "The Risk of Cancer Following High, and Very High, Doses of Ionising Radiation." *J Radiol Prot* 42: 020518.

Wang, K., and J.E. Tepper. 2021. "Radiation Therapy-associated Toxicity: Etiology, Management, and Prevention." *CA Cancer J Clin* 71 (5): 437–54.

Widjaja, N.A., A. Pratama., R.A. Prihaningtyas., R. Irawan., and I. Ugrasena. 2020. "Efficacy Oral Glutamine to Prevent Oral Mucositis and Reduce Hospital Costs During Chemotherapy in Children with Acute Lymphoblastic Leukemia." *Asian Pac J Cancer Prev* 21: 2117–21.

Winter, A.N., E.K. Ross., V. Daliparthi., W.A. Sumner., D.M. Kirchhof., E. Manning., H.M. Wilkins., and D.A. Linseman. 2017. "A Cystine-Rich Whey Supplement (Immunocal®) Provides Neuroprotection from Diverse Oxidative Stress-Inducing Agents in Vitro by Preserving Cellular Glutathione." *Oxi Med Cell Longev* 2017: 10.1155/2017/3103272.

Wischmeyer, P.E., M.W. Musch., M.B. Madonna., R. Thisted., and E.B. Chang. 1997. "Glutamine Protects Intestinal Epithelial Cells: Role of Inducible HSP70." *Am J Physiol - Gastrointest Liver Physiol* 272: G879–84.

Zhao, L., D. Wu., D. Mi., and Y. Sun. 2017. "Radiosensitivity and Relative Biological Effectiveness Based on a Generalized Target Model." *J Radiat Res* 58 (1): 8–16.