Peertechz

Clinical Group

Archives of Otolaryngology and Rhinology

ISSN: 2455-1759 DOI CC By

Tushar Ramesh[1#], Lindsay S Moore[2#], Neel Patel[3], Kiranya Tipirneni[4], Jason M Warram[2,5], Jillian R Richter[4], Erika M Walsh[2], Geoffrey P Aaron[2], Anthony B Morlandt[6], Brian B Hughley[2] and Eben L Rosenthal[7]*

[1]University of Alabama School of Medicine, Birmingham, AL, USA
[2]Department of Otolaryngology, University of Alabama at Birmingham, Birmingham, AL, USA
[3]Department of Psychiatry, University of Alabama at Birmingham, Birmingham, AL, USA
[4]Department of Surgery, University of Alabama at Birmingham, Birmingham, AL, USA
[5]Department of Radiology, University of Alabama at Birmingham, Birmingham, AL, USA
[6]Department of Oral and Maxillofacial Surgery, University of Alabama at Birmingham, Birmingham, AL, USA
[7]Department of Otolaryngology, Stanford University, Stanford, CA, USA
#These authors contributed equally

Dates: Received: 13 April, 2017; **Accepted:** 19 April, 2017; **Published:** 21 April, 2017

***Corresponding author:** Eben L Rosenthal, Professor of Otolaryngology and Radiology, Ann & John Doerr Medical Director, Stanford Cancer Center, Stanford, CA 94305, USA, Tel: (205) 934-9713; E-mail: elr@stanford.edu

Keywords: Reconstructive surgical procedure; Surgical flaps; Head and neck neoplasms; Radiation; Antineoplastic agents

https://www.peertechz.com

Research Article

Effects of Neoadjuvant Chemotherapy and Radiotherapy on Flap Perfusion in a Novel Mouse Model Using Standard Clinical Assessment and Near-Infrared Fluorescence Angiography

Abstract

Purpose: Minimizing surgical morbidity after local flap reconstruction is important in the management of cutaneous defects. Controversy exists in current literature regarding the effects of radiation and chemotherapy on flap perfusion. Neoadjuvant treatments can damage the microvasculature of the surgical bed through fibrosis, endothelial cell damage, and reduced cell proliferation, which collectively increase the likelihood of postoperative flap failure. The aim of this study is to examine the effects of neoadjuvant radiation and chemotherapy on skin flap perfusion.

Methods: Animals were divided into three groups: control (no treatment, n=4), radiation group (36-Gy administered to dorsal skin, n=4), and chemotherapy group (2 mg/kg IP cisplatin, n=4). Treatments were performed 15 days prior to random-pattern dorsal flap surgery, with a flap length-to-width ratio of 4:1 (4x1cm2). Flap perfusion was assessed via laser-assisted (Luna, Novadaq) indocyanine green dye angiography and standard clinical assessment.

Results: Fluorescence imaging was used to quantify flap perfusion as a fraction of healthy skin perfusion. Chemotherapy group flaps had poorer distal end perfusion than radiation or control group flaps (56% vs. 69% and 71%, respectively) on post-operative day (POD) 1. Clinical assessment of flap perfusion by experienced surgeons on POD2 found chemotherapy group flaps to be least viable. By POD5, 100% of chemotherapy group flaps had experienced complete flap loss as measured by clinical evaluation and perfusion imaging.

Conclusion: Flaps receiving neoadjuvant chemotherapy performed worse than those receiving local radiotherapy or no treatment. We demonstrate the detrimental effect of neoadjuvant chemotherapy on flap viability in this preclinical murine model.

Intoduction

The clinical management of locally advanced cancers of the head and neck pose a significant therapeutic challenge [1]. Despite advances in the field, including the routine use of microvascular free tissue transfers in the reconstruction of defects, flap failure persists as a troublesome complication [2]. The underlying factors that influence the development of flap failure are varied and complex, including comorbid systemic diseases, positive surgical margins, lymph node metastases, and prior radiotherapy and/or chemotherapy [1-3].

Radiation and chemotherapy are often administered neoadjuvantly, or prior to surgical intervention, to gauge the degree of tumor response to treatment [3,4] as sufficient downstaging through neoadjuvant treatments may allow an inoperable tumor to become a feasible candidate for surgical treatment [5]. Additionally, therapy-induced tumor regression may also allow for a reduction in wound size and the sparing of critical neighboring structures [4]. There is concern, however, that neoadjuvant therapies may lead to acute vascular injury, which may, in turn, increase the incidence of flap failure [5,6]. This may be due to capillary flow aberrations [6] and angiogenic alterations [7], predisposing flaps to poor perfusion and subsequently to failure.

Clinically, poor perfusion in a flap often signals the need for operative flap revision or salvage surgery. The current

Citation: Ramesh T, Moore LS, Patel N, Tipirneni K, Warram JM, et al. (2017) Effects of Neoadjuvant Chemotherapy and Radiotherapy on Flap Perfusion in a Novel Mouse Model Using Standard Clinical Assessment and Near-Infrared Fluorescence Angiography. Arch Otolaryngol Rhinol 3(2): 038-042. DOI: http://doi.org/10.17352/2455-1759.000042

standard-of-care relies on qualitative markers of poor perfusion to guide surgeons in the decision-making process. This approach is subjective, and thus may be impacted by the surgeon's level of experience and familiarity with the patient population, among other factors. Recent advances in near-infrared imaging technology have made it possible to assess perfusion in a quantitative manner. The LUNA fluorescence angiography system employs an indocyanine green (ICG) dye to allow for intraoperative and post-operative visualization of perfusion [8]. Several groups have shown that perfusion assessments utilizing LUNA fluorescence angiography are reliable and accurate [9,10].

Random-pattern flaps with length-to-width ratios of 2:1 or less are reliably perfused throughout their length [11]. Flaps with ratios greater than 2:1 have been shown to exhibit ischemia in distal regions due to decreased proximity to the pedicled end [12,13]. Studies conducted in rats bearing over-dimensioned 4:1 random-pattern flaps demonstrated ischemia in distal halves of flaps that, over time, led to flap necrosis in those regions [12,13]. In this study we create over-dimensioned 4:1 random-pattern local flaps in a murine model, anticipating a loss of perfusion in the distal halves of flaps. Our intention was to investigate the effects of neoadjuvant chemotherapy and radiotherapy on flap perfusion, which were evaluated by near-infrared imaging technology and standard clinical assessment.

Methods

Animal model

Twelve female athymic nude mice were used in this study. Animals were caged in groups of 4 and fed a standard laboratory diet of food and water. Animal treatment and husbandry was in accordance with IACUC standards. Mice were divided into three treatment groups: control (no treatment, n=4), radiation group (36 Gy administered to dorsal skin, n=4), and chemotherapy group (2 mg/kg cisplatin, n=4). In the radiation group, mice received three 12 Gy treatments administered focally to dorsal skin flaps over a 6-day period. Cisplatin was administered intraperitoneally (IP). Mice were allowed a 15 day recovery period during which they were monitored daily for possible systemic side effects. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards (approved IACUC protocols) of the institution or practice at which the studies were conducted.

Radiation therapy

An X-Ray irradiator (XRAD320, Precision X-Ray, Inc. N. Bradford, CT, USA) designed for use with cell monolayers and small animals provided radiation therapy in this study. The XRAD320 was calibrated to administer a tube voltage of 320 kV and a tube current of 12.5 mA to generate high-energy electrons at a mean dose rate of 1.169 Gy/min to a total dose of 36 Gy. The Isovolt Titan (General Electric, Fairfield, CT, USA) was utilized to monitor exposure time, x-ray production voltage, and current; the Unidos dosimeter (PTW, Freiburg, Germany) recorded total radiation exposure as well as dose rate. A 20 mm thick aluminum collimator was used to effectively eliminate damaging low-energy x-ray exposure. For the procedure, anesthesia was induced using inhaled isoflurane or 2.5 ug/kg ketamine/xylazine. Animals were placed on either side of a shielded, radiation-safe box in the lateral decubitus position (Figure 1A). Target skin was exposed through a slit, allowing for focal delivery of radiation. The entire length of the flap was exposed to radiation treatment. Nose cones on either end ensured oxygen and isoflurane delivery for the duration of radiation exposure. A dosimeter was placed underneath the exposed skin to measure X-Ray dose. The dosimeter and radiation-safe box were secured to one another and to the base of the XRAD320 to insure homogeneity in radiation exposure among different treatment groups (Figure 1B). A three-eighths inch lead sheet was placed atop the radiation-safe box to minimize radiation exposure to the body of the mice (Figure 1).

Operative technique

Under anesthesia, 4:1 (4 cm x 1 cm) quadrangular random-pattern dorsal flaps were elevated superiorly in the suprafascial plane. After hemostasis was obtained, the lifted flaps were sutured into their original anatomic position using a running 5-0 gut suture in a tension-free manner, then stabilized peripherally with additional pexing sutures. Mice received subcutaneous injections of carprofen for pain control.

LUNA fluorescence angiography

The LUNA imaging system (Novadaq Technologies, Toronto, Canada) was used to quantify flap perfusion on post-operative days (POD) 1 and 4. For the dye, 100 uL (0.5 ug) Indocyanine Green (Novadaq Technologies, Toronto, Canada) was administered to each mouse via tail-vein injection. Video acquisition from a camera positioned 15 cm from the flap plane was utilized for analysis. The SPY-Q analysis software (Novadaq Technologies, Toronto, Canada) determined mean ICG fluorescence values in each of four 1 cm x 1 cm regions within the 4 cm^2 flap as well as fluorescence values from surrounding healthy skin. For uniformity, all fluorescence values were recorded 45 seconds after tail-vein injection.

Physician assessment

On POD2, two blinded otolaryngology residents examined the flaps under white light for qualitative signs of poor perfusion, i.e., skin color, turgor, capillary refill, hematoma,

Figure 1: Radiation therapy administration. (A) Animals were placed on either side of a shielded radiation box in the lateral decubitus position. (B) Target area on the mouse was exposed through a small opening then positioned under the XRAD320.

Citation: Ramesh T, Moore LS, Patel N, Tipirneni K, Warram JM, et al. (2017) Effects of Neoadjuvant Chemotherapy and Radiotherapy on Flap Perfusion in a Novel Mouse Model Using Standard Clinical Assessment and Near-Infrared Fluorescence Angiography. Arch Otolaryngol Rhinol 3(2): 038-042. DOI: http://doi.org/10.17352/2455-1759.000042

seroma, wound infection or dehiscence. The physicians graded the proximal and the distal halves of each flap on a scale of 1 to 3, where '1' indicated an optimally-perfused region, '2' indicated a sub-optimally perfused region, and '3' indicated a region that necessitated surgical revision.

Statistical evaluation

Two-way ANOVA tests were performed to determine significant differences in perfusion across treatment groups, as well as between proximal and distal halves of elevated flaps. Unpaired, two-sample t-tests were used to compare two specific flap halves across time or treatment group. Significance is defined as $P < 0.05$.

Results

Flap survival

Mean flap perfusion is expressed as a percentage of healthy skin perfusion to account for anatomical differences in mice. As shown in Figure 1, the distal portion of all four flaps in the chemotherapy group became necrotic and failed by day 5. In contrast, only one flap was fully compromised in the control group, and no flaps were lost in the radiation group. In addition, two-way ANOVA analysis of POD1 flaps demonstrated significantly greater perfusion in the proximal halves compared to distal halves across all treatment groups ($P < .0001$). Additionally, ANOVA analysis showed mean perfusion (as determined by Luna fluorescence angiography) of full-length flaps across the three treatment groups to be significantly different ($P = 0.0002$) (Figure 2).

Post-operative day 1 fluorescence assessment

In the proximal halves of flaps, perfusion was comparable to that of healthy skin (Figure 3A), as determined by Luna fluorescence angiography. However, no statistically significant differences were observed between the proximal halves of control and radiation flaps ($P = 0.1201$) or control and chemotherapy flaps ($P = 0.1562$). In the distal half of flaps, perfusion was notably less than in healthy skin: radiation flaps ($P = 0.0094$) as well as chemotherapy flaps ($P = 0.0294$) were found to be significantly less perfused than control group distal flaps. When comparing proximal halves to distal halves of flaps in each treatment group, all distal halves were significantly less perfused (all $P < 0.002$). Representative fluorescence images are also shown from POD1 of the negative control (Figure 3B), radiation therapy (Figure 3C), and chemotherapy group (Figure 3D).

Post-operative day 4 fluorescence assessment

LUNA fluorescence angiography performed four days following flap elevation exhibited a progression of decreased perfusion in the chemotherapy group (Figure 4A). Perfusion in the proximal half of radiation group flap was, on average, higher but not statistically different ($P = 0.3723$) from the proximal halves of control group flap. However, perfusion of the proximal flaps was less in the chemotherapy group ($P = 0.0296$) than in the control group. Similarly, distal half perfusion was similar in control and radiation groups ($P = 0.5087$) but decreased in chemotherapy groups ($P = 0.0037$). As expected, distal halves of flaps in each treatment group were found to be more poorly perfused than their proximal half counterparts ($P < 0.05$). Overall, flaps on POD4 were more poorly perfused than on POD1. Representative fluorescence images are also shown from POD4 of the negative control (Figure 4B), radiation therapy (Figure 4C), and chemotherapy group (Figure 4D).

Physician assessment

Clinical assessment performed on POD2 revealed the radiation group to have the healthiest-rated flaps when compared to control and chemotherapy groups (Figure 5). Physicians identified the chemotherapy group flaps as least healthy, both proximally and distally. Distal halves of radiation flaps received a mean score of 1.375, being rated much healthier than control group flaps.

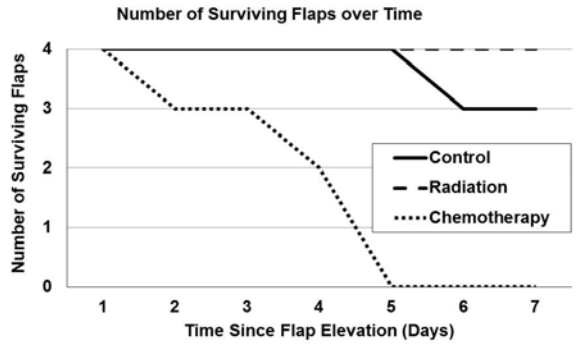

Figure 2: Number of surviving flaps over time. Flap failure was determined by visual presence of necrosis within the local flap. Number of surviving flaps is shown for the negative control, radiation therapy, and the chemotherapy group over seven days.

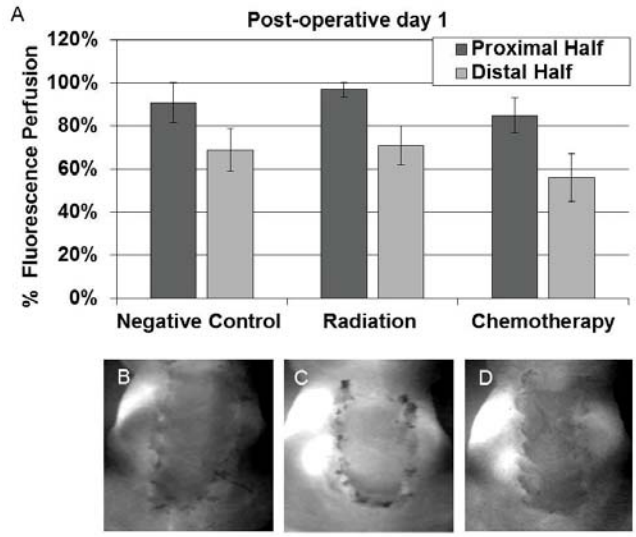

Figure 3: Mean flap perfusion post-operative day 1. (A) The percentage of proximal and distal flap perfusion, as determined by Luna fluorescence angiography, is shown for the negative control, radiation, and chemotherapy groups. Representative Luna fluorescence angiography images are shown for the (B) negative control, (C) radiation, (D) and chemotherapy groups. Values represent mean fluorescence perfusion +/- standard error.

Citation: Ramesh T, Moore LS, Patel N, Tipirneni K, Warram JM, et al. (2017) Effects of Neoadjuvant Chemotherapy and Radiotherapy on Flap Perfusion in a Novel Mouse Model Using Standard Clinical Assessment and Near-Infrared Fluorescence Angiography. Arch Otolaryngol Rhinol 3(2): 038-042. DOI: http://doi.org/10.17352/2455-1759.000042

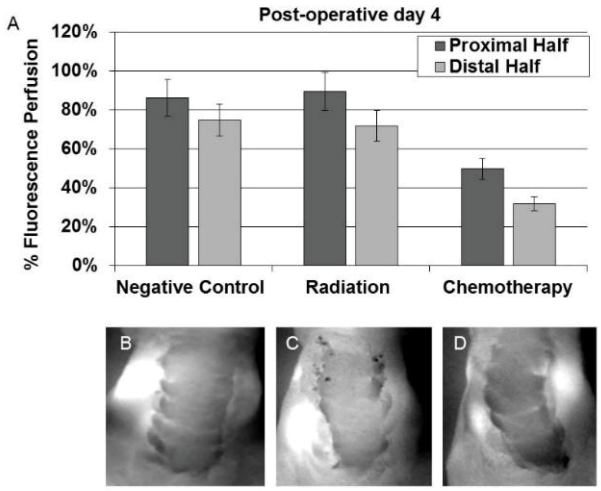

Figure 4: Mean flap perfusion post-operative day 4. (A) The percentage of proximal and distal flap perfusion, as determined by Luna fluorescence angiography, is shown for the negative control, radiation, and chemotherapy groups. Representative Luna fluorescence angiography images are shown from post-operative day 4 for the (B) negative control, (C) radiation, (D) and chemotherapy groups. Values represent mean fluorescence perfusion +/- standard error.

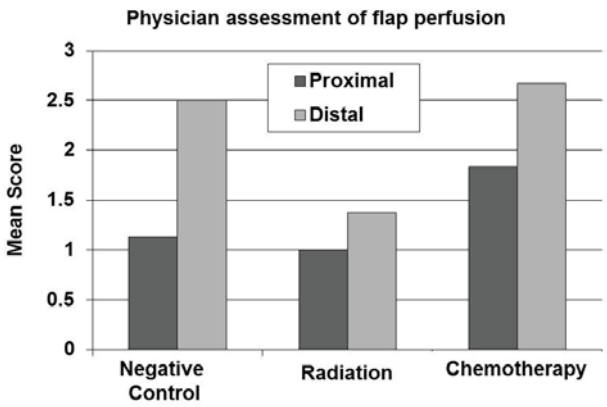

Figure 5: Physician assessment of flap perfusion. Physician assessment (n=2) is shown for the negative control, radiation treatment, and chemotherapy group graded on a 3-point scale at post-operative day 2 (1 - indicated an optimally-perfused region, 2 - indicated a sub-optimally perfused region, 3 - indicated a region that necessitated surgical revision) for the various treatment groups. Both proximal and distal halves were graded on this scale.

Discussion

The tolerance of tissues to radiation and chemotherapy in the perioperative period is not well known. We demonstrate here that the impact of chemotherapy on tissue perfusion after flap elevation is significantly greater in the peri-treatment period. During the study, the local skin flaps exhibited a perfusion gradient with regions proximal to the pedicle having greater perfusion than more distal regions, which was shown to be adversely affected by prior radiotherapy or chemotherapy. This relationship was tested using a preclinical local flap murine model. These perfusion models are useful in eliminating complicating or confounding factors, such as variability in flap geometry and co-morbid disease, which are inherent in clinical cases. In addition, this study design permitted standardized treatment doses and recovery times following neoadjuvant therapy, which often vary widely in clinical studies [14].

Proximal halves of control group flaps—the 2cm x 1cm segments nearest the pedicle—remained adequately perfused as determined by LUNA fluorescence angiography and clinical assessment throughout the experiment, consistent with other groups who employed this model. No statistical difference was found among the treatment groups at POD1, however proximal halves at POD4 of the chemotherapy group flaps were significantly less perfused than control group flaps. Overall, the distal (2cm x 1cm) segments of flaps in this study had poor perfusion. This well-known clinical limitation of random-pattern flaps is why surgeons often select locoregional axial-pattern and perforator flaps, or microvascular free tissue transfers for compromised wound beds [11]. At POD1, a clear perfusion gradient could be visualized in all treatment groups, though the distal-halves of flaps in the chemotherapy group fared the worst. Clinical assessment performed the following day (POD2) and LUNA imaging performed on POD4 reaffirmed these findings, indicating a clear link between neoadjuvant chemotherapy treatments and reduced distal half flap perfusion leading to necrosis.

Chemotherapy hampers the inflammatory response, reducing oxygen delivery to the wound and increasing the likelihood of infection [15]. *Lawrence* et al., found that these delays in wound healing were especially pronounced in cases where chemotherapeutic agents were administered pre-operatively or within 3 weeks post-operatively [7]. Two studies of head and neck cancer patients have established chemotherapy to be among the factors that lead to flap failure [16,17]. Despite this, neoadjuvant chemotherapy remains widely used, albeit in head and neck it is used primarily in advanced stage or surgically unresectable tumors.

Neoadjuvant radiotherapy functions to reduce volume and limit spread of locally advanced head and neck cancers to amend the invasive tumor for surgery [18]. It is unclear, however, whether neoadjuvant radiotherapy may inadvertently complicate microsurgical reconstruction in the process. Animal studies suggest it is detrimental to flap survival [19-21], whereas clinical studies found it to have no appreciable effect [22-24]. In our study, irradiated random-pattern flaps performed similarly to control group flaps in the proximal half and outperformed control group flaps in the distal half. We find the superior performance of radiation group flaps in the distal half to be unremarkable, attributing this finding to human error or limited sample size. The statistical significance of the data may have been limited by the small sample size, suggesting that an increased "n" may allow for more accurate findings. A 36-Gy dose of radiation was selected to produce moderate skin damage with low levels of skin contraction, based on a model developed by *Rifkin* et al. [25]. Higher doses were found to cause increased destruction of epithelial basal cells, leading to desquamation and further reducing viability. It is envisioned that administering greater doses of focal skin irradiation may lead to poorer flap performance.

Numerous conflicting studies can be found in the literature regarding the effects of neoadjuvant treatment on flap survival. These findings may be due to inherent differences in tumor behavior and patient co-morbidities, as well as flap classification by location (local, regional, free), geometric design (advancement, rotation, island), and vasculature

Citation: Ramesh T, Moore LS, Patel N, Tipirneni K, Warram JM, et al. (2017) Effects of Neoadjuvant Chemotherapy and Radiotherapy on Flap Perfusion in a Novel Mouse Model Using Standard Clinical Assessment and Near-Infrared Fluorescence Angiography. Arch Otolaryngol Rhinol 3(2): 038-042. DOI: http://doi.org/10.17352/2455-1759.000042

(random, axial, perforator, myocutaneous, fasciocutaneous). Stricter inclusion criteria in clinical trials would reduce participant heterogeneity and potentially streamline results. Even so, it is difficult to delineate cause and effect in prospective or retrospective studies due to lack of true controls. In this experimental study of mice, we present evidence that 2-mg/kg Cisplatin preoperatively reduces flap perfusion, and that preoperative 36-Gy radiation to the skin does not affect flap perfusion.

The current study employs the novel use of an over-dimensioned random-pattern skin flap in a murine model to study effects of neoadjuvant therapies on flap perfusion. The study utilized two independent metrics, LUNA fluorescence angiography and clinical judgment. Results showed that flaps receiving neoadjuvant chemotherapy fared worse than flaps in the control group. Neoadjuvant radiation had no detrimental effects on flap perfusion. This study can assist clinicians in making informed decisions pertaining to the use of neoadjuvant therapies, obviating the risk of vascular compromise that may accompany such treatments.

References

1. Pattani KM, Byrne P, Boahene, Boahene K, Richmon J (2010) What makes a good flap go bad? A critical analysis of the literature of intraoperative factors related to free flap failure. Laryngoscope 120: 717-723. Link: https://goo.gl/MeO2eo

2. Fujioka M (2013) Factors Predicting Total Free Flap Loss after Microsurgical Reconstruction Following the Radical Ablation of Head and Neck Cancers. ISRN Plastic Surgery 13: 5. Link: https://goo.gl/fhL7Tx

3. Lee S, Thiele C (2010) Factors associated with free flap complications after head and neck reconstruction and the molecular basis of fibrotic tissue rearrangement in preirradiated soft tissue. J Oral Maxillofac Surg 68: 2169-2178. Link: https://goo.gl/7c5p5J

4. Mücke T, Konen M, Wagenpfeil S, Kesting MR, Wolff KD, et al. (2011) Low-dose preoperative chemoradiation therapy compared with surgery alone with or without postoperative radiotherapy in patients with head and neck carcinoma. Ann Surg Oncol 18: 2739-2747. Link: https://goo.gl/HSks09

5. Caudle AS, Gonzalez-Angulo AM, Hunt KK, Pusztai L, Kuerer HM, et al. (2011) Impact of progression during neoadjuvant chemotherapy on surgical management of breast cancer. Ann Surg Oncol 18: 932-938. Link: https://goo.gl/mpC3x4

6. Halle M, Bodin I, Tornvall P, Wickman M, Farnebo F, et al. (2009) Timing of radiotherapy in head and neck free flap reconstruction–a study of postoperative complications. J Plast Reconstr Aesthet Surg 62: 889-895. Link: https://goo.gl/ri1dRJ

7. Lawrence WT, Norton JA, Harvey AK, Gorschboth CM, Talbot TL, et al. (1986) Doxorubicin-induced impairment of wound healing in rats. J Natl Cancer Inst 76: 119-126. Link: https://goo.gl/dnQsxP

8. Green JM, Thomas S, Sabino J, Howard R, Basile P, Dryden S, et al. (2013) Use of intraoperative fluorescent angiography to assess and optimize free tissue transfer in head and neck reconstruction. J Oral Maxillofac Surg 71: 1439-1449. Link: https://goo.gl/1rCiSV

9. Matsui A, Lee BT, Winer JH, Laurence RG, Frangioni JV (2010) Predictive capability of near-infrared fluorescence angiography in submental perforator flap survival. Plast Reconstr Surg 126: 1518-2178. Link: https://goo.gl/OxNwLk

10. Yeoh MS, Kim DD, Ghali GE (2013) Fluorescence angiography in the assessment of flap perfusion and vitality. Oral Maxillofac Surg Clin North Am 25: 61-66. Link: https://goo.gl/M9T4Ya

11. Milton SH (1970) Pedicled skin-flaps: the fallacy of the length: width ratio. Br J Surg 57: 502-508. Link: https://goo.gl/spXIcz

12. Lei Cui, Fa-cheng Li, Qun Zhang, Yun-liang Qian, Wen-xiang Guan (2003) Effect of adenovirus-mediated gene transfection of vascular endothelial growth factor on survival of random flaps in rats. Chin J Traumatol 6: 199-204. Link: https://goo.gl/A2UPaA

13. Giunta RE, Holzbach T, Taskov C, Holm PS, Konerding MA, et al. (2005) AdVEGF165 gene transfer increases survival in overdimensioned skin flaps. The Journal of Gene Medicine 7: 297-306. Link: https://goo.gl/HBrThj

14. Blanchard P, Baujat B, Holostenco V, Bourredjem A, Baey C, et al. (2011) Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): a comprehensive analysis by tumour site. Radiother Oncol 100: 33-40. Link: https://goo.gl/4DEZ06

15. Pasquier E, Tuset MP, Street J, Sinnappan S, MacKenzie KL, et al. (2013) Concentration- and schedule-dependent effects of chemotherapy on the angiogenic potential and drug sensitivity of vascular endothelial cells. Angiogenesis 16: 373-386. Link: https://goo.gl/tZDt2h

16. Bozikov K, Arnez ZM (2006) Factors predicting free flap complications in head and neck reconstruction. J Plast Reconstr Aesthet Surg 59: 737-742. Link: https://goo.gl/XU8vMZ

17. Posner MR, Weichselbaum RR, Fitzgerald TJ, Clark JR, Rose C, et al. (1985) Treatment complications after sequential combination chemotherapy and radiotherapy with or without surgery in previously untreated squamous cell carcinoma of the head and neck. Int J Radiat Oncol Biol Phys 11: 1887-1893. Link: https://goo.gl/2UMvs9

18. Mücke T, Rau A, Weitz J, Ljubic A, Rohleder N, Wolff KD, et al. (2012) Influence of irradiation and oncologic surgery on head and neck microsurgical reconstructions. Oral Oncol 48: 367-371. Link: https://goo.gl/dKW4lD

19. Schultze-Mosgau S, Keilholz L, Rodel F, et al (2001) Experimental model for transplantation of a modified free myocutaneous gracilis flap to an irradiated neck region in rats. Int J Oral Maxillofac Surg 30: 63-69. Link: https://goo.gl/CxRESy

20. Tan E, O'Brien BM, Brennen M (1978) Free flap transfer in rabbits using irradiated recipient vessels. Br J Plast Surg 31: 121-123. Link: https://goo.gl/1a3ics

21. Krag C, De Rose G, Lyczakowski T, Freeman CR, Shapiro SH (1982) Free flaps and irradiated recipient vessels: an experimental study in rabbits. Br J Plast Surg 35: 328-336. Link: https://goo.gl/DZzJiD

22. Bengtson BP, Schusterman MA, Baldwin BJ, Miller MJ, Reece GP, et al. (1993) Influence of prior radiotherapy on the development of postoperative complications and success of free tissue transfers in head and neck cancer reconstruction. Am J Surg 166: 326-330. Link: https://goo.gl/OQAg81

23. Suh JD, Sercarz JA, Abemayor E, Calcaterra TC, Rawnsley JD, et al. (2004) Analysis of outcome and complications in 400 cases of microvascular head and neck reconstruction. Arch Otolaryngol Head Neck Surg 130: 962-966. Link: https://goo.gl/TC4Ysx

24. Klug C, Berzaczy D, Voracek M, Enislidis G, Rath T, et al. (2005) Experience with microvascular free flaps in preoperatively irradiated tissue of the oral cavity and oropharynx in 303 patients. Oral Oncol 41: 738-746. Link: https://goo.gl/xh9EWV

25. Rifkin LH, Stojadinovic S, Stewart CH, Song KH, Maxted MC, et al. (2012) An athymic rat model of cutaneous radiation injury designed to study human tissue-based wound therapy. Radiat Oncol 7: 68. Link: https://goo.gl/IY76XD

Citation: Ramesh T, Moore LS, Patel N, Tipirneni K, Warram JM, et al. (2017) Effects of Neoadjuvant Chemotherapy and Radiotherapy on Flap Perfusion in a Novel Mouse Model Using Standard Clinical Assessment and Near-Infrared Fluorescence Angiography. Arch Otolaryngol Rhinol 3(2): 038-042. DOI: http://doi.org/10.17352/2455-1759.000042