Cancer Research and Oncology: Open Access
Received: Mar 07, 2016, Accepted: May 12, 2016, Published: May 17, 2016
Cancer Res Oncol, Volume 2, Issue 3
http://crescopublications.org/pdf/crooa/CROOA-2-017.pdf
Article Number: CROOA-2-017

Research Article **Open Access**

Breast Radiotherapy does not Raise Cytokine Levels in Humans

Mark Trombetta M.D[1, 2*], Thomas B. Julian M.D[2, 3, 4], D. Lawrence Wickerham[1,3] and David Steed M.D[5]

[1]Department of Oncology; Allegheny General Hospital, 320 East North Avenue, Pittsburgh, PA 15212, USA

[2]Department of Surgery; Allegheny General Hospital, Pittsburgh, PA 15212, USA

[3]Drexel University College of Medicine; Allegheny Campus, Pittsburgh, Pa, 15212, USA

[4]National Surgical Adjuvant Breast and Bowel Project, Pittsburgh, Pa. 15212, USA

[5]Stemnion, Incorporated Pittsburgh, Pa. 15219, USA

***Corresponding Author**: Mark Trombetta, M.D, Department of Oncology, Allegheny General Hospital, 320 East North Ave, Pittsburgh, PA 15212, USA; Tel: 412-359-3400;Fax: 412-359-3981; E-mail: mtrombet@wpahs.org

Citation: Mark Trombetta M.D, Thomas B. Julian M.D, D. Lawrence Wickerham and David Steed M.D (2016) Breast Radiotherapy Does not Raise Cytokine Levels in Humans. Cancer Res Oncol 2: 017.

Abstract:

Objective: To monitor cytokine trends in patients receiving breast radiotherapy and establish whether radiotherapy has an effect on plasma levels of cytokines

Materials and Methods: Twenty female patients with early stage breast cancer were enrolled in two separate cohorts of an Institutional Review Board approved Phase I protocol. Both cohorts received whole breast radiotherapy for 6- 7 weeks. Amnion Derived Cytokine Solution (ACCS) was administered to one half of the breast (double blinded) immediately post radiotherapy as part of this safety/toxicity study. The other half of the breast received 0.9% normal saline solution. Hematologic samples of human cytokines associated with inflammation and repair were sampled immediately prior to radiotherapy, one hour later and then again at six weeks. Blood samples were also tested for routine liver functions and other metabolic parameters.

Results: Whole breast radiotherapy had no effect on the stimulation the production of repair cytokines sampled and no effect on the hematologic indices either acutely or sub-acutely (6 weeks). Additionally, there was no evidence of "cytokine storm." ACCS also showed no significant absorption systemically following topical delivery. No patients developed an adverse event related to ACCS; however there was a noticeable difference in the degree of skin reaction between halves of the breast which did not correlate statistically. There were no patterns of changes in vital signs or clinical laboratory test values related to the treatment regimen.

Conclusions: In this safety/toxicity trial, despite the acute inflammatory nature of radiotherapy, no reactive elevations of serum cytokine levels monitored were noted. Neither ACCS nor radiotherapy had any effect on cytokine levels or routine hematologic parameters. Patient bloodchemistry was not adversely affected, indicating the absence of a reactive response and no evidence "cytokine storm" was identified. The definite and striking difference noted between

the ACCS treated breast half and the untreated half of the breast, while not statistically correlative, was impressive and requires further study.

Keywords: Radiation; Skin reaction; Breast; Cytokine; Dermatitis.

Introduction

Ionizing radiotherapy is known to be highly inflammatory in normal tissues when delivered in even minimal dosages. In general, the higher the biologic dose, the more the resultant acute inflammation. The acute effects of radiotherapy can be mild as exhibited by mild skin erythema, moderate, as evidenced by severe epidermitis of esophagitis, or severe resulting in permanent injury or even death.

Amnion-derived Cellular Cytokine Solution (ACCS), made by Stemnion (Pittsburgh, Pa.), is an acellular multiple cytokine solution derived from naturally occurring cells isolated from the full-term post-partum placenta. Placental donorshave been screened for infectious agents and have consented to tissue usage. There are no living cells in ACCS, only the multiple proteins, and there has been no manipulation of the genetic cellular material. ACCS contains multiple proteins involved in wound healing including VEGF, PDGF, angiogenin (Ang), TGF-β, Tissue Inhibitor of MetalloProteases-1 (TIMP-1), and Tissue Inhibitors of MetalloProteases-2 (TIMP-2) [1, 2].

The selection of ACCS as a vehicle for study occurred because it has been found to be effective in wound healing, including burns [1], and has recently been reported to safe when used topically in humans [3].

Radiotherapy is commonly used in the treatment of cancers of all types in humans. The use of ionizing radiation in the management of cancer creates a highly inflammatory circumstance in the micro and macroanatomy of human organ systems. In humans, an inflammatory response can sometimes be physically measured by the release of cytokines into the peripheral tissues and subsequently reflected in the serum. Clinically, in the worst of circumstances, a life-threatening condition known as “cytokine storm” can develop when the inflammatory reaction and subsequent repair mechanism is initiated. Cytokine storm has been reported in the literature and has been associated with death of subjects in clinical trials [4-6]. We report on our finding in a trial of women who underwent radiotherapy in the treatment of localized breast cancer.

Materials and Methods

Twenty female patients with early stage breast cancer required to be between the ages of 18 and 80 years and who required post-operative radiotherapy were enrolled in two separate but related cohorts of an Institutional Review Board approved Phase I protocol. Patients were excluded if they had abnormal hepatic or renal functions indices (minimum of 2X normal), were on hemodialysis, or were pregnant or lactating post-partum. Cohort 1 consisted of 10 patients who received topical ACCS to the breast immediately following the each of the first 10 fractions of whole breast radiotherapy. Cohort 2 consisted of 10 additional patients who fit the same criteria as the initial cohort but received topical ACCS following the development of at least grade I breast erythema as defined by the Common Terminology Criteria for Adverse Events [7]as a consequence of the whole breast radiotherapy. Both cohorts of patients received 4.0 cc of either ACCS or 0.9% normal saline solution applied to either the lateral or medial breast (determined by double blinded randomization) via an aerosolized spray. The surface of the breast was divided daily by drawing two parallel lines with a semi-permanent marker vertically through the nipple-areolar complex, 1.0 cm apart. The blinded solution application was restricted to each individual side of the breast by using an adhesive surgical drape which was modified to allow only 1.0 cm of drape adhesion placed between the parallel lines.Blood was drawn from each subject immediately before and one hour after treatment with ACCS and again at 6 weeks. The plasma from each sample was tested by antibody array for the presence of proteins in ACCS, including Platelet Derived Growth Factor-BB (PDGF-BB), VEGF, Ang, TIMP-1, TIMP-2, Matrix Metallopeptidase (MMP-9), Epidermal Growth Factor (EGF) and albumin. Also measured were serum levels of nine cytokines involved in inflammation: Interferon Gamma (IFN-g), Interleukin (IL)-1b, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12 p70, and Tumor Necrosis Factor-a (TNF-a). Hematologic indices were also obtained including Hemoglobin (HGB), Hematocrit (HCT), White Blood Count (WBC), Creatinine, Bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase(AST), Alkaline Phosphatase, Glucose, and Albumin. Patients were then observed weekly with breast photography and clinical examination.An extensive quality of life (QOL) patient questionnaire (more than 50 questions related to general, site specific, and both short and long-term features including a self-assessment of the breast cosmesis using the Harvard/National Surgical Breast and Bowel Project (NSABP) Cosmesis Criteria [8]was obtained during treatment and then at 3 month intervals for a total post treatment period of 12 months. The Harvard/NSABP scale was also recorded by the physician investigators.

Prior to entering the study, the subjects underwent lumpectomy for early stage breast cancer. Whole breast irradiation (WBI) was instituted between four to 12 weeks post lumpectomy. For patients receiving chemotherapy, WBI began two to four weeks following the final cycle of chemotherapy. No patient received neoadjuvant chemotherapy. The WBI dose was either 50 Gy at 2.0 Gy per fraction or 50.4 Gy at 1.8 Gy per fraction. Subjects were treated using standard immobilization techniques and three-dimensional conformal, forward planned radiotherapy. A boost to the lumpectomy cavity plus margin of 10-16.6 Gy in 5-9 fractions was used at the discretion of the radiation oncologist.

Statistical Methods

Safety analysis: All safety data, including vital signs, were reported and selected data wereby descriptive statistics. Summaries were prepared for the number (percent) of patients with Adverse Events(AE) and Serious Adverse Events(SAE). Quality of life was assessed globally as well as locally by noting changes in both the medial and lateral aspects breast including breast texture (thickening and hardness), pain, tenderness, shape, sensitivity, swelling, redness, itching, flaking skin, blistering and fluid leak. Follow up was completed at the one year post therapy interval.

Results

The primary endpoint of this safety trial was to determine the safety profile of ACCS as measured by AE and SAE; however we also noticed that breast radiotherapy had no effect on levels of circulating cytokines in the serum. Only two cytokines in a single cohort (IFN- and IL-2) (Table 1) had a mild elevation from pre-treated plasma samples yet still did not differ substantively from levels found in normal plasma samples. Elements of the Complete Blood Count including total lymphocytes, monocytes, eosinophils, neutrophils, basophils, platelets, hemoglobin, red blood cells, and hematocrit were not adversely affected, indicating the absence of a significant hematologically-based reparative response. There was no evidence of acute cytokine level surge or "cytokine storm" that has been previously defined [4]. No patient exhibited any unusual side effect other than those routinely associated with breast radiotherapy (mild/moderate erythema, mild mastalgia, etc.). No evidence of symptomatic fatigue or chronic pulmonary dysfunction was overtly reported by any of our subjects.

Discussion

Acute Effects

Ionizing radiation is an inflammatory and irritative modality frequently used in the treatment of breast cancer, and acute radiation dermatitis is a frequent complication of whole breast radiotherapy. Cytokine release is part of the inflammatory repair process following the delivery of ionizing or even ultraviolet (UVB) radiation [9]. Some authors have reported elevations of cytokines in response to radiotherapy [10-12] and specifically breast radiotherapy [10, 11] noting elevations in multiple cytokines. However, the elevated cytokine levels are not uniformly identified in these studies and have affected multiple elements differently in different publications. For instance, Ryan et al have reported a significant correlation between Il-12/p70 and the degree of radiation dermatitis specifically in breast cancer patients, while in our similar sized cohorts (total n= 20), we did not see any appreciable correlation or elevation of this or any of the measured cytokines. The breast, as a modified sweat gland which is suspended from the chest wall incorporates few supportive structural elements, and varies greatly in volume from woman to woman. Perhaps this variation accounts for some of the incongruity in these reported cytokine values. Organ systems that are, by volume, larger and perhaps more biologically active may respond to inflammatory stimuli more fervently than the breast. Francois et al have demonstrated measurably significant cytokine release following ionizing radiotherapy in the gut mucosa [13]. Additionally, Ong et al have identified that these inflammatory cytokines may be very important in the prevention against or protection from gastrointestinal injury, such as acute mucositis [14]. In their work, upregulation of Il-1 beta, Il-6, and TNF was felt to exhibit a protective effect on both the irradiated jejunum and colon such that only minimal change was noted to the microanatomy post irradiation. This biologically very active organ may reflect a more intensive reparative release due to the inherent dynamic and nearly constant operational function. These reports have used radiotherapy in standard fractionation schedules which would help mitigate super dose fractionation concerns sometimes done to evoke more dramatic and quicker results in animals. Other organ systems may respond similarly.

Chronic Effects: Fatigue

Effects of chronicity related to cytokines are not well described in the literature. One effect that has been related to cytokine release following therapeutic ionizing radiation is fatigue. Multiple authors have reported the correlation [11, 12] and one has related fatigue directly to the cytokine release [12], although we do not see that a direct correlation has been established in this setting. It is also quite possible that this fatigue is more of a global phenomenon secondary to cancer diagnosis and therapy, and not solely due to elevated serum levels of cytokines. No subject in our study related fatigue as a complication following therapy, and all subjects have now passed the one year post treatment mark.

Chronic Effects: Cachexia

At least one author has identified a relationship between cytokineelevations related to cancer therapies in general (including irradiation) [15], however, none of our patients exhibited effects of such. It should be noted, however, that our cohorts of patients were affected by only early stage cancers, with most patients not requiring systemic cytotoxic chemotherapy. In this regard, our population may have been indirectly skewed toward less systemic symptomatology.

Late Effects

Late effects of cytokine release are described, especially in regards to pulmonary effect and toxicity. Multiple authors have reported radiation-induced inflammatory cytokine expression associated with lung toxicity both in pre-clinical and clinical subjects [16, 17].While these reports seem to show correlation of effect, an absolute causal relationship has not been established. Although we did not require formal pulmonary function testing, the patient quality of life as measured in a self-reporting assessment and physician assessment was unaffected, with no self-reported pulmonary symptoms resultant and no physician noted complications.

Breast fibrosis rates have been reported as unassociated with increased levels of cytokines following radiotherapy [18]. We did not notice cytokine elevation or increased fibrosis in any event. This being the case, no determination of effect can be made.

Conclusion

Patient blood/hematology/chemistry (including total lymphocytes, monocytes, eosinophils, neutrophils, basophils, platelets, hemoglobin, red blood cells, and hematocrit) were not adversely affected, indicating the absence of an anaphylactic response in our patient cohorts which differs somewhat from the reported literature, although the overall data reported are scarce.No evidence of increase of the nine inflammatory cytokines sampled was seen and no evidence of "cytokine storm" was identified. We did not see long term effects involving fatigue, cachexia or pulmonary dysfunction, although these findings have been otherwise reported in the literature. Further correlation of the role of cytokines and breast radiotherapy is warranted.

Disclosure: This trial was funded by a grant from Stemnion, Incorporated but was conducted solely by independent clinical investigators who had no other conflicts of interest.

References

1. Uberti M, Ko F, Pierpont Y, Johnson E, Wright T, Smith C, Robson M, Payne M. The Use of Amnion-Derived Cellular Cytokine Solution (ACCS) in Accelerating Closure of Interstices in Explanted Meshed Human Skin Grafts. Eplasty 2009 Mar; 9: e 12.
2. Steed D, Trumpower C, Duffy D, Smith C, Marshall V, Rupp R, Robson M. Amnion-derived cellular cytokine solution. Eplasty 2008 April 7; 8: e 18.
3. Trombetta M, Julian TB, Wickerham DL, Steed D: Safety profile of Amnion-Derived Cellular Cytokine Solution (ACCS) following topical skin application in patients receiving breast radiotherapy ePlasty 2015 (In Press June 2015).
4. Stebbings, Findlay L, Edwards C, et al: Cytokine storm in the phase I trial of monoclonal antibody TGN1412: better understanding the causes to improve preclinical testing of immunotherapeutics. J Immunol 2007; Vol 179: 3325-3331.
5. Attarwala H: TGN1412: From discovery to disaster. J. Young Pharm. 2010 Vol 2(3): 322-336.
6. R. Stebbings, S. Poole, and R. Thorpe. Safety of biologics, lessons learnt from TGN1412. Cur Opin Biotech 2009; Vol (20): 673-677.
7. Common Terminology Criteria for Adverse Events http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf
8. NSABP Protocol B-39 Form COS. http://www.rtog.org/members/protocols/0413/0413.pdf
9. Petit-Frere C, Capulas E, Lyon D, et al: Apoptosis amd cytokine release induced by ionizing or ultraviolet B radiation in primary and immortalized human keratinocytes. Carcinogenesis 2009; 21 (6): 1087-1095.
10. Ryan JL, Keckler AP, Morrow G: Potential plasma biomarkers predicting radiation dermatitis in breast cancer patients. J Clin Oncol 2010;Vol 28 (15) (May 20 Suppl).
11. De Sanctis V, Agolli L, Visco V, et al: Cytokines, fatigue, and cutaneous erythema in early stage breast cancer patients receiving adjuvant radiation therapy. Biomed Res Int 2014:523568. Doi: 10.1155/2014/523568. Epub 2014.
12. Dhruva A, Aouizerat BE, Cooper B, et al:Cytokine gene associations with self-report ratings of morning and evening fatigue in oncology patients and their family caregivers. Biol Res Nurs 2015 Mar;17(2):175-84.
13. Francois A, Milliat F, Guipaud O, et al: Inflammation and immunity in radiation damage to the gut mucosa. Biomed Res Int 2013; http://dx.doi.org/10.1155/2013/123241.
14. Ong Z, Gibson R, Bowen J, et al: Pro-inflammatory cytokines play a key role in the development of radiotherapy-induced gastrointestinal mucositis. Radiat Oncol 2010; http://dx.doi:10.1186/1748-717x-5-22.

15. Laine A, Iyengar P, Pandita TK: The role of inflammatory pathways in cancer associated cachexia and radiation resistance. Mol Cancer Res 2013; 11; 967. Doi: 10.1158/1541-7786.MCR-13-0189.
16. Siva S, MacManus M, Kron T, et al: A pattern of early radiation-induced inflammatory cytokine expression is associated with lung toxicity in patients with small cell lung cancer. PLoS One 2014; Oct 7; 9 (10). Doi: 10.1371/journal.pone.0109560.
17. Ao X, Zhao L, Davis M, et al: Radiation produces differential changes in cytokine profiles in radiation lung fibrosis sensitive and resistant mice. J Hem Oncol 2009; 2:6. Doi: 10.1186/1756-8722-2-6.
18. Westbury CB, Haviland J, Davies S, et al:Cytokine levels as biomarkers of radiation fibrosis in patients treated with breast radiotherapy. Radiat Oncol. 2014 Apr 30; 9:103. Doi: 10.1186/1748-717X-9-103.