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Visible tumor surface response to physical plasma and apoptotic cell kill in head and neck cancer

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ABSTRACT

The aim of the study was to learn, whether clinical application of cold atmospheric pressure plasma (CAP) is able to cause (i) visible tumor surface effects and (ii) apoptotic cell kill in squamous cell carcinoma and (iii) whether CAP-induced visible tumor surface response occurs as often as CAP-induced apoptotic cell kill.

Twelve patients with advanced head and neck cancer and infected ulcerations received locally CAP followed by palliative treatment. Four of them revealed tumor surface response appearing 2 weeks after intervention. The tumor surface response expressed as a flat area with vascular stimulation (type 1) or a contraction of tumor ulceration rims forming recesses covered with scabs, in each case surrounded by tumor tissue in visible progress (type 2).

In parallel, 9 patients with the same kind of cancer received CAP before radical tumor resection. Tissue specimens were analyzed for apoptotic cells. Apoptotic cells were detectable and occurred more frequently in tissue areas previously treated with CAP than in untreated areas.

Bringing together both findings and placing side by side the frequency of clinical tumor surface response and the frequency of analytically proven apoptotic cell kill, detection of apoptotic cells is as common as clinical tumor surface response.

There was no patient showing signs of an enhanced or stimulated tumor growth under influence of CAP.

CAP was made applicable by a plasma jet, kINPen® MED (neoplas tools GmbH, Greifswald, Germany).

1. Introduction

Clinical case reports and select trials have demonstrated that cold atmospheric pressure plasma (CAP) is a useful tool for decontaminating severely infected wounds and ulcerations (Isbary et al., 2010, 2012; Brehmer et al., 2015). For this purpose, it has been applied in our unit as part of the palliative medicine program for patients within the final stages of advanced head and neck carcinoma and grossly contaminated tumor ulcerations. Indeed, head and neck cancers present difficult clinical problems as cancer proximity to significant anatomic structures calls for better local therapy.

1.1. CAP for decontamination

The reduction of microbiological contamination is a result of one of the, actually in fact the most, promising characteristics of CAP, its ability to very effectively inactivate multi-resistant strains of microorganisms (Daeschlein et al., 2014). Whether therapeutic application of CAP might inactivate cancer cells as well is not a

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matter of clinical concern for patients with advanced stages of head and neck cancer, since the effect of CAP is limited to very superficial tissue structures. However, they receive CAP for decontamination and *en passant* the question of cancer cell response has been attracting researchers around the world since Fridman et al. (2007) published their landmark paper entitled "Applied plasma medicine". Keidar et al. (2007) introduced the idea of CAP selectivity for cancer and the possibility of a paradigm shift in cancer therapy. Their idea has been recently supported by clinical observations and findings of immunological interactions (Metelmann et al., 2015a, 2015b; Miller et al., 2015).

CAP is physical plasma generated by adding energy to a gas resulting in ionization and excitation of gas molecules. Biological tissue is primarily affected by two components of physical plasma: 1) electromagnetic radiation (UV, VIS, IR, high-frequency electromagnetic fields, etc.) and 2) ions, electrons and reactive chemical species. The technical possibility of generating physical plasma at low temperatures in an atmospheric environment opens up new chances to use CAP for medical therapies (Isbary et al., 2013).

According to the current state of knowledge, plasma effects on biological systems are mainly caused by reactive oxygen and nitrogen species (ROS and RNS) which influence cellular processes via impacts on the redox balance of cells (von Woedtke et al., 2014) that might be applicable for cancer stem cells, too.

1.2. CAP for inducing apoptosis

The single cancer stem cell is the therapeutic target, when treating malignant tumors with curative intention, as this is the source of clonal tumor growth, metastasis, recurrent cancer and development of cancer disease (VonHoff et al., 1982). The evidence to date suggests that CAP has a significant apoptotic effect on cancer cells as demonstrated in several tumor lines, tumor models in *in vitro* and *in vivo* studies using nude mice (Vandamme et al., 2010; Schlegel et al., 2013; Utsumi et al., 2013). Triggering reactive oxygen and nitrogen species to derange the redox balance and redox signaling of cancer stem cells is considered as a key pathway for understanding CAP-induced apoptosis, since the survival and proliferation signaling network is one of the most essential signaling networks. Two major signaling pathways within this network are the Phosphoinositide 3-kinase (PI3K)-AKT signaling pathway and the Rause-associated sarcoma (RAS)-Mitogen-activated Protein Kinase (MAPK) signaling pathway (Downward, 2003; Martelli et al., 2010). Activation of this signaling network often seen in cancer patients leads to induction of cell growth and inhibition of apoptosis. CAP treatment hinders both pathways (Tanaka et al., 2012) and induces apoptosis of tumor cells due to down-regulation of the survival and proliferation signaling network (Chalhoub and Baker, 2009; Laplante and Sabatini, 2012).

1.3. Tumor cells sensible to CAP

Head and neck cancer cells are of confirmed sensibility to CAP (Guerrero-Preston et al., 2014; Kang et al., 2014; Han et al., 2013; Chang et al., 2014a), and also bladder cancer (Keidar et al., 2011), brain tumor (Tanaka et al., 2011, 2012; Vandamme et al., 2010; Koritzer et al., 2013; Kaushik et al., 2012, 2013), breast cancer (Kim et al., 2010a; Wang et al., 2013), cervical cancer (Leduc et al., 2009; Ahn et al., 2011; Sato et al., 2011; Huang et al., 2013), colorectal cancer (Lupu et al., 2009; Vandamme et al., 2012; Kim et al., 2010b, 2010c; Ishaq et al., 2014), gastric cancer (Torii et al., 2014), leukemia (Thiyagarajan et al., 2012; Barekzi and Laroussi, 2012), liver cancer (Gweon et al., 2010), lung cancer (Huang et al., 2011; Kim et al., 2011; Adachi et al., 2014; Panngom et al., 2013), malignant melanoma (Fridman et al., 2007; Lee et al., 2009; Zirnheld et al., 2010; Daeschlein et al., 2013; Sensenig et al., 2011; Yajima et al., 2014; Iida et al., 2014), ovarian cancer (Iseki et al., 2012; Utsumi et al., 2013, 2014), pancreatic cancer (Brullé et al., 2012; Partecke et al., 2012), prostate cancer (Hirst et al., 2014) and thyroid cancer (Kaushik et al., 2014; Chang et al., 2014b).

The aim of this study is to learn whether clinical application of cold atmospheric pressure plasma (CAP) in head and neck cancer patients is able to cause (i) visible tumor surface effects, (ii) apoptotic cell kill and (iii) whether CAP-induced visible tumor surface response occurs as often as CAP-induced apoptotic cell kill.

2. Material and methods

2.1. Study design

The study is designed as a descriptive evaluation of the clinically visible influence of the intervention in one group of patients (EudraCT number 2014-000416-34) together with a histological analysis of tissue effects in a comparable second group of patients. Patients suffering from advanced squamous cell carcinoma ($n = 21$) were assigned to one of the both groups due to their individual different clinical treatment plan.

2.1.1. Group I

Group I ($n = 12$) was treated with CAP as part of their palliative program, not primarily intended to influence tumor growth but to reduce microbiological contamination of their infected ulcerations. The descriptive clinical evaluation was based upon an intra-individually comparative, prospective, blindly evaluated study protocol. The objectives of the analysis were to prospectively look for and assess changes of the tumor surface as intra-individual differences between a spot treated with CAP and the surrounding area of the same tumor lesion untreated. The assessment of CAP effects was based on photo evaluation by a remote panel of three blinded experts, since blinded, remote photographic analysis is feasible for clinical studies and correlates well with direct clinical assessments (Rennekampff et al., 2015). Additional clinical assessment was performed by the patients themselves and their clinical treatment team evaluating the effects of CAP unblinded. Statistical analysis was not performed, due to the small sample size and cancer-related multi-morbidity of patients causing a very inhomogeneous sample.

The evaluation was conducted in compliance with International Conference on Harmonisation guidelines for Good Clinical Practice and the principles in the Declaration of Helsinki. All blinded viewers and treatment team members received training in the protocol and in the standardized acquisition of photographs. Informed consent was obtained from all patients before inclusion in the analysis.

2.1.2. Group II

Group II ($n = 9$) was treated by curatively intended surgery and received CAP before total tumor resection. Tissue specimens were analyzed for apoptotic cells.

2.2. Patients

The study was conducted between October 2013 and November 2015 and involved 9 female and 12 male Caucasian patients, age of 40–77 years.

2.2.1. Group I

Group I patients were scheduled for palliative care due to the advanced stage of carcinoma disease beyond curative standard cancer therapies. All patients selected were in Karnofsky