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Application of Cold Atmospheric Plasma (CAP) in Cancer Therapy: A Review

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Abstract

Background: Recently the use of cold atmospheric plasma (CAP) for the treatment of cancers called "Plasma Oncology" has gained promising results. The developments have raised the hope that this technology could be an interesting new therapeutic approach in the treatment of cancer.

Methods: The process of this study includes a narrative review in two stages. At the first stage "literature overview", all mechanisms of action related to cold atmospheric plasma have been reviewed. To relevant as much as papers for this review, multiple effective techniques have been applied in our search strategy. An extensive search of the published literature has been conducted.

Results: Results of this study include three sections as follow: the mechanism of action, in vivo and in vitro findings, and studied focused on selectivity effect of CAP. According to several publications, reactive oxidative species (ROS) can be the major cause of the biological effect of plasma. Several publications concerning in vivo and in vitro studies on different cell lines and selectivity effect showed that there are promising results in favor of anti-cancer effect of CAP.

Conclusions: The results of several studies that are summarized within this review show that CAP is effective against cancer which also indicate that CAP seems to be selective for cancer cells compared to non-neoplastic cells. It has been concluded that the feasibility of applying CAP for treating human tissue has already gained momentum although future studies require more studies for clarifying that if CAP is capable to discriminate between normal and malignant cells or not.

Keywords: Cold Atmospheric Plasma, Plasma Oncology, Cancer Therapy, Selectivity

1. Introduction

Surgical oncology can be considered somehow as the important type of cancer therapy which is playing an important role as an effective treatment in cancer therapy (1). In Surgical oncology there is an agreement regarding Tumor R0-resection which is a total excision of a tumor with an adequate surgical margin. Moreover, the most important aspect of successful cancer therapy is the selective eradication of cancer cell without influencing the healthy tissue. This latter issue imposed complications of complete local excision of malignant cells (2-4). Therefore, challenging issues in this field are removing microscopic residues to prevent cancer-positive surgical margins, and simultaneously distance reduction between excised tumor and surrounding normal tissue. For these reasons new therapeutic applications capable of satisfying above conditions are required.

In 1879, William Crookes identified that 99% of the universe is made up of a matter other than liquid, gas, and solid, referred to as the fourth state of matter or plasma (5). Very soon after this discovery the encouraging possibilities of the application of cold plasma, better to say cold atmospheric plasma (CAP) in medicine has been claimed by most researchers. Cold atmospheric plasma (CAP) is a gas which is partially ionized and includes clouds of ions, electrons, and reactive neutral species like reactive oxygen species (ROS), hydroxyl radicals (HO), and nitrogen dioxide (NO) (6). CAP has shown significant potentials for various biomedical applications such as sterilization of infected tissues (7, 8), inactivation of microorganisms (9), wound healing (10, 11), skin regeneration (12), blood coagulation (13), tooth bleaching (14), and the last but not least in cancer therapy (15-17). CAP can directly affect malignant cells and tissue but such direct application with low plasma concentration can just be limited to the narrow depth and so can be invoked as a supplement during open surgery something like Electron Intraoperative radiotherapy (EIORT). Furthermore, a selectivity effect of CAP has been claimed by some researchers. It means cancer cells are more sensitive to its destructive effect than normal cells, which can make cold atmospheric plasma a promising application in cancer therapy. So it can be con-

sidered as a selective treatment option which just affects the cancer cells while leaving normal cells intact. This selectivity has been considered as an interesting ability of CAP in cancer surgery on which free surgical margin playing a crucial role in overall survival after surgical resection. In this study we made an investigation on anti-cancer effects of CAP through reviewing recent works in this field.

2. Methods

The process of this study includes a narrative review in two stages. At the first stage "literature overview", all mechanism of action related to cold atmospheric plasma have been reviewed. In the second- "effectiveness review"-stage, we reviewed the outcome results regarding in vivo and in vitro studies related to anti cancer effects of CAP. To relevant as much as papers for this review, multiple effective techniques have been applied in our search strategy. An extensive search of the published literature has been conducted on the MEDLINE®, Pubmed® and EMBASE® using the following key words (Plasma OR Cold Atmospheric Plasma) AND (Cancer OR neoplasm OR carcinoma OR cancer surgery) AND (mechanism of action OR biology OR in vivo OR in vitro OR anti cancer effect OR selectivity) AND (lung carcinoma OR hepatocellular carcinoma OR neuroblastoma OR skin carcinoma OR melanoma OR colon carcinoma OR pancreatic carcinoma OR bladder carcinoma OR cervical carcinoma OR breast cancer). Also the reference lists of each article were reviewed in detail to find additional articles. Based on the above selection criteria, 75 articles were introduced to this study and finally 60 articles have been used to write this article. Most of these articles have focused on theoretical basis of our search targets. Each article was reviewed independently in full text, the relevance of retrieved articles were evaluated, and recorded the main findings of each study in a table.

3. Results

Mechanisms of action: Table 1 shows a summary of the most stated mechanism of action of CAP on different cancer cells. According to several authors, reactive oxidative species (ROS) produced by plasma can causes morphological changes, depolarization of the membrane, lipid peroxidation, and DNA damage on cells (9, 18). The antineoplastic effects of CAP depend primarily on the delivery of reactive oxygen and nitrogen species (ROS, RNS) (19). It has been verified that ROS can affect the proteins responsible for mitochondrial permeability which consequently will result in triggering the intrinsic apoptotic cascade (20). It has been documented that ROS are generated in cells exposed to a stress condition such as hypoxia, chemicals agents, UV radiation , etc. which cause damages to intracellular organelles and membranes, proteins, DNA, and lipids resulting in cell death in apoptotic way. Beside this, there different mechanisms of CAP in cancer cells were studied in recent papers which include: Activation of genes such as p53 protein (21) and p21 CDK inhibitor (22), Cell cycle arrest at the G2/M and S phase (23), ROS mediated cell cycle arrest (24) and Apoptosis via dysfunction of mitochondria (25). Also studies have shown that mitochondrial enzyme activity and its membrane potential followed by respiration rate are significantly decreased in cancer cells after CAP treatment (26). It has been shown that CAP is capable of controlling the concentrations of intracellular ROS, NO, and lipid peroxide (27). Also alteration of the redox state of Prxs reversibly following induction of peroxides will promote cell growth arrest resulted from CAP-driven alteration of ROS (28). Also essential cellular signaling pathways and functions of proteins may be disturbed or disrupted resulted from a strong and sustained disruption of redox signaling, as result of exposing to plasma (29). General conclusions about the anti-cancer mechanism of CAP can be summarized as below: 1- increasing intracellular ROS, which causes DNA double strands break (DSB) (19, 30). 2- Serious DNA damage which result in the cell cycle arrest (31), apoptosis or necrosis with a dose-dependent pattern (32). 3-producing H2O2 and NO which are key molecules for killing cancer cells (33). 4-stronger resistance of normal cell to CAP than cancer cells which make a killing preference accompanied with the distinct ROS levels and DSB among cancer cells and normal cells (34). Moreover, recently, CAP irradiated media has been considered as effectively as direct application of CAP treatment to kill cancer cells (35, 36).

Based on the above mentioned anti-cancer effects, many studies have shown that CAP had significant success both in vitro and in vivo but mostly in the case of in vitro studies. Many cancer types have been investigated both in vitro (40, 41) and in vivo (42) studies on lung carcinoma. Also other in vitro studies on hepatocellular carcinoma (43), neuroblastoma (44), skin carcinoma (23), melanoma (45), colon carcinoma (46), pancreatic carcinoma (47), bladder carcinoma (40), cervical carcinoma (25, 48) and breast cancer (49) show the same promising result in favor of anti-cancer effects of CAP. Interesting results of anti-cancer effects of CAP have been shown in breast cancer surgery in which nonselective and incomplete tumor ablation impose severe limitations (49).

Also selectively ablation of ablate metastatic BrCa cells in vitro without damaging healthy MSCs impose new era in the application of CAP in cancer treatment. This result suggests a selective treatment of CAP in breast cancerous cells which can result in maintaining healthy cells and the

Table 1. A Summary of the Most Stated Mechanism of Action of CAP on Cancer Cells

Author	Mechanism of Action	Cell Type	Selectivity[a]	Year[b]
Lee et al. (37)	CAP-generated Nitric Oxide (NO) radicals	Squamous cell carcinoma	Yes	2016
Wang et al. (38)	Alteration of BrCa cell surface receptor functions	Human metastatic breast cancer (BrCa) cells	Yes	2013
Preston et al. (39)		HNSCC cells	Yes	2014
Tuhvatulin et al. (21)	Activation of p53 protein	Human Colon Carcinoma Cells	No	2012
Volotskova et al. (23)	Cell cycle arrest at the G2/M and S phase	PAM 212 cells	Yes	2012
Ahn et al. (25)	Apoptosis via dysfunction of mitochondria	HeLa cancer cells	No	2011
Panngom et al. (26)	Decrease of mitochondrial membrane potential, mitochondrial enzyme activity and respiration rate	Human lung cancer cell	Yes	2013
Koritzer et al. (30)	DNA double strands break resulted from ncreasing intracellular ROS	Chemoresistant glioma cells	Yes	2013

[a]Selectivity has been checked or not.
[b]Year of publication.

tissue intact but tumor remediation accompanying near complete ablation (36). In case of normal cells, studies have shown that plasma has minimal impact on normal cellular conditions so it can leave their normal cells unaffected while selectively ablate corresponding cancer cells. For instance, media pH levels (sign for producing reactive agents) and thermal effects associated with cold plasma remain unchanged after CAP treatment (50).

A study showed an important factor that makes cancer cells more sensitive to CAP treatment is their higher percentage of cells in the S phase of the cell cycle (23). In a study by Lee et al. it was shown that CAP-generated nitric oxide (NO) radicals can selectively kill oral squamous cell carcinoma cells (37). Wang et al. showed that human metastatic breast cancer (BrCa) cells can be selectively ablated after CAP treatment using optimized plasma parameters. Also this study showed that healthy human bone marrow mesenchymal stem cells (MSCs) have not been affected (38). The same result has been shown regarding HNSCC cells applying a feasible therapeutic strategy if coupled with endoscopic technology (39). Also in this study the adjuvant application of CAP prior to surgery has been proposed.

4. Conclusions

It has been shown that recurrence-free survival in the management of operable cancers with curative approach depends primarily to surgical free margin or tumor-negative surgical margins. Moreover, this review showed that one of the promising applications of Cold atmospheric plasma (CAP) is a selective eradication of cancer cells. Based on the above mentioned characteristic of plasma, an improving tumor local control while minimizing associated side effects of surgical radicality can be considered after Local Intraoperative CAP treatment during surgery.

Till now CAP has shown a wide range of activity regarding freely suspended cells which can be further extended to systemic applications. The feasibility of applying CAP for treating human tissue has already gained momentum although future studies required for clarifying that CAP is capable to discriminate between normal and malignant cells or not.

Unfortunately this study failed to find a study focusing on investigation on anti-cancer effects of CAP on human volunteers. Also most of the articles were concentrated on positive effects of CAP. For these reasons, future in vivo and human studies will give more clear results companying an insight into probable shortcomings and disadvantages of CAP anti-cancer effects.

Footnotes

Authors' Contribution: Prof Mohamad Esmail Akbari and Dr Reza Reiazi have contributed to the study conception and design, acquisition of data, analysis and interpretation of data, drafting manuscript and revisions. Dr Amir Norozi has contributed to the interpretation of data, drafting manuscript and revisions. Maryam Etedadialiabadi has contributed to acquisition of data, analysis and interpretation of data, drafting manuscript and revisions.

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