

Perspectives of Plasma-treated Solutions as Anticancer Drugs

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INTRODUCTION

In 2018, there were an estimated 9.6 million deaths from cancers and 18.1 million new cancer cases reported [1]. Unfortunately, the classical cancer treatments, such as chemotherapy, radiotherapy and surgery, show various side effects, sometimes with low treatment efficiency. Therefore, in recent years, many new anticancer therapies have been developed, such as photodynamic therapy, sonodynamic therapy, bacterial therapy, as well as Cold Atmospheric Plasma (CAP) therapy. CAP is an ionized gas produced at room temperature, in which heavy particles (such as gas molecules, atoms, radicals) and electrons are in thermal non-equilibrium [2]. CAP is an efficient source of reactive species. Indeed, it is usually produced in a rare gas flowing into ambient air, or directly in air, producing Reactive Oxygen Species (ROS), such as superoxide ($O_2^{\bullet-}$), hydroxyl radicals (OH^{\bullet}), singlet oxygen (1O_2), hydrogen peroxide (H_2O_2), and ozone (O_3), as well as Reactive Nitrogen Species (RNS), such as Nitric Oxide (NO), nitrogen dioxide (NO_2), nitrous oxide (N_2O), and nitrogen trioxide (NO_3). CAP has shown promising anticancer activity against more than 20 cancer types during *in vitro* studies, such as pancreatic carcinoma, melanoma, cervical carcinoma, head and neck carcinoma, neuroblastoma and breast cancer [3]. Additionally, CAP shows inhibitory action against cells that have tumorigenic potential, also known as Cancer-Initiating Cells (CICs) [4]. CICs are a sub-population of cancer cells, responsible for tumor growth and resistance to chemotherapy. However, CAP treatment has limitations for the treatment of internal organs of the body. For these circumstances, CAP-irradiated solutions or Plasma Treated Solutions (PTS) might be a promising alternative.

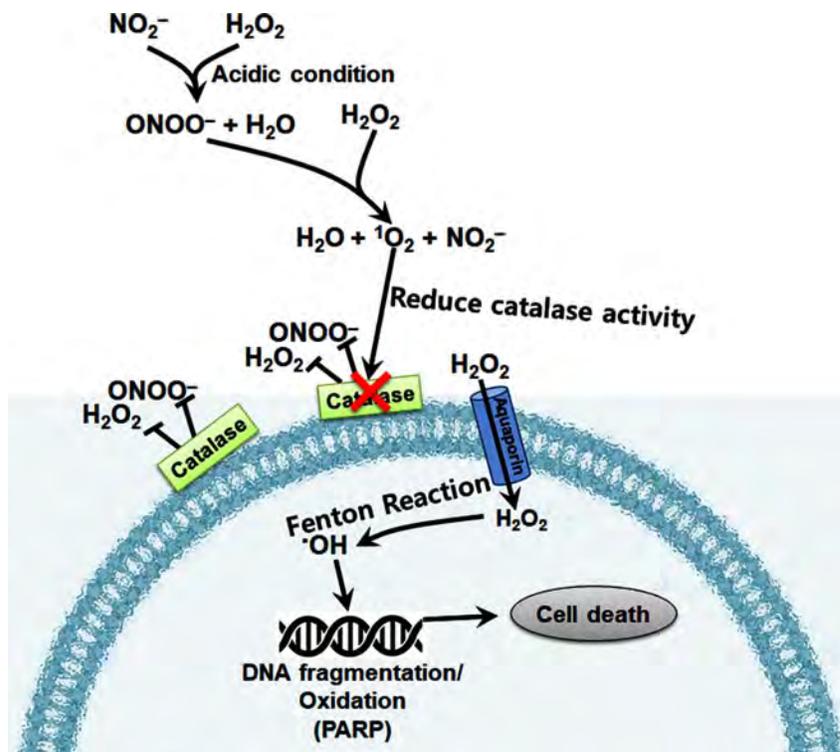
Although most studies in plasma medicine have used direct CAP treatment, PTS have also become a hot research topic over the past 5 years [2]. PTS also show anticancer activity, with equal or slightly lower efficiency than direct CAP treatment, but the latter is compensated by important other advantages. The above statement is supported in the recent results, where authors reveal that direct CAP treatments were more effective than PTS treatments, suggesting the importance of short-lived species generated during direct CAP treatment [5]. Indeed, PTS can be stored for a long time, with the same anticancer activity demonstrated for at least a week [2], hence they can be easily transported to places where a CAP setup is not available. In addition, PTS can be injected directly to tumor tissue, which is not possible for direct CAP treatment. PTS treatment has similar injection modalities as chemotherapy, so we could call it as “*plasma chemotherapy*”. Tanaka *et al.* were the first to demonstrate the anticancer activity of PTS [6]. They referred to PTS as Plasma Activated Medium (PAM) and showed that PAM can selectively kill glioblastoma (U251SP) cells compared to normal astrocytes (ACBRI-371) [6]. They reported that the anticancer capacity of PAM is inversely proportional to the cell density. Additionally, they observed that the PAM effect decreases gradually during storage. Subsequently, a series of studies (*in vitro* and *in vivo*) have been performed using PTS on many types of cancer cells [2]. These experiments showed that PTS can selectively kill cancer cells. Recently, Sato *et al.* showed that Plasma Activated Lactated Ringer's solution (PAL) induces antitumor effects on pancreatic cancer in both *in vitro* and *in vivo* studies [7]. They observed that intraperitoneal administration of PAL suppresses the formation of peritoneal nodules in *in vivo* studies, indicating that PAL intraperitoneal administration can be a new therapeutic option for peritoneal metastases [7]. Another study showed that PAL reduces the viability of A549 cancer cells through mitochondrial dysfunction with the downregulation of NF- κ B- BCl_2 signalling [8].

Bekeschus *et al.* reported that plasma-treated PBS significantly decreases the metabolism and proliferation of pancreatic cancer cells, while plasma-treated culture media (PAM) did not show any effect on the metabolism and proliferation of cancer cells. They also claimed that plasma-treated PBS reduces a 3D pancreatic tumor [9]. Kumar *et al.* compared the effects of PTM and Plasma Treated Water (PTW) on pancreatic ductal adenocarcinomas (MiaPaca-2, BxPc3) and Pancreatic Stellate Cells (PSCs) (hPSC128-SV). It was observed that PTM and PTW have similar anticancer efficacy on both pancreatic cancer cells, but PTW is slightly more effective in killing PSCs compared to PTM [10]. Chauvin *et al.*, reported that PTS are a potentially effective drug for head and neck cancer. They also pointed out that Multi Cellular Tumor Spheroids (MCTS) are a more valuable model than 2D cell cultures for evaluating the PTS anticancer activity [11]. Furthermore, researchers reported that the combination of plasma-treated culture media and chemotherapy drug (cyclophosphamide) treatment induces a reduction in tumor size in *in vivo* studies [12]. Recently, the anticancer effects of PAM on CICs among endometrioid carcinoma and gastric cancer cells were reported [13], although the combined PAM/cisplatin appeared to kill cancer cells more efficiently than PAM or cisplatin alone. It is clear that PTS may serve as a useful anticancer therapy, targeting various cancer cells including CICs, and they can be used in combination with chemo-drugs to increase the anticancer efficiency.

In spite of their promising potential, the efficiency of PTS can be variable, depending on the power of plasma source, the post-treatment storage and treatment time. Boehm *et al.* have reported that manipulation of these parameters affects the concentration of H_2O_2 , which directly affects the cancer cell viability, because H_2O_2 is the main component of PTS with anticancer potential [14]. Van Boxem *et al.* compared the effect of the gap between plasma source and treated liquid, the treatment time and gas flow rate on the anticancer capacity of plasma-treated PBS (pPBS) in different glioblastoma cancer cell lines, and correlated this effect to the chemical composition of pPBS. They concluded that H_2O_2 plays a more important role than NO_2^- in the anticancer capacity of pPBS [15]. These findings were also confirmed by other studies [16]. On the other hand, Bauer reported that the presence of both NO_2^- and H_2O_2 in PTS yields multiple reactions, resulting in the formation of a singlet oxygen, which can inactivate membrane-associated catalase through an auto-amplificatory mechanism, resulting in apoptosis of cancer cells [17]. Despite the central role of H_2O_2 for the anticancer effect of PTS, their mechanism of action is still not clear. So far, it has been reported that PTS treatment could change the intracellular pathways and increase the intracellular ROS content in cancer cells,

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which may cause DNA damage, as well as trigger apoptosis-related pathways. The probable mechanism is shown in Scheme 1, based on literature [2, 17, 18]. Adachi *et al.* observed the generation of intracellular OH radicals, which might be due to the Fenton reaction between H_2O_2 and intracellular ferrous ions [18]. The OH-induced DNA injury was followed by the activation of poly (ADP-ribose) polymerase-1 and elevations in the intracellular Ca^{2+} content [18]. An oxidative DNA damage cascade was also observed in our previous work using PTS treatment [19]. In addition, apoptosis was observed in chemo-resistant ovarian cancer cells for *in vitro* and *in vivo* experiments after PTS treatment through the activation of caspase-3/7 [20]. Thus, in spite of these interesting investigations that might reveal some underlying mechanisms, the actual mechanism of PTS in the selective killing of cancer cells still remains a mystery.



Scheme 1. The probable reactions produced in PTS that can reduce the activity of membrane-associated catalase of tumor cells, which results in the uptake of H_2O_2 increases into the cells through aquaporins. In addition, through Fenton reaction, the hydroxyl radical generated that oxidized or fragmented the DNA, results in cell death.

CONCLUSION

PTS has some extra value above CAP treatment, for tumors inside the body, which are not easily accessible by direct CAP treatment. Currently the mechanism of PTS is mainly linked to the presence of H_2O_2 , but PTS are more than a H_2O_2 solution, because their effect is larger than a H_2O_2 solution at the same concentration, indicating that it is the chemical cocktail of species that induces selective cancer cell death. Further progress on the understanding of the anticancer mechanisms and the degradation mechanism of PTS will be crucial for its future clinical application.

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