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Review Article

Butyric Acid Fermented in Aloe Vera Gel Exerts an Anti-Tumor Effect as a Histone Deacetylase Inhibitor and an Immune Checkpoint Inhibitor Case Reports: Mitigation of Cervical Cancer and a Small Cerebral Neoplasm with Aloe Vera Juice as an Adjuvant

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Abstract

As one of histone deacetylase inhibitors, butyrate may be overcome by several approaches with anti-multiple myeloma agents and as an adjuvant. Butyrate may mediate its anti-cancer effects as a potential therapeutic prodrug for multiple-myeloma cells apoptosis, partly through microRNA (miRNA) or in collaboration with miRNA. Butyrate fermented in aloe vera gel exerts an anti-tumor effect as a histone deacetylase inhibitor and as an immune checkpoint inhibitor. Case report 1, 2 and 3 showed that the long-time ingestion of aloe vera juice possibly mitigated cervical cancer and a small cerebral neoplasm as an adjuvant.

Keywords: *Butyric Acid; Aloe Vera; Histone Deacetylase Inhibitor; Cervical Cancer; Cerebral Neoplasm*

Introduction

Butyric acid in the treatment of cancer, special uterus cervix cancer, was demonstrated in case reports by Watson and Glasg on 1933 [1]. Butyric acid is miscible with water in any proportion. Mixed with a solution of serum albumin of human origin, it gives pearly white precipitate, while a flare solution of bovine albumin, gives definitely yellow precipitates. Butyric acid-albumin thus formed is active when applied to a cancerous ulcer.

Butyrate has been an essential agent for determining the role of histone-acetylation in chromatin structure and function. Inhibition of histone deacetylase (HDAC) activity affects the expression of only 2% of mammalian genes. Promoters of butyrate-responsive genes have butyrate response elements, and the action of butyrate is often mediated through Sp1/Sp2 binding sites. Davie., *et al*. [2] demonstrated that the potential of butyrate and HDAC inhibition in the prevention and treatment of cancer.

The colonic epithelial cells near the top of the crypt have shown to undergo apoptosis. Because butyric acid (BA) produced by fermentation of dietary fiber in the large bowel and Aloe vera gel, it may be an important regulator of apoptosis in colorectal cancer. Mandal., *et al*. [3] investigated which signaling pathway is triggered by BA to undergo apoptosis in human colorectal cancer cells. Human DIFI and FET colorectal cells were treated with BA to undergo apoptosis and were assayed for activation of c-Jun N-terminal kinase (JNK), transcription factor activation protein 1 (AP1) and NF-κB, and the proapoptotic molecular Bax (Bcl-2 associated X protein). BA-mediated DNA fragmentation and Bax induction were preceded by early stimulation of JNK, and the DNA-binding activities of AP1 and NF-κB. BA-induced enhancement of DNA fragmentation and stimulation of Bax promotor activity were blocked by the expression of dominant-negative mutants of JNK1 or AP1 but not NF-κB. These findings suggested that apoptosis triggered by BA involves transcriptional stimulation of the Bax gene via activation of the JNK/AP1 pathway in colonic epithelial cells. Entin-Meer., *et al*. [4] explored the efficacy of a novel class of histone deacetylase inhibitors in the treatment of malignant gliomas. Treatment of glioma cell lines with two butyric acid derivatives, pivaloylomethyl butyrate and butyroyloxymethyl, induced hyperacetylation, increased p21Cip1 expression, inhibited proliferation, and enhanced apoptosis. The data suggested that novel butyric acid prodrugs provided a promising treatment strategy for malignant glioma as single agents and in combination with radiation therapy. Sodium butyrate (SB) increased endoplasmic reticulum stress by altering intracellular calcium levels a well-known autophagy trigger. Mahyar-Roemer., *et al*. [5] investigated the effects of butyrate on the human wild-type p53 and p21 expressing HCT116 colon carcinoma cell line and on HCT116 cells with either p53 or p21 alleles inactivated by homologous recombination. The effects of butyrate were compared with those elicited by cytotoxic drugs and the natural chemo-preventive phytoalexin of wine and grapes, resveratrol. The author documented that physiological concentrations of butyrate stimulate p21 expression and induce apoptosis independently of p53, and that the absence of p21 increased apoptosis drastically. The apoptosis is mediated through the mitochondria and is accompanied by mitochondrial proliferation and membrane potential changes. The control of p21 expression may support chemoprevention and certain tumor therapies. Zhang., *et al*. [6] investigated whether sodium butyrate (SB)-induced endoplasmic reticulum stresses mediated autophagy, and whether there was crosstalk between autophagy and the SB-induced apoptotic response in human colorectal cancer cells. The obtained results suggested that SB-induced autophagy was mediated by endoplasmic reticulum stress, and that preventing autophagy by blocking the endoplasmic reticulum stress response enhanced SB-induced apoptosis. The results provided novel insight into the anti-tumor mechanisms of SB. Nakagawa., *et al*. [7] focused on the mechanisms underlying the effect of SB on human glioblastoma (GB) A172 cells, proliferation, motility and invasion. The author demonstrated that SB inhibits GB cells proliferation, induces cells to senescence and inhibits tumor cell invasion, indicating that it may be developed as a novel therapeutic strategy to treat GB cells. In our previous paper butyrate was shown to induce cell cycle arrest, differentiation, and apoptosis in a variety of cancer cells. Non-pathogenic anaerobic butyrate-producing bacteria with aloe vera fermentation may be a versatile tool in tumor therapy, as these bacteria can grow in anoxic and hypoxic regions of tumors and influence tumor cells by producing a potent butyric acid [8].

The intestinal microbiota is now accepted as a potent modulator of immune responses, especially in the context of immune checkpoint inhibitor treatment of cancer. In a study published in Cell Metabolism, He., *et al*. [9] showed the role of butyrate in the gut microbial metabolite in CD^+8T cells immunity and cancer immunotherapy. Butyrate can directly modulate antitumor CD^+8T cell response and improve the chemotherapy efficacy through ID2-dependent IL-2 signaling, suggesting that manipulation of gut microbial metabolites could be effective as a part of cancer therapy. Butyrate directly boosts the anti-tumor CD^+8T cell response via ID2 and butyrate supplementation improves the antitumor therapy efficacy. Several lines of evidence from preclinical to clinical research have gradually established the gut microbiota can modulate antitumor immunity and affect the efficacy of cancer immunotherapies, especially immune checkpoint inhibitors (ICIs). Lu., *et al*. [10] reviewed the potential mechanisms by which the gut microbial metabolite short chain fatty acids augment the efficacy of ICIs. The author expressed the mechanistic exploration provides novel insights for developing rational microbiota-based therapeutic strategies by manipulating gut microbiota, such as fecal microbiota transplantation, probiotics, engineered microbiomes, and specific microbial metabolites, to augment the efficacy of ICI and advance the age utilization of microbiota precision medicine.

Short chain fatty acids（SCFAs）inhibit human deacetylase (HDAC) and serve as energy substances to connect dietary patterns and activate G-protein-coupled receptors, inhibit histone deacetylase (HDAC), and serve as energy substrates to connect dietary patterns and gut microbiota, thereby improving the intestinal health. A significantly lower abundance of SCFAs and SCFA-production bacteria has been demonstrated in colorectal cancer (CRC), and the supplementation of SCFAs-producing probiotics can inhibit intestinal tumor development. Hou., *et al*. [11] reviewed SCFAs-guided modulation in both mouse and human CRC, which might inspire new approaches for the diagnosis, treatment and prevention of CRC on the basis of gut microbiota-derived metabolites SCFAs.

In present review we discussed butyrate as a potential therapeutic prodrug for multiple myelomas (MM) cells apoptosis and in case reports the efficacy of butyrate for patients with recurrent malignant glioma and the mitigation of cervical cancer and a small cerebral neoplasm with the long-time ingestion of aloe vera juice as an adjuvant were positively expressed.

Butyrate affects pancreatic and gemcitabine response in *in vitro* and *in vivo* models

Pancreatic ductal adenocarcinoma (PDA) is an aggressive cancer. The characteristic excessive stromatogenesis accompanying the growth of this tumor is believed to contribute to chemoresistance which, together with drug toxicity, results in poor clinical outcome. Panebianco., *et al*. [12] provided *in vitro* evidence that, beside slowing proliferation, butyrate enhanced gemcitabine effectiveness against two human pancreatic cancer cell lines, mainly induing apoptosis. In addition, the author observed that, when administrated to a PDA mouse model, alone or combined with gemcitabine treatment, butyrate markedly reduced the cancer-associated stromatogenesis, presented intestinal mucosa integrity and affected fecal microbiota composition by increasing SCFAs producing bacteria and decreasing some pro-inflammatory microorganisms. The results supported that butyrate supplementation, in addition to conventional therapy, can interfere with pancreatic cancer biology and response to treatment and can alleviate some damages associated to cancer itself or to chemotherapy.

Butyrate improves intestinal integrity and microbiota composition in pancreatic cancer mice, and butyrate with or without gemcitabine affects serum metabolome and lipidome in pancreatic cancer mice.

Clinical report in continuous intrathecal or intracavitary administration of sodium butyrate for patients with recurrent and progressive malignant glioma

The continuous intrathecal or intracavitary administration of 60 milli-molar sodium butyrate (SB) for recurrent and progressive malignant glioma (MG) 23 patients (19 glioblastoma and 4 anaplastic astrocytoma patients) between October 2001 to February 2006 and followed until October 2006. was clinically attempted by Nakagawa., *et al*. [13]. The author evaluated the clinical potential of intrathecal use of SB for malignant glioma. The therapy was well tolerated and resulted in long-term inhibition of the growth of the tumor in some patients and showed therapeutic safety.

Gene modulating butyrate metabolism for assessing clinical prognosis and responses to systematic therapies in hepatocellular carcinoma

Butyrate, one of the major products of the gut microbiota, has played notable roles in diverse therapies for multiple tumors. Chuanbring., *et al*. [14] determined the roles of genes that modulate butyrate metabolism (BM) in predicting the clinical prognosis and responses to systemic therapies in hepatocellular carcinoma (HCC). The genes modulating BM were available from the Gene-Card database, and gene expression and clinical information were obtained from TCGA-LIHC, GEO, ICGC-JP, and CCLE databases. Candidate genes from these genes that regulate BM were then identified by univariate Cox analysis. The role of BM-related gene signature (BMGs) in identifying high-risk patients of HCC, assessing the prognoses, and predicting systematic therapies were determined in various datasets. The author has identified BMGs based on eight genes that modulate butyrate metabolism. BMGs may be served as novel promising biomarkers for early

identifying high-risk groups of HCC, as well as assessing prognoses, drug sensitivity, and the responses to immunotherapy, transcatheter arterial chemoembolization, and ablation therapy in patients with HCC.

Short-chain fatty acid-releasing nano-prodrugs for attenuating growth and metastasis of melanoma

Short chain fatty acids (SCFAs) have been reported to possess anti-neoplastic effects; however, rapid renal clearance and high dose-based side effects limit their clinical translation. Shahni., *et al*. [15] has designed a new self-assembling nano-prodrugs that can effectively supply SCFAs: endogenous enzyme metabolizable block copolymer poly (ethylene glycol) block-poly (vinyl ester) possessing several units of SCFAs conjugated as side chains via ester linkages. The author showed the therapeutic efficacy of SCFAs nanoparticle (NanoSCFA) in a mouse model of metastasis (melanoma). Ad libitum intake of NanoSCFA markedly demonstrated a decrease in the metastatic tumor modules in the lung compare with the effect observed after low-molecular-weight (LMW)-SCFA administration with no discernible toxicity to the GI tract. In contract, LMW-SCFA, even at a low concentration than that of the NanoSCFA, facilitated villus atrophy. The findings suggested that the use of NanoSCFA as a therapeutic intervention for metastatic cancer is preferable over typical LMW-SCFAs.

Can mRNA vaccines help treat cancer?

When the pandemic struck, mRNA vaccine technology had an unexpected opportunity to demonstrate its promise. Winsted [16] reviewed that the production of mRNA vaccine today is easy, fast and can be scaled up as needed. For more than a decade, cancer researchers have been developing a type of treatment known as a personalized cancer vaccine using various technologies, including mRNA and protein fragments, or peptides. The investigational mRNA vaccines are manufactured for individuals based on the specific molecular features of their tumors. Some companies investigating mRNA cancer vaccines that are based on collections of a few dozen neoantigens that have been linked with certain types of cancer, including prostate cancer, gastrointestinal cancers, and melanomas. Some investigators believe the success of the mRNA Covid-19 vaccines could help accelerate clinical research on mRNA vaccines to treat cancers. Insights about the composition of mRNA is packaged that emerge from studies of viruses could potentially inform work on cancer vaccines. Cancer research led to speedy development of mRNA vaccines.

The potential of miRNAs to act as enhancers of anti-cancer effects of histone deacetylase inhibition

Ali., *et al*. [17] investigated that the gut fermentation product butyrate displays anti-cancer properties in the human proximal colon, including the ability to inhibit proliferation and induces apoptosis in colorectal cancer cells. A natural histone deacetylase inhibitor, butyrate can alter global gene expression, including the non-coding transcriptome and microRNA (miRNA). A high-throughput functional screen was employed to identify miRNA, including miR-125b, miR-181a, miR-593, and miR-1227, enhanced apoptosis, decreased proliferation, and promoted cell-cycle arrest in the presence of butyrate. The author reported that several cancer-associated miRNA targets were synergistically regulated by the combination of cognate miRNAs and butyrate to act as enhancers of anti-cancer effects of HDAC inhibition and identified specific miRNAs that might be exploited for therapeutic benefit.

Are short chain fatty acids (SCFAs) associated with clinical outcomes in patients with solid cancer tumors treated with programmed cell death-1 inhibitors?

To evaluate fecal and plasma SCFAs in patients with solid cancer tumors treated with programmed cell death-1 inhibitors (PD-1i), Nomura., *et al*. [18] investigated a prospective cohort biomarker study of patients with cancer who planned therapy with PD-1i at Kyoto University Hospital between July 2016 and February 2019. The result of the study suggested that fecal SCFAs concentrations may associated with PD-1i efficacy: SCFAs may be the link between the gut microbiota and PD-1i efficacy. Because fecal examinations are completely noninvasive, they may be applicable for routine monitoring of patients. The findings suggested that fecal SCFAs concentration may be a potential biomarker to identify patients with solid tumors who could benefit from treatment with programmed cell death-1 inhibition.

Butyrate in both prevention and supportive treatment of colorectal cancer

Sodium butyrate (NB) is characterized by a wide range of beneficial properties/activities. It influences the function of the immune system, maintains intestinal barrier integrity, positively affects the efficiency of anti-cancer treatment, and may reduce the risk of mucositis induced by chemotherapy. Kazmierczak-Siedlecka., *et al*. [19] analyzed NB and its impact on gut microbiota as well as anti-tumor activity by describing molecular mechanisms. NB is available as food with special medical purposes, and its administration seems to be a promising option for colorectal cancer patients.

Case reports in previous paper

The case reports in our previous paper [20] showed efficacy of autologous peripheral blood stem cell transplantation treatment, cancer therapy drugs, and adjuvant aloe vera juice (AVJ) supplementation to multiple myeloma of 51-years old male patient, acute leukemia of 12-years-old boy patient, and acute myeloid leukemia of 43-years-old lady patient without any side effect. Beneficial use of HDAC inhibitor, butyrate may be overcome by several approaches with anti-multiple myeloma agents and an adjuvant AVJ. Aloe polysaccharide, acemannan, as a promising vaccine adjuvant could ensure maintenance of homeostasis for hematologic malignancy and butyrate may evaluated to clinical potential intrathecal or intracavitary use for malignant glioma.

Case report 1

A 44-years-old female had an abnormal on 2011, and was diagnosed as cervical cancer and endometrial cancer. She was denied full uterine surgery and ingested Aloe vera juice (AVJ, 1000 ml/day) for two months. The endometrial cancer was disappeared but the cervical cancer still remained, and she had well-being QOL. On March 20, 2022 when she was 55-years old, she had the fourth times vaginal bleeding, and was taken in the ambulance and hospitalized. After two-times of transfusion, anti-biotic intravenous and iron preparations medicines intravenous drip, she left the hospital after10-days. She was diagnosed the reason of the virginal bleeding to be cervical cancer and glandular flat epithelial carcinoma at stage IV. Then, she was hospitalized into department of radiology, because her cancer was so much larger for treatment of resection surgery, she had chemo-radiation therapy from May, 24 2022. Since then, she was hospitalized and left the hospital on November, 2022. The comparison of examination value before/after 6 months'-hospitalizing expressed that the improvement of anemia and kidney function, and the recovery to standard value of tumor marker. After 11-years ingestion of AVJ, the side effect due to chemoradiation therapy was decreased and the therapeutic effects on cervical tumor increased in prognosis. She has a well-being QOL on March, 2023.

Case report 2

A 42-years-old female who had an abnormal and large amount of uterine blooding, was hospitalized on 2010. She was diagnosed as a cervical cancer and early-stage of endometrial cancer. She started to ingest Aloe vera juice (AVJ, 250 ml/day) after the ingestion of cervical cancer drug. During for the ingestion of AVJ for 1.5 month, the cervical cancer disappeared but the endometrial cancer still remained. Then she was operated the endometrial cancer. After the operation she was diagnosed no-spread of endometrial cancer into lymph nodes. When she was 55-years-old, she recovered well-being QOL continuing the drug administration and ingestion of AVJ on March, 2023.

Case report 3

A 33-years-old female who had dizziness with double appearance for ~6 months, consulted doctor and was found a small cerebral neoplasm in MRI examination on December, 2022. She decided to ingest 7200ml/month with the drug. After 2 months AVJ ingestion, she visited the second MD. MRI examination showed a clear data and a small cerebral neoplasm was disappeared. She has a well-QOL on March, 2023.

Summary

Since 1933 [1], butyric acid, one of the essential agents for determine the role of histone-acetylation in chromatin structure and function, was demonstrated in the treatment of special uterus cancer, special uterus cervix cancer.

Gene modulating butyric acid could play notable roles in diverse therapeutics for multiple tumors.

Butyric acid induces apoptosis triggered by butyric acid in human colorectal cancer cells (CRC). Butyric acid fermented in Aloe vera gel may participate to expert anti-cancer efficacy as an adjuvant.

Present review showed the gut fermented butyrate displays anti-cancer properties in the human proximal colon, including the ability to inhibit proliferation and induces apoptosis in colorectal cancer cells (CRC). MicroRNA (miRNA) vaccine technology after pandemic struck advanced the science of miRNA cancer vaccines. miRNA plays the butyrate-mediated inhibition of CRC. Butyrate and miRNA can regulate similar cell growth and death signaling pathways, suggesting that butyrate may mediate its anti-cancer effects partly through miRNA regulation or in collaboration with miRNAs [17]. And butyrate facilitates anticancer therapy efficacy by modulating cytotoxic $CD^{+}8T$ cell immunity [9].

In case report 1, 2, and 3, AVJ long-time ingestion showed the possible mitigation to cervical cancer and a small cerebral neoplasm as an adjuvant. Fermented butyrate during a long-time ingestion of AVJ could mitigate a cervical tumor and a small cerebral neoplasm on immune modulation and intestinal homeostasis.

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