Anticarcinogenic Actions of Tributyrin, A Butyric Acid Prodrug

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Abstract: Bioactive food compounds (BFCs) exhibit potential anticarcinogenic effects that deserve to be explored. Butyric acid (BA) is considered a promising BFC and has been used in clinical trials; however, its short half-life considerably restricts its therapeutic application. Tributyrin (TB), a BA prodrug present in milk fat and honey, has more favorable pharmacokinetic properties than BA, and its oral administration is also better tolerated. In vitro and in vivo studies have shown that TB acts on multiple anticancer cellular and molecular targets without affecting non-cancerous cells. Among the TB mechanisms of action, the induction of apoptosis and cell differentiation and the modulation of epigenetic mechanisms are notable. Due to its anticarcinogenic potential, strategies as lipid emulsions, nanoparticles, or structured lipids containing TB are currently being developed to improve its organoleptic characteristics and bioavailability. In addition, TB has minimal toxicity, making it an excellent candidate for combination therapy with other agents for the control of cancer. Despite the lack of data available in the literature, TB is a promising molecule for anticancer strategies. Therefore, additional preclinical and clinical studies should be performed using TB to elucidate its molecular targets and anticarcinogenic potential.

Keywords: Bioactive food compounds, butyrate, butyric acid, cancer, cellular targets, chemoprevention, molecular targets, tributyrin.

INTRODUCTION

Cancer is a major health problem worldwide, with approximately 12 million new cancer cases in 2008 [1, 2] and approximately 8 million deaths. Approximately 13.1 million deaths are predicted to occur in 2030 [3]. According to recent estimates, the risk of developing cancer may be reduced 30-40% when preventive measures such as the promotion of appropriate nutrition including the use of specific foodstuffs and regular physical activity are adopted [4]. In this regard, several epidemiologic and preclinical studies have convincingly argued for a definitive role of bioactive food compounds (BFCs) for the prevention and treatment of some cancers [5-10]. BFCs generally exhibit low toxicity, are well tolerated by humans, and are pleiotropic i.e., they act on multiple targets involved in carcinogenesis [11]. Some BFCs may be toxic in high doses [12]. However, its use can be considered as a therapy option for individuals that are prone to developing cancers, whereas the toxic effects may be tolerated [13, 14]. Therefore, future translational studies focusing on the clinical relevance and mechanism of BFCs are needed to further assess the applicability of dietary factors as chemopreventive agents and/or adjuvant chemotherapeutic agents [15].

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BUTYRIC ACID (BA) AND CANCER

The hypothesis that the intake of the dietary fiber present in fruits and vegetables may modulate the development of several pathological conditions is not new. In the 1960s, Burkitt found a lower incidence of diseases such as diverticulitis, appendicitis, and colon cancer among populations that consumed large amounts of fruits and vegetables compared with populations that consumed refined foodstuffs [16, 17]. This dietary pattern of consuming large amounts of fruits and vegetables is associated with a greater production of short-chain fatty acids (SCFA) such as acetic, propionic, and BA in the colon. Each of these acids has been tested in transformed human cell lines, and BA exhibited the strongest inhibitory effects on cell growth compared to other SCFAs [18]. In the colon, BA is the main source of energy and provides 70 to 90% of the energy consumed by colonocytes [19], thus playing a major role in the normal development of this organ [20]. The remarkable anticarcinogenic potential of BA was further observed in several in vitro and in vivo cancer models including prostate [21, 22], breast [23, 24], stomach [25], and liver [26] cancer. Leder and Leder observed that BA and/or its salt sodium butyrate (NaBu) induced cultured erythroleukemic cell differentiation due to their structural characteristics rather than through an exclusive function of their role as energy sources or the action of their metabolites [27]. Because it exhibits potential for chemotherapy use, NaBu has been considered a potential molecule for cancer therapy molecule [28]. Such observations led to the development of pharmacologic-clinical studies using parenteral NaBu in patients with acute leukemia [29, 30] that resulted
in partial remission [29]. It is worth emphasizing that the intravenous administration of BA up to 1.5 g/kg/day during 17 days [29] is not associated with noticeable toxicity [29, 30]. Nevertheless, the therapeutic efficacy of NaBu may be affected when ideal serum concentrations similar to those observed in in vitro studies (i.e., 0.5 mM) are not achieved [30]. NaBu is quickly absorbed [31] through the plasma membrane by simple diffusion [32], SCFA/HCO$_3^-$ co-transport [33], and the active transport of dissociated forms. Effective butyrate blood concentrations are difficult to maintain because it is quickly metabolized into ketone bodies, which mainly include 3-hydroxybutyrate [34] and butyryl-CoA, enoyl-CoA, L-hydroxyacyl-CoA, acetoacetyl-CoA, and acetyl-CoA [35] as well. Bioavailability studies have demonstrated that the butyrate half-life exhibits a biphasic behavior as a consequence of its pattern of elimination from the body. During the first minutes, butyrate clearance is fast and results in a serum half-life of just 30 seconds. Butyrate then binds to serum proteins, its clearance decreases, and its half-life increases up to 14 minutes [36]. In rodents, the butyrate serum half-life was less than five minutes after intraperitoneal or intravenous administration [36].

The butyrate half-life may be considerably increased when it is administered as its natural produg, namely tributyrin (TB) [37-39]. TB, which is found in a variety of food-stuffs such as milk fat and honey [40, 41], is a triacylglycerol composed of three BA molecules esterified with glycerol. Therefore, the full hydrolysis of 1 mole of TB may generate 3 moles of BA. After oral administration to rodents, the TB half-life was approximately 40 minutes [42]. In addition, the oral administration of TB to rodents produced detectable serum butyrate levels five minutes later, and the levels reached their peak 15 to 60 minutes after administration [39] and could be maintained above 0.1 mM for up to 120 minutes [39, 41]. Mice exhibited serum butyrate levels between 0.8 and 1 mM for up to one hour after administration of a 7.75 g/kg body weight (b.w.) dose of TB. A 8.2 g/kg b.w. dose of TB did not exhibit toxic effects in rodents treated via the oral or intraperitoneal routes [39]. The oral administration of 2 g/kg b.w. of TB to animals subjected to an experimental hepatocarcinogenesis model increased the BA level in the liver up to five times, compared to animals that did not receive TB, but were submitted to the same experimental procedure [43, 44]. The fact that TB may be administered per the oral route represents an advantage because, in addition to more favorable pharmacokinetic properties, it exhibits minimal toxicity [45], is better tolerated than butyrate [39, 42], and is better accepted by patients [38, 39]. Thus, TB is quite promising, and its use as a food additive has been approved in several countries including the United States, Canada, and Brazil [46-50]. In addition, the administration of TB to mice reduced the circulating levels of triacylglycerols and cholesterol and attenuated the increase in serum leptin and resistin. These effects indicate that TB can protect against diet-induced obesity and the associated insulin resistance [51].

**TB CELLULAR AND MOLECULAR TARGETS FOR CANCER CONTROL**

The anticarcinogenic potential of TB was shown in several in vitro and in vivo studies (Table 1). Among its mechanisms of action in cells Fig. (1), the induction of apoptosis [21] and cell differentiation [52] are notable. In addition, TB also modulates epigenetic mechanisms such as histone acetylation, whose dysregulation is currently acknowledged as an important molecular feature of carcinogenesis [15, 43, 44].

**TB and Apoptosis Induction**

The induction of apoptosis is an important mechanism for the inhibition of carcinogenesis and cancer growth by TB. Numerous studies have documented the ability of this compound to induce several programmed cell death mechanisms in different cancer cell lines [48-53] even more intensely than BA [21]. The intrinsic apoptosis pathway is induced by TB in a caspase-3-dependent or caspase-3-independent manner. Thus, in HT-29 colon cancer [46] and B16-F10 melanoma cells [53], TB increased apoptosis by activating caspase-3 activity. In MCF-7 human mammary carcinoma cells, TB induced a transient increase in mitochondria-associated Bax, dissipation of the mitochondrial membrane potential, and caspase-3-independent cleavage of PARP [54]. These events were followed by a transient increase in cytosolic cytochrome c, and finally, through the generation and accumulation of cells with subdiploid DNA content, a terminal apoptosis event [54]. TB inhibited the growth of SGC-7901 gastric cancer cells by decreasing DNA synthesis and inducing cell death, which was associated with the down-regulation of Bcl-2 and up-regulation of Bax expression [55]. NaBu increased the susceptibility of hepatoma cells to apoptosis by inducing the signaling of tumor necrosis factor [56]. In an experimental hepatocarcinogenesis study in rats, TB inhibited the development of persistent preneoplastic lesions (pPNLs), which are considered sites of hepatocellular carcinoma (HCC) progression. In addition, TB increased PNL remodeling, which tends to make the PNLs disappear. This protective effect was partially associated with the induction of apoptosis in PNLs undergoing remodeling [43].

Alterations in the p53 tumor suppressor gene have been linked to reduced apoptosis in cancer cells and chemotherapy resistance [57]. In three phenotypically and genotypically divergent human prostate cancer cell lines, PC-3 (p53-deficient), TSU-PR1 (p53-negative), and LNCaP (p53-normal), TB strongly induced growth inhibition, cell cycle arrest, and apoptosis. These data indicated that the compound acts independently of the p53 status [22]. Similarly, TB administration for four consecutive weeks to mice after the implantation of prostate cancer cell lines resulted in strong cell growth inhibition and increased apoptotic activity. These effects also appeared to be independent of the p53 status [21]. The pathologic accumulation of cytoplasmic wild-type p53 has been related to alterations in differentiation, an increase in malignancy, tumor metastasis, and poor cancer prognosis [58]. Hepatic pPNLs, considered to be more aggressive and the sites of origin for HCC, exhibit a cytoplasmic accumulation of p53 [59], which may be associated not only with increased genomic instability during the early stages of hepatocarcinogenesis but also with the evasion of apoptosis [60]. Interestingly, TB reduced the frequency of hepatic pPNLs with aberrant cytoplasmic p53 accumulation, indicating that TB may act on lesions exhibiting more aggressive phenotypes and greater potential to evolve.
Table 1. The anticarcinogenic potential of TB.

<table>
<thead>
<tr>
<th>Study Type</th>
<th>Feature of the Study</th>
<th>Most relevant Findings</th>
<th>Literature</th>
</tr>
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<tbody>
<tr>
<td>In vitro</td>
<td>Use of human myeloid leukemia cells (HL-60) and murine erythro-leukemia cells (MEL-cells)</td>
<td>TB induced differentiation</td>
<td>Chen and Breitman. 1994 [37]</td>
</tr>
<tr>
<td>In vitro</td>
<td>Use of transformed human liver cells (Hep G2)</td>
<td>TB was a potent apoptotic agent</td>
<td>Watkisn, et al. 1999 [20]</td>
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<tr>
<td>In vitro</td>
<td>Use of human prostate cancer cells lines (LNCaP, PC-3, TSU-PrI)</td>
<td>TB inhibited cell growth and induced apoptosis</td>
<td>Maier, et al. 2000 [22]</td>
</tr>
<tr>
<td>In vitro</td>
<td>Use of human colon cancer cells (HT-29)</td>
<td>TB induced apoptosis mediated through the activation of caspase-3</td>
<td>Clarke, et al. 2001 [46]</td>
</tr>
<tr>
<td>In vitro</td>
<td>Use of human colonic adenocarcinoma cells (LS 174T)</td>
<td>TB reduced the cell proliferation and induced apoptosis by up-regulation of caspases 3 and 8</td>
<td>Schröder, et al. 2002 [68]</td>
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<tr>
<td>In vivo</td>
<td>Use of prostate cancer cell lines (PC3 and TSU-PrI) seeded on the chorioallantois membrane and implanted in a xenograft model using nude mice</td>
<td>TB reduced the cell tumor cell proliferation and possibly involved a p21/Rb/c-myc pathway</td>
<td>Kuefer, et al. 2004 [21]</td>
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Fig. (1). TB Anticarcinogenic Actions. The full hydrolysis of 1 mole of TB generates 3 moles of butyrate. Once absorbed butyrate can exert different inhibitory effects on cancer cells. The mechanisms of action may involve: 1) Apoptosis induction through a caspase-3 dependent or independent pathway involving Bcl-2 down-regulation and Bax up-regulation; PARP cleavage and p53 cytoplasmic-nuclear translocation; 2) Cellular differentiation acting in ERK inhibition, p38 MAP kinase activation, up and down regulation of NF-xB dependent genes, and in NDRG1, β-casein and c-myc regulation; and 3) Epigenetic mechanisms acting as a HDACi, in induction of histone hyperacetylation on the promoting region of some tumor suppressor genes, such as p21; as well as, in the alteration of miRNAs profile.
into cancer [43]. Unpublished results by our group suggest that TB modulates proteins such as chromosomal-region maintenance 1 (Crm1), apoptosis repressor with caspase recruitment domain protein (Arc), parkin-like ubiquitin ligase (Parc), and importin-a, which are involved in the regulation of p53 nuclear-cytoplasmic translocation. Such nuclear-cytoplasmic machinery may become a viable and promising chemopreventive (therapeutic) TB target [61, 62].

Interestingly, multiple cell signaling pathways leading to apoptosis are induced by TB at concentrations to which normal cells are not affected [43, 44]. The mechanisms that determine the resistance against apoptosis, induced by TB, in normal cells, is still unknown. However, it is suggested that the rapid reversion of the AB linkage to the histone deacetylase (HDAC) of classes I and II may provide normal cells with the ability to compensate for the inhibitory effects of these agents [63]. The transformed cells do not present the same type of compensatory response, because they often present a defect on the Checkpoint kinase (Chk1). The Chk1 plays an essential role in the correction of damages in normal cells, which in part gives them resistance to the HDACi (histone deacetylase inhibitor)-induced apoptosis [64]. As a result, cells harboring neoplastic and preneoplastic lesions would be more susceptible to the cytotoxic effects of TB. These actions represent important features for establishing safe and clinical relevant strategies for the prevention and treatment of cancer.

**TB and Cellular Differentiation Induction**

The induction of cell differentiation is considered an attractive mechanism for cancer control. NaBu was classified as a powerful inducer of cell differentiation in several transformed lines including K562 and HL60 leukemia cells, PANCl pancreatic carcinoma cells [65], HT-29 colorectal cancer cells [65, 66], SKG-IIIa uterine cervical cancer cells [67], androgen-sensitive and androgen-resistant human prostate cancer cells [22], and PLC/PRF/5 hepatoma cells [56]. TB treatment induces cell differentiation in HL60 and MEL leukemia cells at a level that is three times more powerful than that induced by BA [37]. In addition, TB induced differentiated phenotypes for LS 174T colon cancer cells [68] and prostate cancer cell lines [22] regardless of androgen sensitivity [52], and it is considered a promising compound for clinical trials in patients with prostate cancer [22]. Thus, phase I clinical trials were performed mostly based on the notion that butyrate and its derivatives may play a role in the treatment of cancer by inducing cell differentiation [65]. Nevertheless, the exact mechanism by which such compounds induce the differentiated phenotype of neoplastic cells is poorly understood.

Thus, for example, the induction of the differentiation of K562 leukemia cells was associated with extracellular signal-related kinase (ERK) inhibition and activation of the p38 MAP kinase pathway [69]. In addition, p38 activation combined with the induction of stress signaling pathways plays an important role in the butyrate-induced differentiation of PANCl, HT29, and HL-60 cells [69]. It was reported that NaBu induces breast cancer cell differentiation by regulating beta-casein and N-myc downstream-regulated gene 1 (NDRG1) in breast cancer cells [70]. A substantial number of up- and down-regulated genes were associated with the TB-induced cell differentiation process in a prostate cancer line, some of which are nuclear factor kappa-B (NF-kB)-dependent [52]. Treatment with NaBu induced the in vitro differentiation of U138B human glioblastoma cells as denoted by alterations in their morphology, inhibition of cell proliferation, decreased c-myc expression, and the increased expression of glial fibrillary acidic protein [71]. These positive results reappeared in preclinical studies of brain tumor models with rodents [72].

The assessment of PNL persistence or remodeling is used in chemoprevention studies in experimental hepatocarcinogenesis [73-75]. Although the cellular and molecular mechanisms involved in both phenotypes i.e., PNL persistence and remodeling, have not been sufficiently elucidated, it is believed that remodeling may in fact involve the redifferentiation of the hepatocytes in the nodules into a phenotype similar to that of the adult liver [76, 77]. TB inhibited the development of persistent PNLs that evolve into cancer and induced the remodeling of PNL towards a “normal” liver. Thus, the induction of cell differentiation also appears to be involved in the mechanism of the chemopreventive activity of TB in experimental hepatocarcinogenesis [43].

**TB and Epigenetic Mechanisms Modulation**

Cancer is considered a genetic and epigenetic disease [78], and increased attention has been given to the role of epigenetic alterations during cancer development [79]. Epigenetics, which was first described in 1942 [80], generally refers to heritable changes in gene expression and chromatin organization that are not due to alterations in the DNA sequence [78, 79]. Epigenetic modifications, including changes in DNA methylation, histone covalent modifications, and small RNAs, are of particular interest in the field of cancer research because their impact on the epigenome is involved in cell proliferation, differentiation, and survival [78, 79, 81]. The reversibility of epigenetic chromatin modifications by synthetic and natural compounds has opened new avenues for the control of cancer [82-85] with great potential for clinical translation [86].

Vorinostat (suberoylanilide hydroxamic acid, SAHA, Zolinza®, Merck & Co Inc., Whitehouse Station, New Jersey) was the first commercially available HDACi approved for oncology use [87]. It has been approved for use in patients with cutaneous T-cell lymphoma who have progressive, persistent, or recurrent disease, or those following two systemic therapies [87]. NaBu was the first compound found to cause an increase in histone acetylation [88, 89]. Butyrate acts as a weak ligand for HDAC and inhibits most HDACs as class I HDAC and class II HDAC 4, 5, 7 and 9 [90, 91], most likely because it contains a short three-carbon spacer attached to a carboxylic acid group, which likely enters into the active site of the enzyme and forms a bidentate ligand with a zinc atom [85]. Two butyrate molecules could possibly occupy the hydrophobic pocket and inhibit the enzyme [90].

Histone hyperacetylation was shown in a variety of vertebrate cell lines after butyrate treatment [37]. NaBu induces the sensitization of HeLa cells towards the action of cisplatin, and it was associated with hyperacetylation of the
Histone core and abrogation of the cisplatin-imposed cell cycle arrest [92]. Genome-wide analyses demonstrated that NaBu-induced enterocytic differentiation is associated with hyperacetylation on lysine 9 of histone 3 (H3K9) at some gene promoters, indicating that this epigenetic pattern seems to be an important marker of distinct differentiation pathways [93]. BA administration reduces the incidence of colorectal cancer induced by 1,2-dimethylhydrazine (DMH) in ICR mice, and this action is involved with the epigenetic mechanism of histone acetylation [94]. TB suppressed hepatocarcinogenesis in rats by increasing histone acetylation [43, 44] specifically in the nuclei of PNL hepatocytes, which reflects a specific and direct epigenetic action on such PNL that may be relevant for its chemopreventive activity [43]. In addition, this epigenetic modulation occurred specifically H3K9, which is considered an important target for the reactivation of gene expression [43, 44]. Similar actions were observed in transformed MCF-7 breast cells treated with NaBu [95].

Several studies have shown that TB, as an HDACi, strongly activated p21cip/waf expression [96, 97], a tumor suppressor gene that influences multiple functional processes such as cell division, cell death, repair, and differentiation. p21cip/waf-deleted cells failed to undergo growth-inhibition by NaBu in the HT29 human colon carcinoma cell line [98]. Histone acetylation in the promoter of this gene after butyrate treatment has been reported [99]. Butyrate administration increases the expression of p21cip/waf in the colon mucous membranes of rats, and this effect was associated with increased histone acetylation [100]. Increased p53-independent p21cip/waf induction and G2-M arrest was observed in the TB-mediated growth arrest and apoptosis of wild-type p53 MCF-7 cells [54]. The chemopreventive actions of TB in hepatocarcinogenesis were also accompanied by the restoration of p21cip/waf expression, which indicates the potential of this substance for gene reactivation [43, 44]. In this regard, studies indicate that as much as 10% of genes may be affected by NaBu either directly or indirectly. Interestingly, a similar number or even more genes are down-regulated than up-regulated by this drug. This can be explained by the specific genomic regions that can be deacetylated after butyrate exposure [101]. The promoter regions of butyrate-responsive genes contain butyrate response elements that control genes that are induced or repressed by this compound [24, 102-105]. In this context, a bipartate butyrate-responsive element was identified in the human promoter region of calretinin (CALB2), a gene negatively regulated by butyrate and expressed in the majority of poorly differentiated colon carcinoma cells [106].

However, histones are not the only substrates that can undergo post-translational modification. High-mobility group proteins, including multiple transcription factors, can be acetylated, which has a wide range of effects on their function. HDACis including NaBu reverted the resistance to chemotherapy by inducing p53 acetylation and its subsequent migration towards the nucleus, thus restoring its function in neuroblastaoma cells [107]. Unpublished data obtained by our group suggest that the chemopreventive activity of TB is not only associated with histone acetylation but also with p53 acetylation, which increases the nuclear stability of this transcription factor and induces the expression of genes involved in the apoptotic pathway induced by p53.

miRNA expression can contribute to cancer development and progression, and miRNAs are differentially expressed in normal tissues and cancers [108]. Growing evidence documents the emerging role of miRNAs in the identification of therapeutic targets of specific cancers, and this may be a promising approach for molecular cancer therapy [109]. NaBu treatment induced the differentiation of human embryonic stem cells, which was related with alterations in the miRNA profile [110]. In addition, NaBu inhibited several miRNAs induced in HCT-116 human colon cancer cells. One such miRNA is mir106b, whose target is p21cip/waf [111]. The clinical anticancer potential of molecules that act by modulating the miRNA expression must be explored [112]. Thus, the identification of microRNAs modulated by TB represents another interesting molecular mechanism to investigate.

**BIOMARKERS**

TB acts in several cellular mechanisms, and hence it is necessary to find a biomarker that is capable of monitoring its clinical effectiveness. Moreover, once the HDAC activity is linked to carcinogenesis, it is possible that the measurement of its expression or activity may be considered as a response biomarker. Therefore, patients that present distinct patterns of HDAC expression and activity may be divided into subgroups so that the therapy with HDACi, such TB, may be more potent [113, 114].

The biomarker which is the most used in clinical studies is the acetylation of histones H3 and H4, once this epigenetic modification is directly regulated by HDACs. However, the acetylation of H3 and H4 does not seem to be related to the treatment response [113]. The utilization of the p21 expression as a biomarker of HDAC inhibition has been investigated [115].

HDACis are involved with the transcriptional regulation of a small number of genes; it is possible that a group of genes is identified as biomarkers of therapy response. One of these genes, HR23B, has been validated as a promising biomarker [116]. Studies are being conducted in this way and will allow a better comprehension of the HDACi clinical effects.

**PHARMACEUTICAL PERSPECTIVES**

Translational cancer research aims to translate scientific discoveries into new methods of cancer control. Thus, TB may be considered a promising molecule because it often acts on known critical mechanisms of carcinogenesis [109]. In addition, TB exhibits better bioavailability compared to BA and can be administered via the oral route; thus, its clinical potential should be better explored.

Conley et al. (1998) conducted the first TB clinical trial with the following aims: to establish its tolerable single daily dose; to establish its toxic and therapeutic effects; to document its pharmacokinetics; to establish the dose that results in effective serum butyrate concentrations (i.e., 0.5-3 mM); and to define the dose that may maintain such serum concentrations [38]. Thus, 13 patients with different types of cancer
were given TB gelatin capsules in a single daily dose varying between 50 and 400 mg/kg b.w. The patients did not exhibit any significant toxicity signs and symptoms. With a 200 mg/kg TB dose, the serum concentration reached a level of 0.45 mM, which is close to the concentration used in in vitro studies (i.e., 0.5 mM). Based on the serum butyrate concentrations measured every 30 minutes during 4 hours after administration of the compound, it was concluded that a single daily dose of TB is unable to maintain serum concentrations at a constant and sufficient level needed for its therapeutic activity, and the dose suggested was 200 mg/kg three times per day [38].

A subsequent clinical trial [45] performed with 20 patients with different types of cancer administered 200 mg/kg TB gelatin capsules to patients three times per day, and no relevant toxic effects were observed. In addition, the large number of capsules each patient needed to take did not reduce the compliance with treatment, which lasted over several months and succeeded in stabilizing the disease in several cases [45]. The average serum butyrate concentration in such individuals was higher compared with leukemia patients treated with intravenous NaBu [30, 45].

These TB studies found significant variations in the serum butyrate concentration between the individuals who were given TB capsules, which may be related to individual metabolic variations or an inherent instability of the prodrug [38, 45]. Therefore, several different strategies aimed at increasing the TB bioavailability are currently being investigated. The administration of a TB lipid emulsion to rats increased the bioavailability of butyrate and/or TB up to four times compared with the administration of pure TB [42]. This TB emulsion was synthesized with synthetic low-density lipoprotein (LDL) fractions that exhibited serum kinetic behavior similar to native LDLS, i.e. the ability to bind to LDL receptors and intracellular incorporation [117]. Thus, a TB emulsion exhibiting such properties has the potential to act on neoplastic cells hypoxpressing LDL receptors and to induce apoptosis in transformed colon and liver cells [42]. An oil-in-water emulsion with TB as internal oil phase inhibited the growth and colony formation of melanoma cells in vitro and in vivo [53]. Because the internal phase of such an emulsion provides an environment appropriate for the solubilization and transport of lipophilic molecules, these molecules can be combined with the emulsion, thus producing a drug delivery system with anticarcinogenic and even additive properties [53]. Celecoxib, which is a selective cyclooxygenase-2 (COX-2) inhibitor, exhibits several anticancer effects. However, because celecoxib is a highly hydrophobic molecule, its clinical use is problematic. Incorporation of celecoxib into TB-based emulsions allowed overcoming limitations inherent to its solubility. In addition, the anticarcinogenic properties of celecoxib in transformed cells improved when it was incorporated into the TB-based emulsion, which was due to its improved solubility and the addition of its anticarcinogenic effects with those of TB [118]. Emulsions containing TB are unstable due to its low molecular weight, and they induce the Ostwald ripening (OR) phenomenon [119], which makes its liposolubility and incorporation in foodstuffs difficult. In this regard, TB incorporated into different emulsions containing long-chain triacylglycerols mostly derived from corn oil reduced the tendency for OR and inhibited the viability of HT-29 colon cancer cells more efficiently than an emulsion containing TB alone [120]. That same study further observed that TB, but not the other components of the system, is responsible for the effects of such emulsions on cell viability. These results are important for the development of systems that increase and improve the organoleptic characteristics and bioavailability of TB to facilitate its incorporation in foods and beverages [119] and its use in food/drug delivery systems for anticancer agents [47].

Recently, nanotechnology has become an interesting technology applied to cancer treatment [121-124], particularly for the development of drug delivery systems that may improve the bioavailability of lipophilic molecules such as TB [120]. Nanoparticles obtained from cholesterol and butyrate ester i.e., cholesteryl butyrate, inhibited the proliferation of lung carcinoma cell lines [125] and induced apoptosis in melanoma cells in a dose-dependent manner [126]. It was further observed that the treatment of myeloid or lymphocytic leukemia cells with these nanoparticles more powerfully inhibited the cell proliferation and expression of the c-myc oncogene than NaBu [127]. Thus, the incorporation of TB into nanoparticles may enhance the anticarcinogenic effects of BA and consequently be used in strategies for cancer chemoprevention [124].

The combination of TB with other agents also exhibiting anticarcinogenic potential may increase biological responses and thus be used as a promising anticancer strategy. A combination of TB and all-trans-retinoic acid (ATRA) enhanced the effects of the retinoid on NB4 acute promyelocytic leukemia cells [128]. In addition, ATRA-loaded TB submicron emulsions with affinity for apolipoprotein(s) could improve the uptake and exert a larger antiproliferative effect than that observed with ATRA in Caco-2 and HepG2 cells that express LDL receptors [42]. A combination of retinoids and HDACis was assessed in preclinical and clinical trials [129-131]. TB and its analogs restored the response to retinoids in cases of leukemia that were resistant to retinoid acid [128] and in prostate [132] and breast [133] cancer by inducing the hyperexpression of the retinoic acid receptor β (RAR-β), a tumor suppressor gene. The combined administration of TB and vitamin A (VA) to rats subjected to a model of experimental hepatocarcinogenesis exhibited inhibitory effects on PNL, induced H3K9 acetylation, and restored the expression of p21waf1. In addition, this combination induced apoptosis in PNLs more intensely than TB administered alone [44]. The epigenetic reactivation of the genes involved in the metabolism of retinoids, which are silent in several types of preneoplastic and neoplastic lesions, may possibly explain the synergism between those compounds and their use in strategies for the control of cancer. It has been observed that treatment with VA and NaBu inhibited the growth of MCF-7 breast cancer cells in addition to restoring RAR-β expression [95]. The effect of the combination of ATRA and TB was investigated in the FTC-133 poorly differentiated follicular thyroid carcinoma cells, which do not respond to radioiodine ablation. This study found that the combination of ATRA and TB synergistically induced the expression of sodium-iodide symporter (NIS), which is one of the most important thyroid differentiation markers regulating iodide trapping. In addition to increasing the NIS expression in a dose-
dependent manner, this combination also significantly increased the radioiodine uptake in FTC-133 cells compared with ATRA treatment alone, which points to a novel treatment to restore radioiodine uptake and inhibit cell proliferation in poorly differentiated thyroid carcinomas [134]. The data reported by those studies support the effectiveness of TB as a HDACi, which should be considered for cancer control combination strategies including those with retinoids [44]. A combination of TB and dihydroxycholecalciferol (OH)_2D_3 inhibited the proliferation and induced the differentiation of Caco-2 colon cancer cells. That effect was attributed to an increased expression of vitamin D receptors, which exhibited higher affinity for their ligand [135]. These data likely suggest a novel therapeutic option for patients with colon cancer. A combination of NaBu and folic acid (FA) was tested on DMH-induced colon carcinogenesis in mice. This study found that the investigated combination inhibited the development of colon cancer compared with its separate components. In addition, epigenetic mechanisms including DNA methylation and the post-translational modification of histones explained the protective action of the NaBu and FA combination [94]. Unpublished results obtained by our group indicate that the combination of TB and FA exhibits chemopreventive effects in hepatocarcinogenesis during the promotion stage in rats. In this regard, the involvement of epigenetic mechanisms is currently being investigated.

The physicochemical properties of triacylglycerols may be altered by restructuring or modifying the composition and/or positional distribution of fatty acids on the glycerol molecule. Specific lipases allow for the synthesis of structured lipids containing fatty acids derived from different triacylglycerols within a single glycerol molecule [136-138]. These may be considered as a new generation of substances with chemopreventive or therapeutic potential for several pathological conditions including cancer [139]. In this regard, rats were given Yoshida sarcoma cell xenografts and treated with structured lipids obtained through the interesterification of fish oil and medium-chain fatty acids. Treatment with structured lipids resulted in smaller tumors compared with rats treated with short-chain fatty acids or fish oil alone [140]. Recently, triradlyglycerols were suggested as potential vehicles for BA in the form of alkylglycerols such as 2,3-dibutyroil-1-O-octadecyl glycerol (D-SCAKG) [141]. It is believed that the products of the hydrolysis of D-SCAKG exhibit bioactive properties [141, 142]. In addition, unpublished results by our group demonstrate that structured lipids may be obtained by the enzymatic interesterification of TB with linoseed oil. The resulting triacylglycerols contain BA and alpha-linolenic acid molecules esterified with glycerol. The treatment of rats subjected to a hepatocarcinogenesis model with such structured lipids exhibited inhibitory effects on PNL and the induction of histone acetylation in addition to the accumulation of significant amounts of butyrate in the liver. Thus, the synthesis of structured lipids from TB may represent an important strategy for releasing BA, which further minimizes the dose needed to induce its anticarcinogenic effects by modulating the release speed and duration of the butyrate effect.

CONCLUSIONS

TB, the natural BA prodrug, is quickly absorbed into the serum and is chemically stable. The hydrolysis of TB gives rise to butyrate molecules that exert inhibitory effects on several types of cancer. Pathways involving the induction of apoptosis and cell differentiation and the modulation of epigenetic mechanisms represent the main cellular and molecular TB targets. In addition, TB can specifically target multiple pathways in preneoplastic and neoplastic cells, and it has no deleterious effects on normal cells. This feature may play an important role in its anticarcinogenic properties. Options for improving the organoleptic characteristics and bioavailability of TB aiming at its clinical application are being investigated, e.g., the development of structured lipids. In addition, TB may be used alone or in combination with other compounds as adjuvant therapy alongside conventional cancer treatments. Therefore, the use of TB may become a promising strategy in the fight against cancer. More preclinical and clinical studies should be conducted using TB to better elucidate its anticarcinogenic potential.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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REFERENCES

Anticarcinogenic Actions of Tributyrin


Ong TP, Heidor R, de Conti A, Dagli ML, Moreno FS. Farnesol and geraniol chemopreventive activities during the initial phases of hepatocarcinogenesis involve similar actions on cell proliferation and DNA damage, but distinct actions on apoptosis, plasma cholesterol and HMGCAR1A reno- expression. Cancer Genesis 2006; 27: 1194-96.


Espíndola RM, Mazzantini RP, Ong TP, de Conti A, Heidor R, Moreno FS. Geranylgeraniol and beta-ionone inhibit hepatic preneoplastic lesions, cell proliferation, total plasma cholesterol and DNA damage during the initial phases of hepatocarcinogenesis, but only the former inhibits nuclear factor-kappaB activation. Carcinogenesis 2005; 26: 1091-9.


Davie JR. Inhibition of histone deacetylase activity by butyrate. J Nutr 2003; 133: 2485S-2493S.


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