Life Sciences, Vol. 27, pp. 1351-1358 Pergamon Press
Printed in the U.S.A.

MINIREVIEW

BUTYRIC ACID: A SMALL FATTY ACID WITH DIVERSE BIOLOGICAL FUNCTIONS

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Summary

Butyric acid, a 4-carbon fatty acid, affects morphology, growth rate and gene expression in mammalian cells in culture. Sodium butyrate (0.5 to 3 mM) produces reversible growth inhibition in several mammalian tumor cells in culture, but it causes cell death only in human neuroblastomas and human glioma cells in culture. Sodium butyrate in combination with currently used tumor therapeutic agents produced a synergistic, an additive or no effect on growth of mouse neuroblastoma cells and rat glioma cells in culture. At least in NB cells, the cell death and growth inhibition may be related to the reduction in anaerobic glycolysis. Sodium butyrate increases the expression of one or more differentiated functions in mouse NB cells, mouse erythroleukemic cells, human epidermoid carcinoma, human colon carcinoma cells and Chinese hamster ovary cells. The induction of differentiation by butyrate may in part be related to an increase in the cellular cyclic AMP level. Sodium butyrate increases the activities of several enzymes, whereas, it decreases the activities of some. The increase of some enzymes appears to be correlated to hyperacetylation of histones. *In vitro* studies suggest that sodium butyrate may be useful in the management of neoplasms by causing selective cell death, and/or cell differentiation and by increasing the cell killing effect in conjunction with currently used tumor therapeutic agents. Sodium butyrate can also be used as a tool to study the regulation of gene expression in mammalian cells.

Butyric acid, a 4-carbon fatty acid, occurs naturally in the body and is formed by the hydrolysis of ethylbutyrate. Recent studies on mammalian cells in culture show that this fatty acid affects morphology, growth rate and gene expression. The first review (1) on the effect of sodium butyrate on mammalian cells in culture appeared in 1976. Since then, many new studies revealing new functions of butyric acid have been published. For example, *in vitro* studies suggest that butyric acid can be used as an anticancer agent, as an adjuvant in combination with current tumor treatment modalities, and as a biological tool in the study of

0024-3205/80/411351-08$02.00/0

regulation of gene expression. The purpose of this minireview is to discuss recent data, and to point out the unique role of sodium butyrate in a variety of biological studies.

In 1967 Pace et al. (2) were first to show that sodium butyrate at higher concentrations (10 mM and above) is toxic to human cells in culture. This study on the effect of butyric acid began with the investigation of the role of adenosine 3',5'-cyclic monophosphate (cyclic AMP) in mammalian cells in culture, using N^6O_2'-dibutyryl adenosine 3',5'-cyclic monophosphate (dibutyryl cyclic AMP). Since butyric acid is formed in the solution of dibutyryl cyclic AMP, it became customary to use sodium butyrate as a control whenever the effect of dibutyryl cyclic AMP was studied. However, it was found that sodium butyrate produces many effects on mammalian cells in culture, some of which are unique to this agent, whereas, others are similar to those produced by dibutyryl cyclic AMP. The effect of sodium butyrate on mammalian cells in culture markedly varies depending upon the cell type. The role of sodium butyrate in regulating the biological functions of mammalian cells in culture will be discussed under the following categories: (a) sodium butyrate as a tool in the study of regulation of gene expression; (b) sodium butyrate as a potential anticancer agent; and (c) modulation of the effect of pharmacological agents by sodium butyrate.

(a) Sodium Butyrate as a Tool in the Study of Regulation of Gene Expression

The treatment of mammalian cells in culture by sodium butyrate increases the activities of several enzymes. These include tyrosine hydroxylase, choline acetyltransferase, acetylcholinesterase, catechol-o-methyltransferase and adenylate cyclase in NB cells (1); sialyl transferase in Hela cells (3), alkaline phosphatase in Hela cells (4-5); CMP-sialic acid:lactosylceramide siolosyltransferase in Hela cells (6); guanosine and adenosine monophosphate kinases, adenine and hypoxanthine phosphoribosyltransferase, guanase, adenosine deaminase and adenosine kinase in human colon cancer cells (7). Sodium butyrate inhibits lactate dehydrogenase and pyruvate kinase activities in neuroblastoma cells (8); hexokinase and glucokinase activities in hepatoma and normal liver cells (9); and tyrosine transaminase activity in hepatoma cells in culture (10). In addition, butyrate stimulates ganglioside G_{M3}1 biosynthesis (3,11), causes increased production of gonadotropins (12-13), induces new β-adrenergic receptors and their coupling to adenylate cyclase (14-15), and new cholera-toxin receptors in Hela cells (16). In addition, sodium butyrate (1 mM) increases the synthesis of prostaglandin D_2, E_2 and $F_{2\alpha}$ (17).

Some of the sodium butyrate-induced alterations in gene expression may be due to its effect on the translational level, whereas, others may be due to its effect on the transcriptional level. The latter is supported by the observations that sodium butyrate increases acetylation of histones in a variety of mammalian cells in culture, due to an inhibition of deacetylase activity (18-21). It has been proposed that acetylation of histones provides an enzymatic mechanism of modulation of interaction between histones and DNA, and that increased acetylation releases constraints upon the DNA template, and alters the struc-

ture and function of chromatin (22-23). Some of the sodium butyrate-induced increase in enzyme activities may be due to hyperacetylation of histones by butyrate. That sodium butyrate-induced hyperacetylation of histones causes an increase in transcription is further supported by the following observation (24). Hyperacetylation of histones, particularly of H_3 and H_4, was observed in sodium butyrate treated Friend erythroleukemic cells in culture. This was associated with an increase in the accumulation of about 38% new RNA transcripts synthesized from unique sequences of mouse DNA. These new RNA transcripts were not present in control cells. In addition, sodium butyrate-treated cells contained many new species of proteins which were not present in control cells (24). These data show that butyric acid may be a useful tool in the study of regulation of gene expression and enzyme induction in mammalian cells. The reasons for sodium butyrate-inhibition of certain enzyme activities are unknown.

(b) Sodium Butyrate as a Potential Anticancer Agent

An ideal tumor therapeutic agent must satisfy the following conditions: (a) it should be nontoxic to normal proliferating cells; and (b) it kills and/or differentiates tumor cells in large numbers either directly or by stimulating the host's immune system. At present, most of the currently used tumor therapeutic agents do not possess the above properties of an ideal tumor therapeutic agent. There is no doubt that extensive use of multiple drugs and/or ionizing radiation has produced an increased number of 5-year survivors in certain types of human tumors, but the risk of second tumors and other toxic side effects of treatment remains in these survivors. Therefore, the present use of aggressive therapy involving near lethal doses of immunosuppressive agents cannot be considered desirable. Recent studies indicate that sodium butyrate possesses both properties of an ideal tumor therapeutic agent.

Cell lethality and inhibition of cell division - Sodium butyrate (0.5-2.5 mM) causes reversible growth inhibition in a variety of mammalian tumor cells in culture (2,6,9,25-39). Among cell lines studied, only mouse neuroblastoma cells (25-26), human neuroblastoma cells (27) and human astrocytoma cells (28) in culture show a varying degree of cell lethality after treatment with sodium butyrate (0.5-1.0 mM). However, sodium butyrate (2.5-5.0 mM) does not inhibit the growth of the rat myoblast line L6D (40). Sodium butyrate at a lower concentration (1.0 mM) does not inhibit the growth of slow growing hepatoma (8999) cells in culture (9), XC rat sarcoma cell line (41), and mammary tumor cells in vivo (42). From these data it appears that sodium butyrate can cause lethality, and/or reversible growth inhibition in many mammalian tumor cells in culture. The extent of the effects may depend upon the concentration of sodium butyrate, and type of tumor cells. The mechanism of sodium butyrate on cell lethality or growth inhibition is unknown. The data obtained from mouse neuroblastoma suggests that the sodium butyrate-induced cell lethality and growth inhibition may in part be due to an inhibition of anaerobic glycolysis (8). Sodium butyrate inhibits lactic acid dehydrogenase, a rate limiting enzyme in anaerobic glycolysis (converts pyruvate to lactate). A previous study has shown (43) that neuroblastoma cells in culture depend heavily on

anaerobic glycolysis because DL-glyceraldehyde which is an inhibitor of anaerobic glycolysis, inhibited the growth of mouse NB cells more markedly than Chinese hamster ovary cells, or baby hamster kidney cells; although it inhibited the anaerobic glycolysis to the same extent in all cell types. Based on this, we have proposed a working hypothesis that sodium butyrate-induced cell lethality may, in part, be due to the inhibition of anaerobic glycolysis in those cells which rely primarily on this pathway as a source of energy. The fundamental question arises as to whether the cells relying primarily on anaerobic glycolysis exist in tumor tissue. It is well established that varying proportions of hypoxic tumor cells exist in a tumor mass, and they must rely on anaerobic respiration for survival. However, the dependence of these hypoxic tumor cells on anaerobic glycolysis may be a transient adaptive phenomenon, since these cells can shift to aerobic glycolysis when they enter the cell cycle following a proper stimulus. The tumor cells may also rely anaerobic glycolysis for survival because of mutational events. Indeed, Warburg suggested that the high rate of anaerobic glycolysis in vitro is a unique requirement of neoplastic cells. However, his hypothesis has been a subject of controversy since it was originally proposed. The fact that DL-glyceraldehyde, a potent glycolytic inhibitor (44), reduces the growth of many types of neoplasms (45-49) supports this hypothesis. However, some studies show that this metabolic anomaly is not due to a fundamental difference between normal and tumor cells, but merely reflects their particular growth rate in vitro (50-55). This general statement has been disputed by another study (43) in which it was found that neuroblastoma cells have a much higher rate of glycolysis than Chinese hamster ovary (CHO-K) cells, although the former cell type has a longer doubling time than the latter. We suggest that some of these differences in results may be due to the fact that the number of cells relying primarily on anaerobic glycolysis for survival may vary from one tumor type to another and from one individual to another for the same tumor type. If this is the case, the effect of sodium butyrate and possible effect of other inhibitors of anaerobic glycolysis would vary depending upon the number of cells relying on anerobic glycolysis for survival.

Cell differentiation - In addition to cell death, sodium butyrate can exert its antitumor effect by inducing morphological and biochemical differentiation in certain tumor cells. Sodium butyrate causes morphological and biochemical differentiation in neuroblastoma cells in culture (1). Unlike human neuroblastoma cells in culture (27), mouse neuroblastoma cells do not show morphological differentiation (26) after treatment with sodium butyrate. However, mouse NB cells do exhibit an increase in biochemical differentiated functions (1). For example, sodium butyrate (0.5-1.0 mM) treated mouse NB cells exhibit the following features: Increase in size of soma and nucleus associated with a rise in total RNA and protein contents; elevation in tyrosine hydroxylase, choline acetyltransferase, acetylcholinesterase, catechol-o-methyltransferase, and adenylate cyclase activities; and elevation of the intracellular level of cyclic AMP which is not due to the inhibition of cyclic AMP phosphodiesterase activity.

Sodium butyrate also induces one or more differentiated functions in erythroleukemic cells (35,56), human epidermoid carcinoma (6), human colon carcinoma cells (39), and in Chinese hamster ovary cells (33). However, it delays the expression of differentiation in myoblast (L6 line) cells in culture (34). Sodium butyrate (3.0 mM) reversibly inhibits the formation of myotubules without affecting the normal program of biochemical differentiation (57). This effect is not related to the reduction of cell growth, but may be due to the modification of cell membranes by butyric acid (57).

The exact mechanism of sodium butyrate-induced cell differentiation is unknown. From the studies on neuroblastoma (1), erythroid lekemia (58) and Chinese hamster ovary cells (33), it appears that sodium butyrate-induced cell differentiation may be related to a rise in cellular cyclic AMP. The rise in the intracellular level of cyclic AMP after treatment with sodium butyrate is not seen within a few minutes of treatment, rather it is seen after a prolonged treatment. At least in neuroblastoma cells (1), the rise in cellular cyclic AMP is due to an increase in basal activity of adenylate cyclase in sodium butyrate-treated cells, since the activity of cyclic AMP phosphodiesterase does not change. The effect of sodium butyrate on cell differentiation varies depending upon the cell type.

Clinical trial of sodium butyrate - Dr. Tom Voûte of Amsterdam was first to use sodium butyrate in patients with advanced metastatic neuroblastomas, because of unresponsiveness to current treatment modalities. This study was followed up by Dr. L. Furman of Children's Hospital, Denver, Colorado. Although the clinical effectiveness of sodium butyrate remains to be evaluated at this time, sodium butyrate at high doses (up to 10 g/day) produces no clinically detectable toxicity in patients with neuroblastoma (personal communication, Dr. Voûte). Sodium butyrate is, however, known to produce a bad odor; therefore, this drug may not be socially acceptable.

(c) Modulation of the Effect of Pharmacological Agents and Ionizing Radiation by Sodium Butyrate

Recent studies show that sodium butyrate modulates the effects of a variety of pharmacological agents and ionizing radiation on mammalian tumor cells in culture. The extent of modification depends upon the cell type and upon the particular agent. For example, sodium butyrate (0.5 mM) in combination with x-irradiation, 5-fluorouracil, 1-(2-chloroethyl)-2-cyclohexyl-2-nitrosourea (CCNU), vincristine, adriamycin, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidenone (R020-1724), papaverine and prostaglandin E_1 (PGE_1) produces a synergistic effect on growth inhibition (due to cell death and inhibition of cell division) of mouse neuroblastoma cells in culture (59). The combined effect of sodium butyrate with 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC) or methotrexate was less than additive (59). Sodium butyrate in combination with theophylline produces an effect which is similar to that observed with theophylline alone (59). Sodium butyrate and cyclic AMP stimulating agents (Prostaglandin E_1, papaverine, theophylline and R020-1724) caused a reduction in growth (Primarily due to inhibition of cell division) when added

individually to human amelanotic melanoma cells in culture (60). However, the combination of sodium butyrate (1 mM) with one of the cyclic AMP stimulating agents produced a synergistic effect on growth inhibition (Primarily due to cell death).

Sodium butyrate also enhances the sensitivity of MSV transformed murine cells to interferon, but does not influence the response of normal cells (61). This phenomenon could be attributed in part to the butyrate-induced elaboration of the microtubules and microfilaments network (62). However, the exact mechanism of the modification of the effect of pharmacological agents by sodium butyrate on mammalian tumor cells in culture is unknown. It is also unknown whether sodium butyrate can modify the effect of pharmacological agents *in vivo*. If it does, the addition of a nontoxic physiological substance such as butyrate may markedly enhance the therapeutic effectiveness of current tumor therapeutic modalities. Systematic preclinical studies are needed to substantiate the observations made in *in vitro* systems.

Activation of viruses - Sodium butyrate (3 mM) is a powerful inducer of the Epstein-Barr virus (EBV) in the P3HR-1 Burkitt lymphoma line (63). Retinoic acid reduces the butyrate induction of the early antigen and viral capsid antigen by 26-41% (64). At this time it is not clear whether activation of EBV viruses by sodium butyrate occurs only in transformed lymphocytes or whether sodium butyrate would enhance the EBV-induced transformation rate of normal lymphocytes by activating the viruses. If the former event occurs, the potential usefulness of sodium butyrate as a tumor therapeutic agent is not compromised. However, if the latter event occurs, sodium butyrate may not be useful in the management of tumors like Burkitt's lymphoma.

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