Efficacies of plant phenolic compounds on sodium butyrate induced anti-tumour activity*

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The ability of the differentiation inducing agent sodium butyrate (NaB) alone or combined with plant-derived phenolic compounds to produce growth inhibition in human erythroleukemic cells was investigated. As a single agent, curcumin produced a marked inhibition of proliferation indicated by its low concentration used. The effect of phenolics on the cell cycle could probably contribute to the augmented antiproliferative activity of NaB. The present data show that quercetin produced synergistic effect in terms of cell killing in association with NaB. Both curcumin and ferulic acid potentiated NaB-induced reduction of cell number. When NaB was added before exposure to graded doses of quercetin it did induce a greater inhibitory effect. The combination of NaB and quercetin seems less effective on S180 ascites tumour cells. As a single agent quercetin was found to be the most efficacious on S180 tumour model.

Keywords: Antitumour agents, Erythroleukemic cell, Plant phenolics

Sodium butyrate, a short-chain fatty acid produced in the mammalian gastrointestinal tract from the bacterial metabolism of ingested soluble fiber, is a primary oxidative fuel for colonocytes. Sodium butyrate (NaB) has been shown to inhibit cancer cell proliferation and to induce cellular differentiation and programmed cell death (i.e. apoptosis) in certain cell types. Furthermore, recent studies suggest that derivatives of NaB inhibit the development or progression of breast, colorectal and hematologic malignancies in experimental animal models and kill multidrug-resistant breast, gastric, ovarian and colon cancer cells in vivo. Collectively, these studies suggest that NaB may be an effective chemotherapeutic agent in the adjuvant treatment of multiple types of cancer. Additional advantages are it is not toxic unlike well established anticancer drugs.

Because of their safety and the fact that they are not perceived as “medicine” food-derived products are highly interesting for development as chemotherapeutic agents. Numerous diet-derived agents are included for major cancer targets including breast, prostate, colon and lung. Examples include green and black tea polyphenols, soy isoflavones, curcumin, caffeic acid phenethyl ester (CAPE) etc. Among the various classes, plant phenolics are a class of antitumour agents whose beneficial effects have been characterized in several cell culture and animal cancer models.

The purpose of this study was to investigate NaB along with quercetin (QC), curcumin (CCM) and ferulic acid (FER), QC is a bioflavonoid whereas CCM and FER have characteristics of plant phenolics that are structurally related to 3,4-dihydroxy cinnamic acid. Cytotoxicity was determined against human erythroleukemic cell line and in mice S180 ascites tumour cells in vitro. Anti tumour effects were studied in vivo on S180 ascites tumour model as an extended investigation.

Cell culture — The K562 erythroleukemic cell line was grown as a suspension culture in IMDM medium supplemented with 10% FBS, penicillin (100 IU/ml) and streptomycin (50 µg/ml). Sarcoma 180 tumour cells were grown for a short duration. S180 tumour cells were withdrawn from the ascites fluid of tumour bearing Swiss mice for this purpose.

Cytotoxicity studies — K562 cells (1×10⁵ cells/ml) were incubated with NaB, CCM, QC or FER alone or in combination for 72 hr at 37°C in a humidified 5% CO₂ atmosphere. S180 tumour cells were exposed to
the agent for a period of 4 hr. Cells were washed with cold PBS and centrifuged at 2000 rpm for 10 min. Viable cells were counted by trypan blue dye exclusion.

Cell morphology—May Grunwald Giemsa staining was performed on smears of control and treated cells after the end of treatment. Around 200 cells were scored in each field. Cells in ten fields were counted for morphological observation.

Flow cytometric analysis—Following treatment, the cells were washed twice with ice cold PBS and fixed in suspension in ice cold 70% ethanol and stored at −80°C. The fixed cells were rehydrated in PBS, centrifuged and resuspended in PBS (1 ml) containing 10 μg/ml propidium iodide and 100 μg/ml RNase A. Cell cycle phase distribution and the percentage of cells in each phase were determined with a FAC scan flow cytometer using Cell Fit Software5.

Tumour induction and treatment—The S180 tumour cells were harvested, washed, counted and injected (ip) at 5 x 10⁶ cells/0.2 ml PBS/mouse. Swiss mice were randomized after tumour cell inoculation into control and treated groups. Each group consisted of six mice. Treatment was initiated after 24 hr to S180 cell injected mice on days 1, 5, 9 (intermittent treatment, every 4th day). NaB (50 mg/kg), QC (100 mg/kg), CCM (50 mg/kg), FER (400 mg/kg) were administered by ip route on the day of treatment. The combined treatment groups received both NaB and one of the phenolic compounds. Body weights were recorded regularly. All mice were followed for survival11.

In this study, we compared the patterns of cell proliferation, cell death and cell cycle arrest in established K-562 human erythroleukemic cells. Viability assessment performed by microscopical enumeration in the presence of trypan blue dye. IC₅₀ of QC and CCM are below 10 μg/ml with the exception of FER which shows a higher IC₅₀ value (data not incorporated). These three phenolics are dietary constituents and hence the expected IC₅₀ values should be above 100 μg/ml. Consequently QC and CCM become two of the most important micronutrients in our ongoing study. The IC₅₀ of NaB is within range like any other cancer chemotherapeutic. NaB shows antiproliferative effect right from 24 hr of K562 cell incubation (Fig. 1).

Marked reduction in cell number was observed when QC was combined with NaB (Table 1). NaB was shown previously by earlier workers to cause induction of topoisomerase IIα expression in K-562 cells12. Increased topo IIα expression is associated with enhanced efficacy of topo II-poisoning antitumour drugs such as etoposide13. Because QC is reported as a topo II inhibitor14 we tested it further with NaB, in the present experimental setup. QC was added to K-562 cells keeping a gap of 24 hr or 48 hr after the cells were exposed to NaB so as to give

![Image](https://example.com/image.png)

**Fig. 1**—K562 cells were exposed to different concentrations of NaB for 72 hr (A). Time course for the growth inhibition caused by NaB in K562 cells (B). Cell viability was determined by trypan blue exclusion.

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration (μg/ml)</th>
<th>% Cell kill*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NaB</td>
<td>100</td>
<td>61 ± 0.75</td>
</tr>
<tr>
<td>QC</td>
<td>10</td>
<td>41.4 ± 2.0</td>
</tr>
<tr>
<td>NaB + QC</td>
<td>100 + 10</td>
<td>85.8 ± 3.4</td>
</tr>
<tr>
<td>CCM</td>
<td>2.5</td>
<td>50.9 ± 6.4</td>
</tr>
<tr>
<td>NaB + CCM</td>
<td>100 + 2.5</td>
<td>77.3</td>
</tr>
<tr>
<td>FER</td>
<td>300</td>
<td>26.6 ± 0.1</td>
</tr>
<tr>
<td>NaB + FER</td>
<td>100 + 300</td>
<td>68.3 ± 1.1</td>
</tr>
</tbody>
</table>

Cells were seeded at 1 x 10⁵ cells/ml in IMDM containing 10% bovine calf serum in presence of drugs. The percentage of viable cells was determined at 72 hr of treatment.

*Values are mean of three experiments.
sufficient time duration for the release of topo IIα. However, total cell kill was not observed by sequential QC treatment (Table 2).

On microscopic examination NaB induced differentiation in K562 cells was not observed. Consequently mediation of differentiating effects of butyrate on K562 cell line by phenolic compound did not arise. A higher concentration and longer exposure is required to induce differentiation in these cells.

From the flow cytometric data it is obvious that among the three phenolic compounds FER show G2/M cell cycle arrest to some extent. Otherwise marginal differences in the accumulation of cells in the various phases of cell cycle were observed with the test agents (Fig. 2).

Enhanced response observed for various combinations indirectly points to the possibility that NaB induced effects were brought about by a cooperation between the histone deacetylase inhibitor activity of NaB12 and the ability of the plant phenolics to modulate several critical growth-regulating signaling pathways15.

The studies were extended to S180 ascites tumour model. The tumour appears to be more responsive to plant derived agents. The IC₅₀'s of NaB and FER could not be determined as the S180 ascites tumour cells were exposed for a short duration of 4 hr. QC and CCM showed IC₅₀ of 100 and 7.5 μg/ml respectively. Co exposure was carried out taking of a fixed dose of NaB (400 μg/ml). QC and CCM alone show antiproliferative effects but their combinations with NaB were not effective (Data not shown).

QC prolonged the survival period of tumour bearing Swiss mice significantly when injected three times during the treatment period. However, its combination with NaB was ineffective (Data not shown). CCM, FER so also NaB did not prolong the life span of S180 ascites tumour bearing mice. Although CCM is most effective in vitro its failure in animal tumour model is attributed to its bioavailability. CCM either gets chemically reduced, degraded or forms glucorodinates. CCM needs greater indepth study to be clinically effective. We already made a beginning by making its cyclodextrin inclusion complexes for better therapeutic gains and have obtained promising results. The problem with NaB is its short half life (5 min). It is an ideal candidate for liposomal encapsulation and we propose to undertake suitable formulation.

Thus, on the basis of our in vitro data it is conceivable that CCM show potent antiproliferative effect and is superior to QC and FER in inhibiting the growth of K-562 and S180 tumour cells. QC in combination with NaB is most effective against the leukemic cells. Animal studies indicate that QC has a potential to arrest the growth of S180 ascites tumour.
References


