A Study of a Cancer-Cell Growth Inhibiting Ingredient in Modified Arabinoxylan from Rice Bran (BioBran /MGN-3)

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Introduction

Various cells in the blood are derived from pluripotent haematopoietic cells in the bone marrow, which ultimately differentiate into erythrocytes, leukocytes, platelets, and lymphocytes. During the process of differentiation into leukocytes, haematopoietic cells change sequentially into myeloblasts, promyelocytes, myelocytes, and then metamyelocytes, finally developing into granulocytes including neutrophils, eosinophils, and basophils with their respective functions. Cells can repeat cycles of growth division a finite number of times at each stage before finally becoming terminally differentiated. Normal blood cells maintain a balance between growth and differentiation. However, if cells deviate from such a normal balance or if abnormal differentiation occurs at any stage and cells are unable to reach the terminally differentiated stage, they will become cancerous (leukaemia), where they will remain undifferentiated and continue to grow. If it were possible to discover a way to inhibit the growth of undifferentiated leukaemia cells and induce them to differentiate into terminally differentiated cells, this could provide a therapy for leukaemia.

In the early 1980s, Wang et al. in China reported that treatment of leukaemic cells from leukaemia patients

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with all-trans retinoic acid induced differentiation into normal cells. They used this in treatment of leukaemia although the number of patients was small. In 1988, it was reported that the differentiation inducer vitamin A compound (all-trans retinoic acid: ATRA) alone induced differentiation and allowed complete clinical remission[1]. These reports showed that differentiation-inducing therapy is feasible and highlighted studies of differentiation induction[2]. Complete remission implies that no leukaemic cells can be detected in peripheral blood and that the patient's physical condition returns to normal. This was tested in France[3], the U.S.[4] and Japan[5] again and the results were confirmed. However, acute promyelocytic leukaemia accounts only for 15% of all leukaemias. A differentiation inducer effective against all types of leukaemia as well as other types of cancer is needed.

The differentiation of leukaemic cells has been actively studied to understand the mechanism of leukaemia development and the nature of the cells and to develop therapies. Many types of leukaemic cell lines with different modes of differentiation have been established. Representative examples include HL60 (derived from acute promyelocytic leukaemia cells), K562 (from chronic myelocytic leukaemia cells), and U937 (from monocytic leukaemia cells). Growth potential and tumour-forming ability decrease depending on the level of differentiation and are completely lost in some cell lines. Differentiation inducers reported to date include vitamin A compounds, carcinogenesis promoters such as TPA (12-o-tetradecanoyl phorbol-13-acetate)[6], vitamins such as active vitamin D3 (1 α ,25-dihydroxy vitamin D3)[7] and vitamin K_2[8], polar compounds such as DMSO (dimethylsulfoxyde)[9], cytokines including rTNF- α (recombinant tumour necrosis factor- α)[10], camputothecin[11], daidzeinn[12], VP16 (4'-dimethylepipodophyllo-toxin ethyliden- β -D-glucoside)[13], bufalin[14], GGA(geranyl gerranyl acetone)[14], and actinomycin D[15].

Treatment with a growth/differentiation factor for normal haematopoietic stem cells causes normal stem cells to differentiate as they grow. This suggests that a coordinate regulatory mechanism is involved in the growth and differentiation of normal stem cells.[16] In leukaemic cells, however, the mechanisms of growth and differentiation are abnormal and uncoordinated, and growth becomes separated from differentiation in many cases[16-18]. The aforementioned differentiation inducers, such as TPA and active vitamin D3, are considered to cause differentiation of leukaemia cells through their particular differentiation-inducing functions rather than direct inhibition of growth. On the other hand, it has been reported that the addition of GM3 ganglioside to the HL 60 cell line caused inhibition of cell growth and differentiation into monocytes and macrophages[19]. These differentiation inducers are important compounds for differentiation-inducing therapy.

Compounds used in cancer treatment are designed to injure and kill cancer cells and many conventional chemotherapeutic agents are reported to induce apoptosis in cancer cells. Apoptotic cells were detected in cancer tissue of patients administered cisplatin, VP16, or retinoic acid[20,21]. Apoptosis is gene-programmed cell death that is not accidental and is reproducible. This cell death is physiologically meaningful and is frequently induced by a certain type of stimulation. Morphologically, the cell volume decreases without destruction of the cell membrane, accompanied by DNA fragmentation, nuclear chromatin aggregation, cytoplasmic swelling, and formation of apoptotic bodies. Subsequently, apoptotic cells are phagocytosed by macrophages and granulocytes and disappear without inducing inflammatory reactions[22]. A differentiation inducer that inhibited cancer cell growth by inducing differentiation and then caused apoptosis could be an ideal therapeutic agent.

In recent years, disorders due to immunodeficiency have increased and foods with biophylactic activity are now considered important for maintaining health. Dietary fibre not only has a mechanical action of

cleansing the gastrointestinal tract but also various physiological actions including an immunomodulatory effect, which is attracting attention. The present study was designed to search for a cell-growth inhibitor in BioBran/MGN-3, a commercially available complex product obtained by partial hydrolysis of a hemicellulose fraction from rice bran with a carbohydrase complex in a culture filtrate of shiitake fungi, a basidiomycete. In addition, the types of cancer cells on which the target compound exerts its effect were examined.

Materials and Reagents

BioBran/MGN-3 powder was provided by Daiwa Pharmaceutical. The human acute promyelocytic leukaemia cell line (HL60) was from Japan Health Sciences Foundation. The human chronic myelocytic leukaemia cell line (K562), liver cancer cell line (HepG2, HLE), pancreatic cancer cell line (PANC1), uterine cervical cancer cell line (HeLa), a colon cancer cell line (SW480), and a normal gingival fibroblast cell line (Gin1) were from Dainippon Pharmaceutical Co., Ltd. The cell culture medium for cell suspension, RPMI1640, was from Sigma and Dulbecco's Modified Eagle's Medium was obtained for adhesive cells. To these media were added foetal bovine serum (Intergen) at a final concentration of 10% and kanamycin at 0.2g/10m*l*. Culture conditions were temperature 37℃, carbon dioxide gas 5%, and humidity 100%.

Commercially available special-grade reagents were used for other reagents unless otherwise specified.

Methods

1. Preparation of BioBran/MGN-3 crude extract ingredient

BioBran/MGN-3 powder was suspended in distilled water at 0.2g/m*l* and stirred for about 30 mins. The suspension was centrifuged at 10,000×g for 20 mins and the supernatant was used as the crude extract. This was freeze-dried and used as the crude extract ingredient.

2. Partial purification of BioBran/MGN-3

BioBran/MGN-3 was suspended in 100% ethanol at 1g/m*l*, stirred for about 30 mins, and centrifuged at 10,000×g for 20 mins to obtain the precipitate, which was then dried and dissolved in distilled water at 0.2g/m*l*. The solution was centrifuged and the supernatant was applied to a Sephadex G-25 column for partial fractionation using distilled water as a separating solvent. The solution was separated into 3 fractions using blue dextran (mw about 2,000,000) and DNP-alanine (mw 269) as markers. The fraction in which blue dextran was eluted was Fraction A and that in which DNP-alanine was eluted was Fraction C. The intermediate fraction between Fractions A and C was Fraction B. These fractions were freeze-dried.

3. Evaluation of growth inhibiting effect

The initial cell density in the culture medium was set at 2.0×10^5 cells/m*l*. Each powdered fraction of BioBran/MGN-3 was dissolved in distilled water at 50mg/m*l* regardless of the crude extract fraction or partially purified fractions. The solution was added at 0-15% of the total volume of each cell culture medium and total volume was adjusted with phosphate buffered saline (PBS) to the same volume. The viable cell count at 3 days of culture was measured with a Cedex Analyze System (Innovatis). Taking as 100% the viable cell count for cell culture medium without addition of fractions of BioBran/MGN-3 (con-

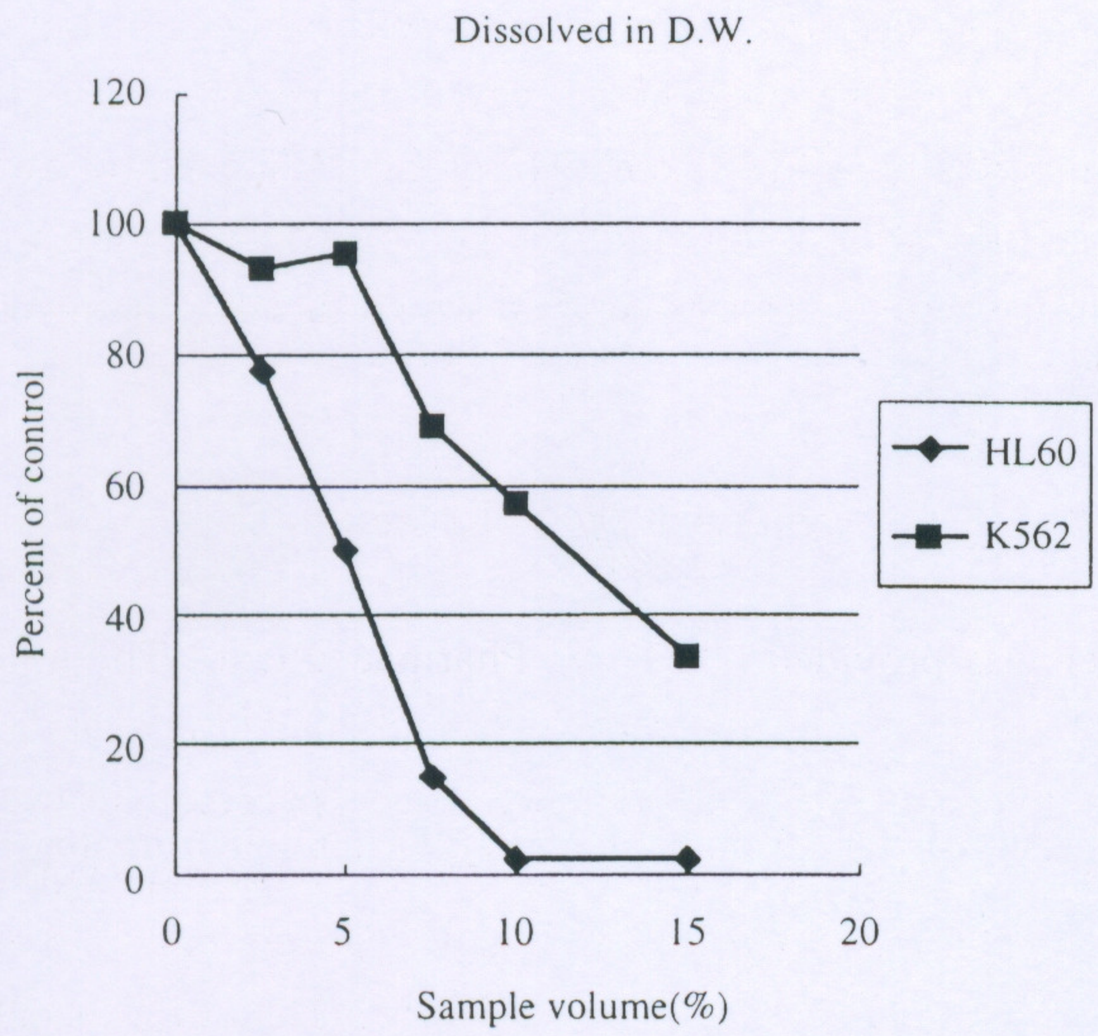

Fig.1 Effects of BioBran on the proliferation of HL60 and K562.

trol), the viable cell count for each added volume was expressed as the relative value and plotted as a graph.

Results and Discussion

After a solution other than culture medium and foetal bovine serum is added to cell cultures, the concentration of the culture medium decreases. The larger the volume of added solution, the lower the required concentration of culture medium. To confirm this, PBS was added to cell culture media and the effect of the additional volume on cell growth was studied. As a result, even if about 35% of the volume of culture medium was added, there was no effect on cell growth without a large difference in growing cell number (data not shown). This indicates that the addition of solutions at 0-15% of the culture medium volume had no effect on cell growth.

Figure 1 shows the results of adding BioBran/MGN-3 crude extract ingredient in distilled water to the HL60 and K562 cell lines in different volumes. The HL60 cell line showed a growth inhibition of about 85% after addition of 7.5% of the total volume (final concentration: 3.75 mg/m*l*), while the K562 cell line displayed growth inhibition of about 60% after addition of 15% of the total volume (final concentration: 7.5 mg/m*l*). Although both cell lines were derived from the same blood cells, the effect of growth inhibition on each was different. This may have been due to the type of cell line, i.e. the original cancer cells.

Next, a fraction obtained by treating BioBran/MGN-3 powder with ethanol was partially purified using Sephadex G-25 column chromatography and the fractions obtained at different molecular weights were investigated for growth inhibitory activity. The yield from 1 g of powder obtained after ethanol treatment was 75 mg for Fraction A, 513 mg for Fraction B, and 138 mg for Fraction C. The cell lines tested were HL60 and K562 and the volumes added were 5% of the total culture (final concentration: 2.5 mg/m*l*) and 10% (final concentration: 5.0 mg/m*l*). **Figure 2** shows the results obtained with the HL60 cell line and

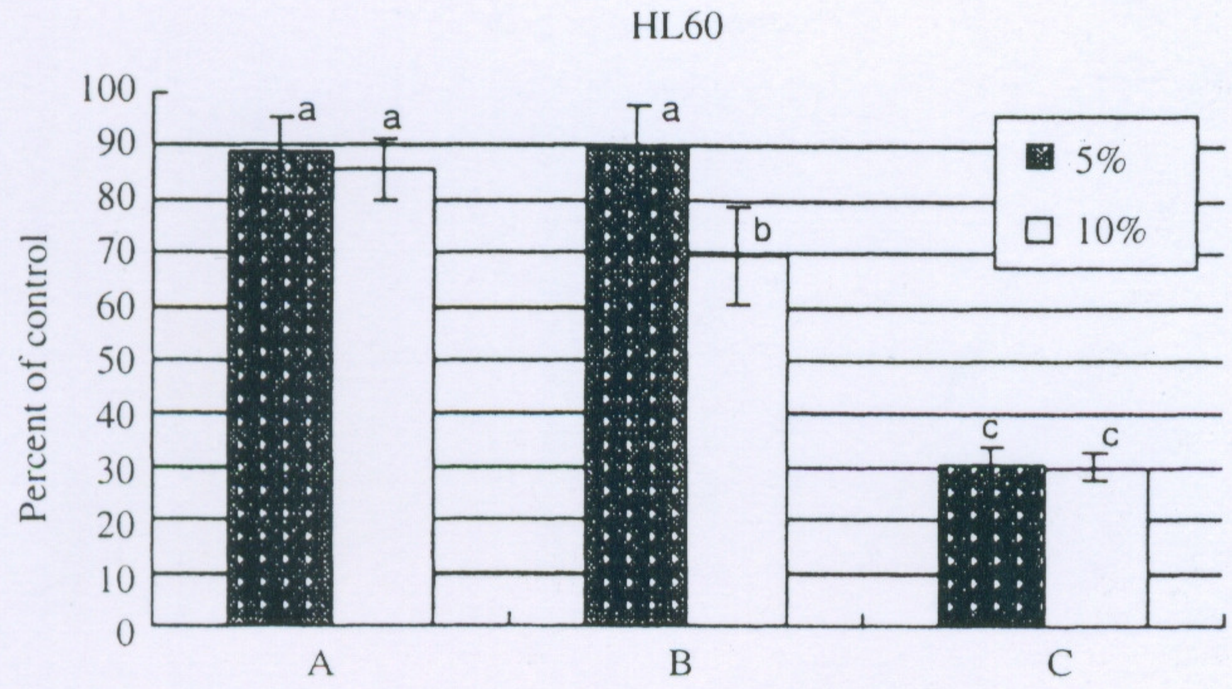

Fig.2 Effects of fractions from Sephadex G25 column chromatography on the proliferation of HL60.

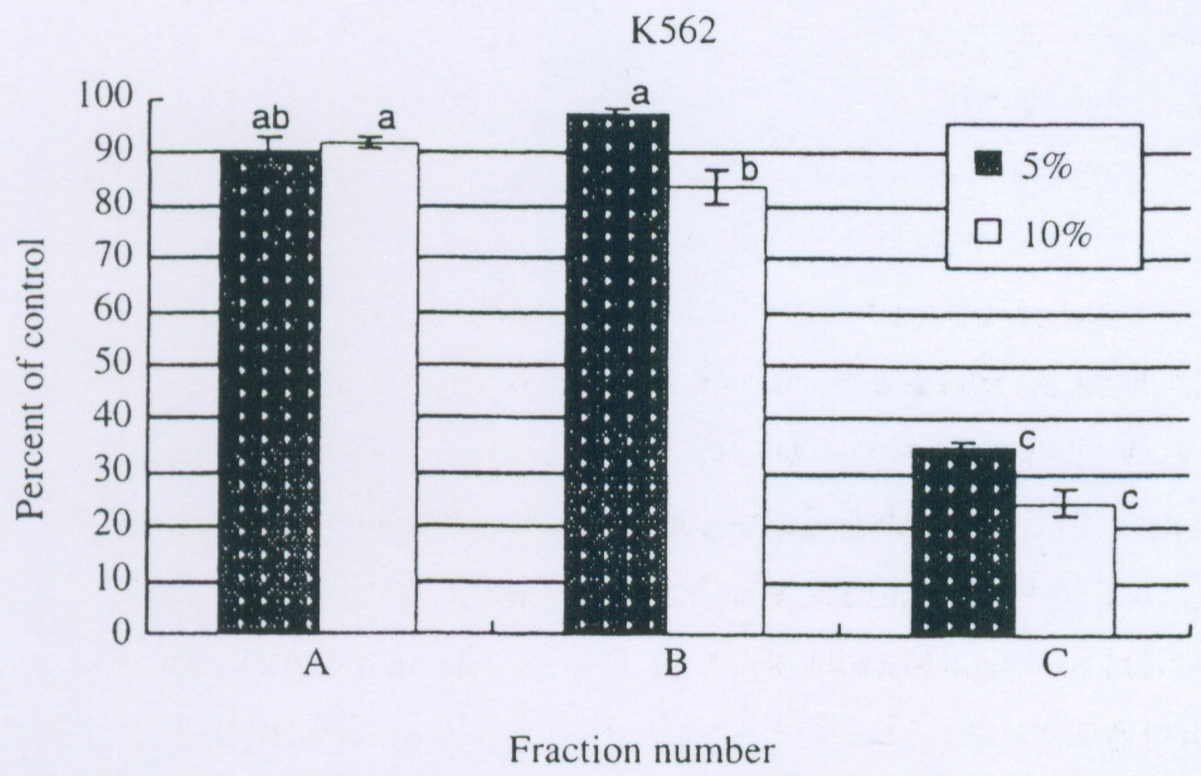

Fig.3 Effects of fractions from Sephadex G25 column chromatography on the proliferation of K562.

Figure 3 those with K562 cells. Both clearly show that Fraction C has a growth inhibitory activity. They also show that other fractions (Fractions A and B) have weak growth-inhibitory action on one cell line. However, the activities of Fractions A and B are very weak compared with that of Fraction C and they have almost no effect on the K562 cell line. The results for the other cancer cell lines, HepG2, HLE, PANC1, HeLa, and SW480, were almost the same although the magnitude of effect varied. Based on these results, Fraction C was used in subsequent evaluations of the cell-growth inhibitory component.

The cell-growth inhibitory effect of Fraction C was studied at concentrations of 0-15% with the above cancer cell lines and normal gingival fibroblast cell line, Gin1. The results in **Figure 4** show that growth inhibition is low in the normal cell line Gin1. This result could be interpreted in two ways: one possibility is that although the effect was weak, Fraction C inhibited the division of normal cells, while the other is

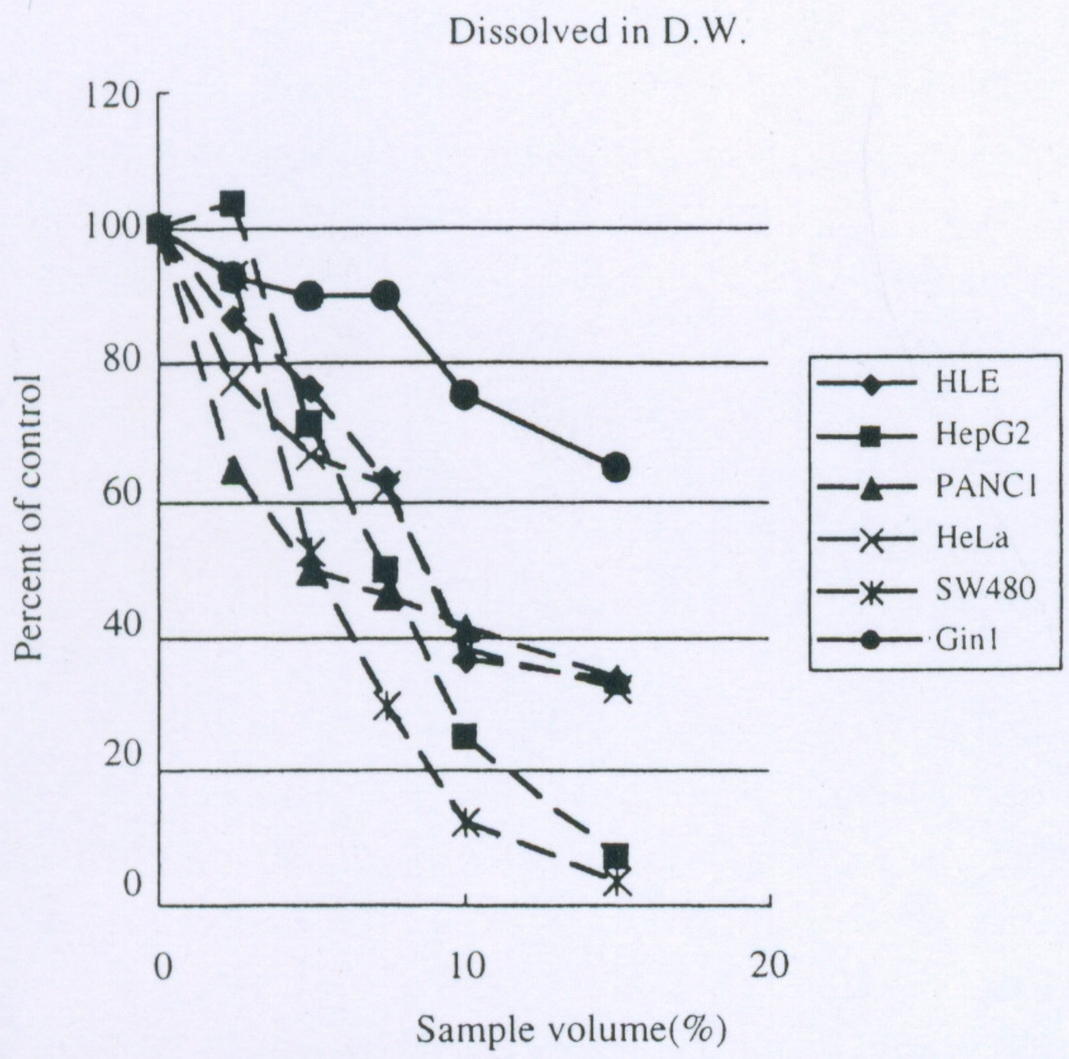

Fig.4 Effects of BioBran on the proliferation of HLE, HepG2, PANC1, HeLa, SW480 and Gin1.

that this normal cell line that exhibits division growth has some characteristics of cancer cells. Lymphocytes from human blood have been cultured with Fraction C but no adverse effects were observed. It cannot be ascertained whether Fraction C inhibits the growth of normal cells from this result, because although lymphocytes are normal cells, they were non-proliferative. To elucidate this point requires evaluation of the effect on normal proliferating cells. Fraction C had an inhibitory effect on all cancer cell lines studied, although the magnitude varied.

The growth-inhibitory component in Fraction C remains to be studied and identified. However, the results of the present study, together with fragmentary results obtained previously, show that the growth-inhibitory material mainly contains sugars or is a low-molecular weight oligosaccharide. The ingredient is likely to have the ability to induce partial differentiation of cancer cells. It is clear that its presence in culture medium inhibits cancer cell growth but it is unknown what effect it may have on the growth of normal cells. When cells with inhibited growth were separated from the culture, washed and recultured in medium without this ingredient, the cells grew at the usual rate.

Even if the substance has only the effects mentioned above, the fact that it can inhibit cancer cell growth and activate immune cells during the inhibition suggests a poteintial use in cancer therapy.

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