Modified Arabinoxylan from Rice Bran (BioBran/MGN-3) Beneficial for Weight Loss of Mice as a Major and Acute Adverse Effect of Cisplatin

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Alkylating agents and antimetabolites remain prominent in a wide variety of cancer chemotherapy protocols on the basis of more selective effects on the faster than normal mitotic cycles of malignant cells. Among new platinum-containing anticancer molecules, the lead compound cisplatin (Cis-platinum (II) diammine dichloride) has been known to possess beneficial anticancer properties in terms of specific interaction of cisplatin and DNA strands. Cisplatin (Rosenberg *et al.* 1969) has been encouraged for treatment of head and neck, bladder, and cervical cancers (Loehrer & Einhorn 1984), as well as breast cancer (Smith & Talbot 1992). Although the prevalent incidence of lung, gastric, breast, colorectal and prostate cancers place them in top ranks of the most common cancers in the civilized countries, many of these advanced cancers are unresponsive to chemotherapy. Platinum-based drugs would be a potent choice in these situations, but they frequently cause substantial side effects, such as nausea, vomiting, nephropathy and hypomagnesaemia due to damage of renal tubules (Lajer & Daugaard 1999). Furthermore, in addition to hearing loss and peripheral neuropathy, myelosuppression is one of the most devastating suppressive side-effects (Prestayko *et al.* 1979) leading to immunocompromized states. Therefore, any reduction of

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the side effects of cisplatin would be valuable. Thus, we explored the effect of BioBran/MGN-3 on protection of weight loss of mice under tolerable maximal doses of cisplatin. Others have briefly commented on the antistress and antifatigue effects of fermented rice bran (Kim *et al.* 2001) or some beneficial effect of BioBran/MGN-3 on some adverse actions of anticancer drugs in rats (Jacoby *et al.* 2000).

The experiment protocol was approved by the Animal Research Ethics Board at McMaster University in Ontario, Canada and as follows. BALB/c female mice (4 week-old) purchased from Charles River Canada were acclimatized for a week. They were weighed and separated into seven groups of five in accordance with minimal variation of weight difference within each group. The average weight of all mice studied (17.43g with standard deviation±0.51g) at the beginning of the present experiment was designated as 100% of body weight. Five mice were housed in each standard metabolic cage in a conventional air-conditioned room. Free access to lab chow (LabDiet®) and water was provided. Light and darkness cycles were 12 hr.

Cisplatin and dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich (Oakville, Ontario, Canada). Since cisplatin is less soluble in water or phosphate-buffered saline (PBS), DMSO was used as a vehicle for solubilization of cisplatin (0.1% V/V).

Characteristics of BioBran/MGN-3 used in the present experiment were as follows. Defatted extract of rice bran was modified by digestion with glycosidases such as amylase, galactosidase and glucuronidase derived from Shiitake mushroom (*lentis edodes*) mycelia. The end-product of BioBran/MGN-3 was highly water-soluble. It was composed of polysaccharides such as mainly arabinoxylan hemicellulose and of protein (13.2%) determined by the method of Lowry *et al.* (1951). It has been produced by the Daiwa Pharmaceutical Company, Tokyo, Japan and sold by the name of BioBran/MGN-3 (Ghoneum 1998) in North America. The product (US Patent 5560914) was a kind gift from Dr. Hiroaki Maeda of Daiwa Pharmaceutical Company.

One week before cisplatin administration, BioBran/MGN-3 was administered to two groups of mice daily by gavaging in a volume of 0.1 m*l* at a concentration of 10 mg/m*l* of BioBran/MGN-3 (dry weight) in water or by intraperitoneal injection in a volume of 0.1 m*l* at the same concentration of BioBran/MGN-3 in phosphate-buffered saline. The dose of 1 mg of BioBran/MGN-3 per mouse was calculated from that recommended for human usage (50 mg/kg). One shot of cisplatin was administered in a volume of 0.1 m*l* at the concentration of 15 mg/kg of cisplatin in phosphate-buffered saline containing 0.5% DMSO as a vehicle intraperitoneally. Two groups of mice received a gavage of water or intraperitoneal administration of phosphate-buffered saline and one week later cisplatin was administered to the both groups. Mice given tap water for oral administration and mice given phosphate-buffered saline with or without DMSO as a vehicle for cisplatin as intraperitoneal administration groups were designated as three separate control groups. The body weight of individual mouse was monitored daily. In comparison with the average weight of all mice at the start of the experiment at an arbitrary 100%, the percentage of body weight of individual mice was plotted.

Weight loss after intraperitoneal injection of cisplatin occurred next day in both groups with and without administration of BioBran/MGN-3. At the 5th day after cisplatin treatment, the greatest of weight loss was obseved in both groups with or without BioBran/MGN-3 orally as well as intraperitoneally. The most of weight loss occurred in mice given cisplatin without BioBran/MGN-3 administration. Although loss of body weight appeard to be close to 20% of standard body weight of mice in the groups of cisplatin treatment, no mice died, nor did any show diarrhoea or rectal bleeding both frequent side effects of cisplatin.

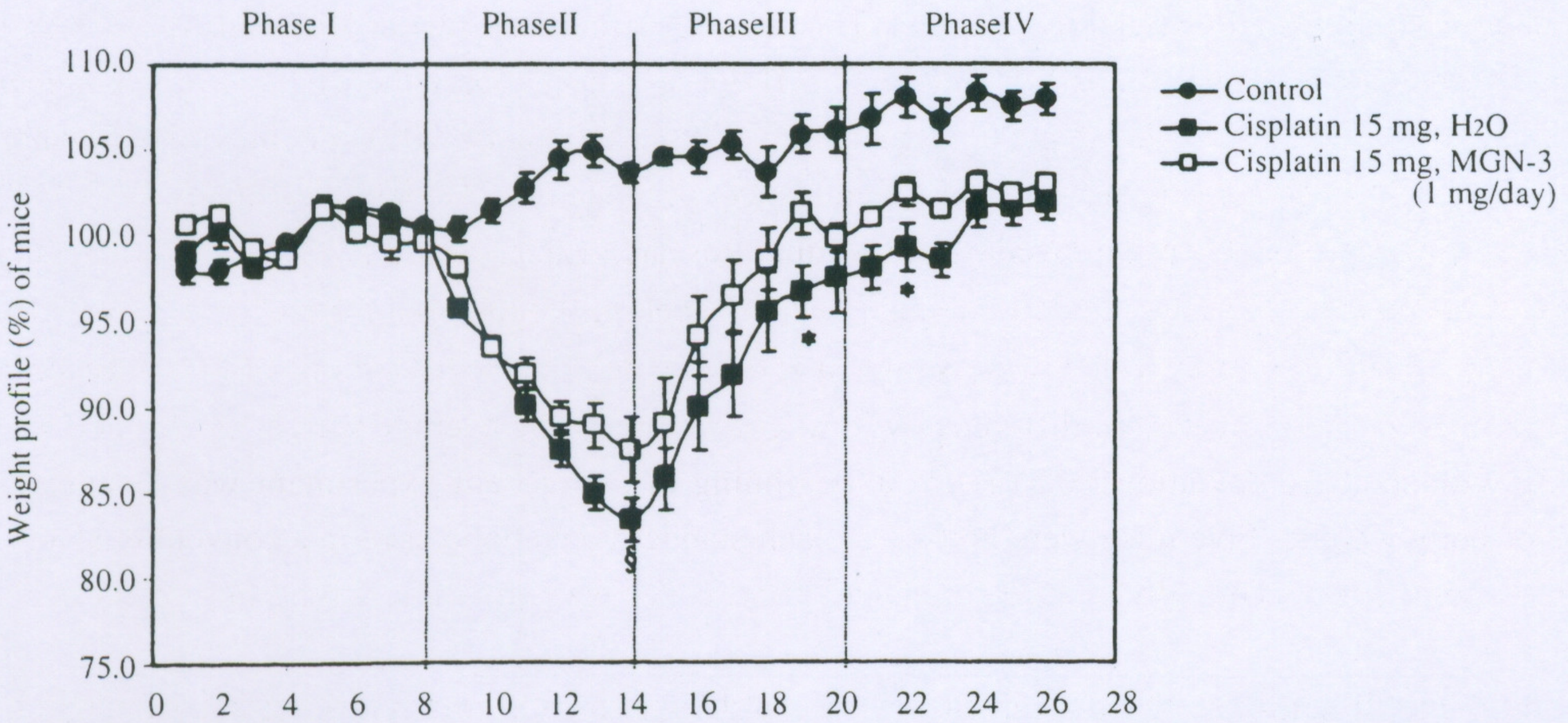

Fig. 1 Comparison of profile of reduced percentage of body weight of mice given cisplatin (15 mg/kg) intraperitoneally on the eighth day with (open rectangles) or without oral intake of MGN-3 (1 mg/day) since the first day (closed rectangles).

Hundred percent of mouse weight of the ordinate means an average weight of whole mice used at the start of the experiment (17.43 g±0.51 g).

Control means neither treatment of cisplatin nor MGN-3.

The abscissa shows experimental days from the start of administration of MGN-3.

Reduced percentage of body weight of mice shows statistically significant differences between the groups with and without oral intake of MGN-3 on the point of maximal reduction of body weight and in the late recovery phase.

Bars = the mean and standard errors of five mice in each group. §P<0.001, *P<0.05.

As shown in **Fig. 1** and **2**, weight gain of mice started on the same day 14 after a shot of cisplatin in both groups and recovery pace of weight gain of groups of mice given BioBran/MGN-3 orally as well as intraperitoneally was faster than that of the groups of control.

The severity of weight loss of mice by cisplatin was dose-dependent (data not shown). BioBran/MGN-3 given orally as well as intraperitoneally showed accelerated protection against severe loss of body weight of mice due to cisplatin. **Fig. 1** shows statistically significant difference between the curve of weight loss due to cisplatin in oral intake group of BioBran/MGN-3 and that in water intake group by ANOVA analysis ($P<0.05$) in phase II, III and IV. **Fig. 2** shows statistically significant difference ($P<0.05$) between the curve of weight loss due to cisplatin in the intraperitoneal administration group of BioBran/MGN-3 and that in phosphate-buffered saline group by ANOVA analysis in phase II, III and IV. There was no significant difference in protective effect of BioBran/MGN-3 on weight loss when the groups of oral and intraperitoneal administration of BioBran/MGN-3 were compared by ANOVA analysis. These results indicate that beneficial substances in BioBran/MGN-3 in terms of protective effect on weight loss of mice due to cisplatin were equally effective by oral administration of BioBran/MGN-3 as by intraperitoneal administration.

In order to detect absorbed compounds with BioBran/MGN-3 in tha serum from the gut mucosa of mice treated with BioBran/MGN-3, we raised polyclonal antibodies against BioBran/MGN-3 in BALB/c mice. A volume of 0.1 m*l* of BioBran/MGN-3 solution (1 mg/m*l*) emulsified with the same volume of complete

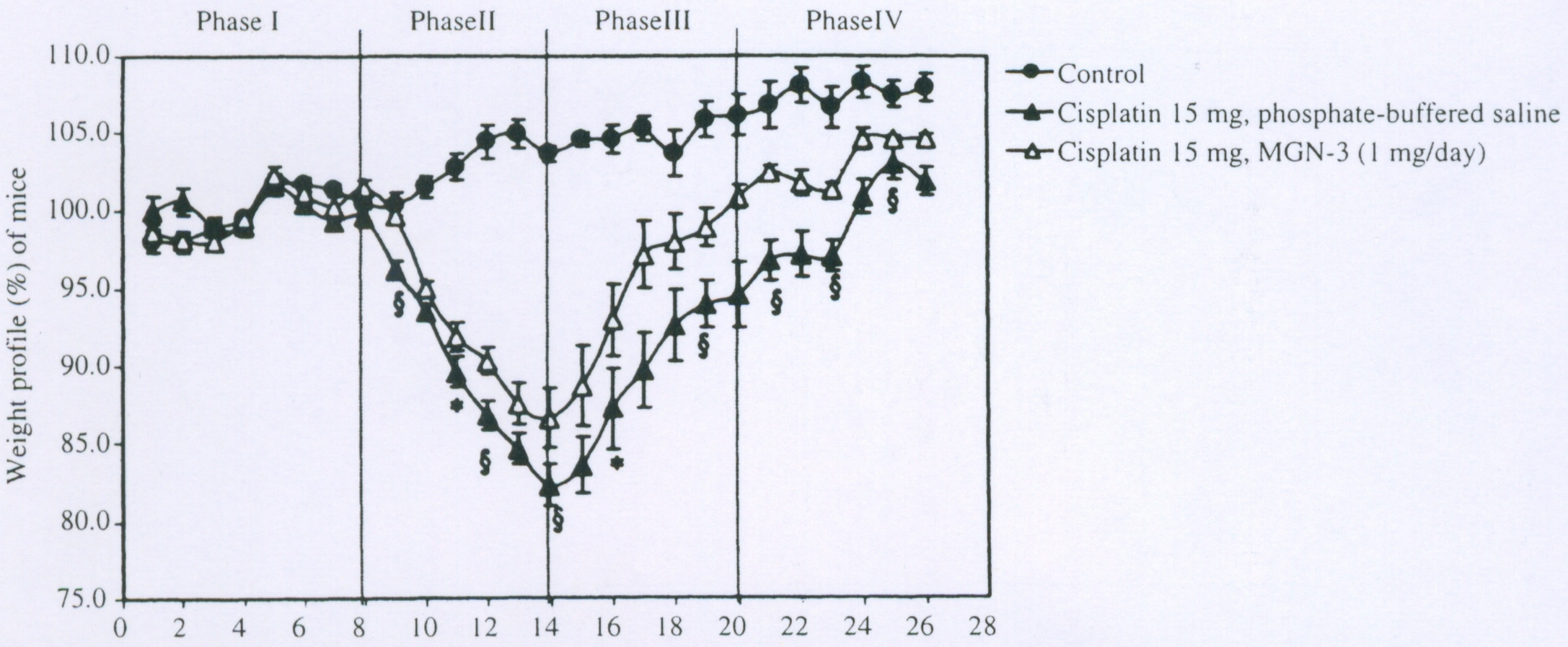

Fig. 2 Comparison of profile of reduced percentage of mouse weight of mice given cisplatin (15 mg/kg) intraperitoneally on the eighth day with (open triangles) or without intraperitoneal administration of MGN-3 (1 mg/day) since the first day (closed triangles).

Hundred percent of mouse weight of the ordinate means an average weight of whole mice used at the start of the experiment (17.43 g±0.51 g).

Control means neither treatment of cisplatin nor MGN-3.

Reduced percentage of body weight of mice shows statistically significant differences between the groups with and without intraperitoneal administration of MGN-3 in the reducing phase after cisplatin treatment and through the recovery phase.

Bars = the mean and standard errors of five mice in each group. §$P<0.001$, *$P<0.05$.

Freund's adjuvant was injected intraperitoneally followed by a boost immunization of 0.1 m*l* of BioBran /MGN-3 solution (1 mg/m*l*) emulsified with the same volume of incomplete Freund's adjuvant. Since BioBran/MGN-3 may be composed of polysaccharides and polysaccharides, immunoreactive substances of both combounds among BioBran/MGN-3 in the serum should be detected in mice treated by BioBran /MGN-3.

To do this, we purchased Covalink microwell plates for quantitation of polypeptides as well as polysaccharides, and conventional polystylene plates (Polysorb) for quantitation of polypeptides from NUNC (Missisauga, Ontario, Canada) for enzyme-linked immunosorbent assay (ELISA) in accordance with the method reported by Zielen *et al.* (1996). To assure that this ELISA system is valid, **Fig.3** shows significant difference in titers of mouse polyclonal antibodies against BioBran/MGN-3 with (+) or without (−) digestion of BioBran/MGN-3 by proteinase K for polypeptides. Peroxidase-conjugated rabbit antibody against mouse IgG antibodies as a second antibody for optical density by 492 nm of wave length by a ELISA reader was purchased from Medical Biological Laboratories (Nagoya, Japan). These data permitted detection of polypeptide as well as polysaccharide compounds of BioBran/MGN-3 on the Covalink microtiter plate.

Immunoreactive compounds of absorbed BioBran/MGN-3 in the mouse serum treated orally by the bran can be detected by absorption procedure of immunological method by using these polyclonal antibodies against BioBran/MGN-3. Thus, immunoreactive substances of BioBran/MGN-3 in sera of mice must be absorbed by the polyclonal antibodies against BioBran/MGN-3 after incubation at 37℃ for 60 min. and centrifugation (15,000 rpm) for 30 min at 4℃. Serial sera were taken from three mice before oral adminis-

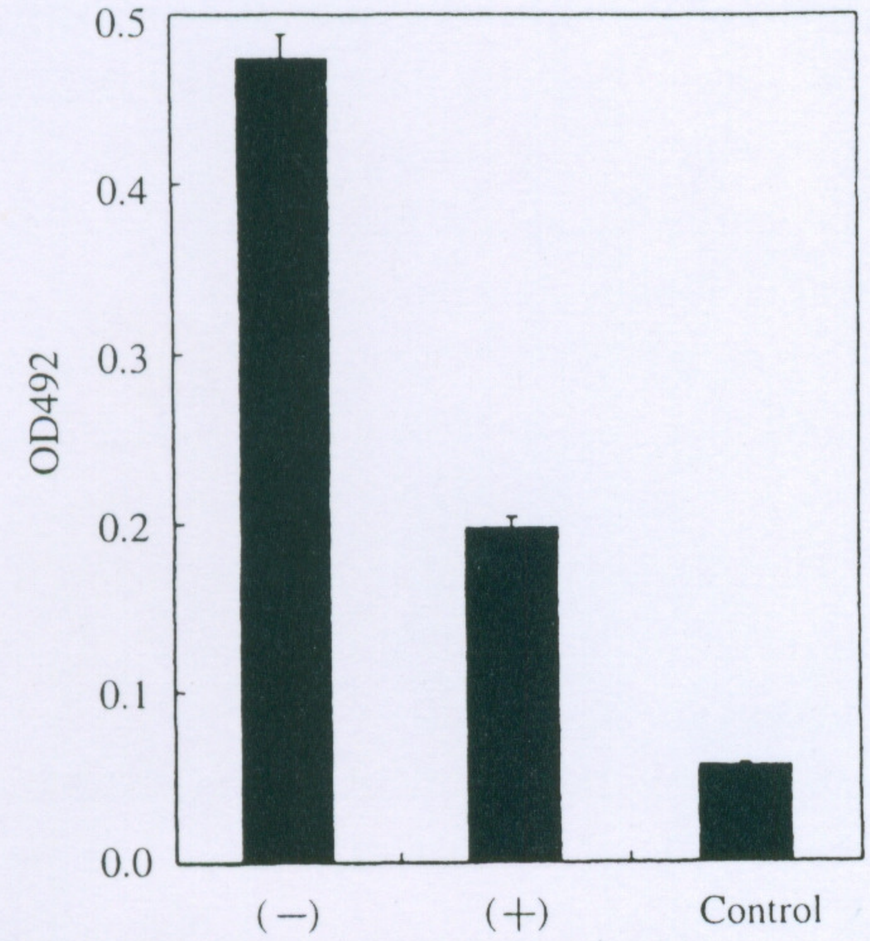

Fig. 3 Polyclonal antibodies against MGN-3 raised by us were tested by ELISA using Covalink microtiter plates.

MGN-3 was digested by proteinase K to eliminate protein antigens.
The left histogram (-) shows titer of polyclonal antibodies against whole MGN-3 without proteinase K digestion and indicates that of both protein and polysaccharide antigens in MGN-3.
The middle histogram (+) shows titer of polyclonal antibodies against MGN-3 after proteinase K digestion and indicates that of only polysaccharide antigens in MGN-3.

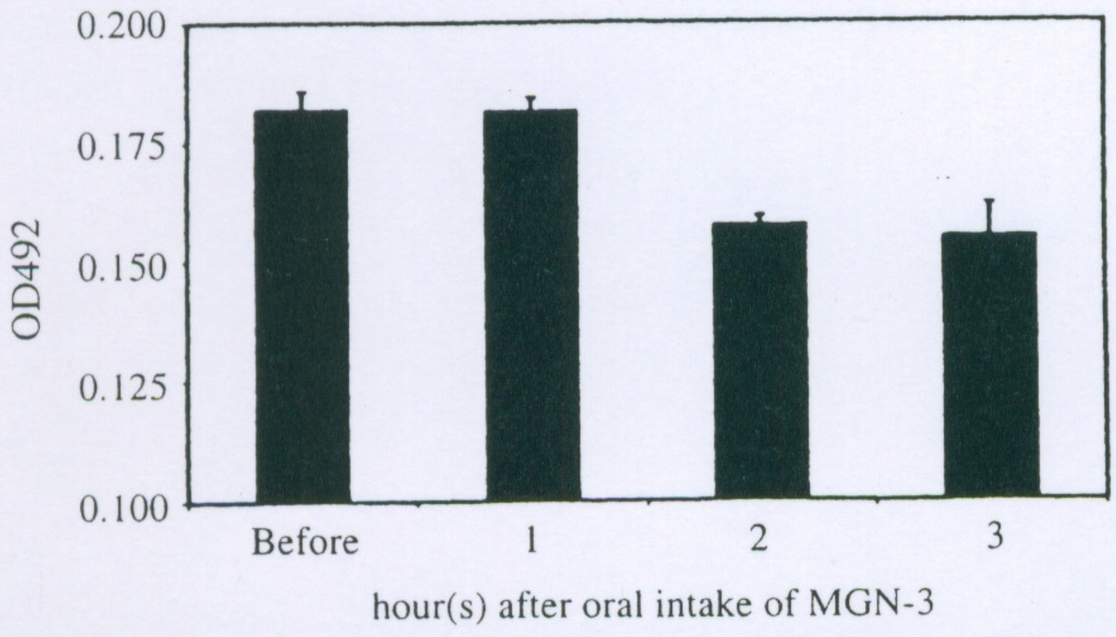

Fig. 4 To summarize absorption data of immunoreactive substances of MGN-3 in the mouse serum after oral intake of MGN-3, OD values before and 1 hr after oral intake are the same level indicating absence of immunoreactive substances of MGN-3 in the serum followed by decrease of OD value indicating existence of them in the mouse serum at 2 and 3 hr after oral intake of MGN-3.

The decrease of serum titers of polyclonal antibodies against MRB was statistically significant (Wilcoxon test $P<0.01$).

tration of BioBran/MGN-3 (10 mg/kg) and three points of time sequence 1 to 3 hr after oral intake of BioBran/MGN-3. Immunoreactive substances of BioBran/MGN-3 in sera of mice treated by BioBran /MGN-3 in two different dilution (100× and 1000×) before and after absorption with the polyclonal anti-

bodies against BioBran/MGN-3 were measured by ELISA in terms of consistency of adsorption procedure for Covalink microtiter plates. **Fig. 4** shows significant decrease of titers of the polyclonal antibodies against BioBran/MGN-3 after absorption with each serum at 2 and 3 hr after oral intake of BioBran/MGN-3. This decrease of titers of polyclonal antibodies against BioBran/MGN-3 was statistically significant (Wilcoxon test $P<0.01$) and indicated that immunoreactive substances of BioBran/MGN-3 must exist in the sera of mice treated orally with BioBran/MGN-3. Whether these substances were the active components responsible for the protective effect on weight loss due to cisplatin remains to be determined. Nevertheless, the present results clearly show that oral intake of BioBran/MGN-3 was effective on reduction of weight loss of mice due to cisplatin. The polyclonal antibodies against BioBran/MGN-3 developed in the present study could identify promising contents of BioBran/MGN-3 closely related to protective function against adverse effect of anticancer drugs.

It has been reported that defatted rice bran hemicellulose increases the peripheral blood lymphocyte count in rats and that the ratio of helper/inducer T cells to suppressor/cytotoxic T showed a decrease in rats given 10% hemicellulose diet (Takenaka & Itoyama 1993). Modified arabinoxylan from rice bran (BioBran /MGN-3) has been shown to enhance the activity of natural killer cells after oral intake of BioBran/MGN-3 in man (Ghoneum 1998). α-Glucan extracted from rice bran by ethanol has potent antitumour activity (Takeo *et al.* 1988), whereas various polysaccharides belonging to β-glucan from mushroom, such as *Lentinus*, *Schizophyllum* and *Grifola*, are resposible for the antitumour effects (Borchers *et al.* 1999).

These reports did not show that components of rice bran in the serum from the gut were absorbed. This is the first demonstration that immunoreactive components of BioBran/MGN-3 in terms of polypeptides and polysaccharides were definitely absorbed from the gut into the blood after oral intake of BioBran/MGN-3.

It has previously been reported that BioBran/MGN-3 is effective in lowering serum lipids and on taste preference in streptozotocin-induced diabetic rats (Ohara *et al.* 2000). This is a potential benefit of BioBran /MGN-3 compated to emetic side effects of cisplatin. Despite not knowing how BioBran/MGN-3 may play a beneficial role in the protection against cisplatin-induced weight loss, our results encourage the performance of a clinical trial of BioBran/MGN-3 as a so-called functional food to verify its protective effect on the quality of life of advanced cancer patients (Harrap 1995). Further characterization of the effective components of BioBran/MGN-3 is needed to achieve the best anticancer effect as well as protective effect against anticancer drugs.

Acknowledgements

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