

Short Communication

Modified Rice Bran 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 the side effects of cisplatin would be valuable. Thus, we explored the effect of modified rice bran 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 modified rice bran 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 (17.43 g with standard deviation ± 0.51 g) 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 modified rice bran 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 modified rice bran 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 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, modified rice bran was administered to two groups of mice daily by gavage in a volume of 0.1 ml at a concentration of 10 mg/ml of modified rice bran (dry weight) in water or by intraperitoneal injection in a volume of 0.1 ml at the same concentration of modified rice bran in phosphate-buffered saline. The dose of 1 mg of modified rice bran 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 ml 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 ga-

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vage 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 modified rice bran. At the 5th day after cisplatin treatment, the greatest of weight loss was observed in both groups with or without modified rice bran orally as well as intraperitoneally. The most of weight loss occurred in mice given cisplatin without modified rice bran administration. Although loss of body weight appeared 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. 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 modified rice bran 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). Modified rice bran 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 modified rice bran 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 modified rice bran 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 modified rice bran on weight loss when the groups of oral and intraperitoneal administration of modified rice bran were compared by ANOVA analysis. These results indicate that beneficial substances in modified rice bran in terms of protective effect on weight loss of mice due to cisplatin were equally effective by oral administration of modified rice bran as by intraperitoneal administration.

In order to detect absorbed compounds with modified rice bran in the serum from the gut mucosa of mice treated with modified rice bran, we raised polyclonal antibodies against modified rice bran in BALB/c mice. A volume of 0.1 ml of modified rice bran solution (1 mg/ml) emulsified with the same volume of complete Freund's adjuvant was injected intraperitoneally followed by a boost immunization of 0.1 ml of modified rice bran solution (1 mg/ml) emulsified with the same volume of incomplete Freund's adjuvant. Since modified rice bran may be composed of polysaccharides and polypeptides, immunoreactive substances of both compounds among modified rice bran in the serum should be detected in mice treated by modified rice bran.

To do this, we purchased Covalink microwell plates for quantitation of polypeptides as well as polysaccharides, and conventional polystyrene plates (Polysorb) for quantitation

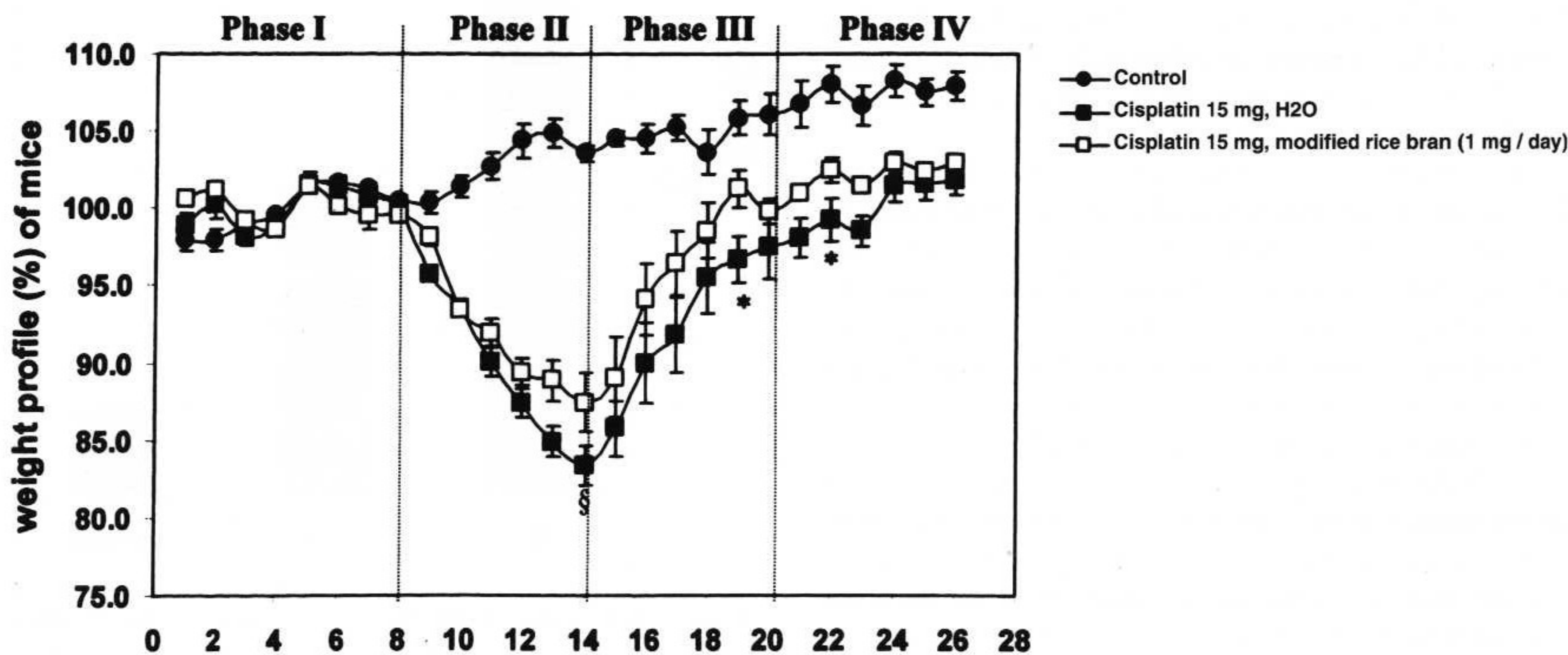


Fig. 1. Comparison of profile of reduced percentage of body weight of mice given cisplatin (15 mg/kg) intraperitoneally on the eighth day with (open rectangles) or without oral intake of modified rice bran (1 mg/day) since the first day (closed rectangles). Hundred percent of mouse weight of the ordinate means an average weight of whole mice used at the start of the experiment ($17.43g \pm 0.51g$). Control means neither treatment of cisplatin nor modified rice bran. The abscissa shows experimental days from the start of administration of modified rice bran. Reduced percentage of body weight of mice shows statistically significant differences between the groups with and without oral intake of modified rice bran 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. $^{\$}P < 0.001$, $^*P < 0.05$.

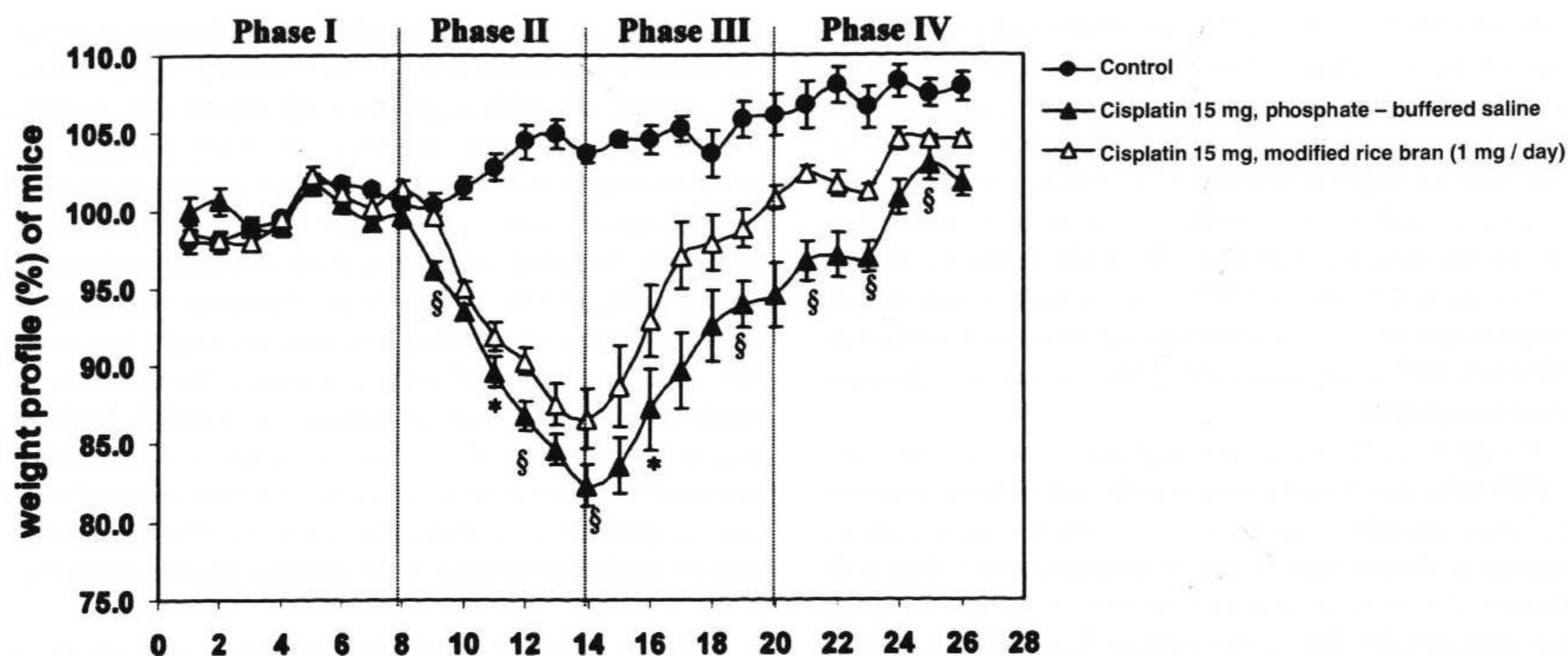


Fig. 2. Comparison of profile of reduced percentage of mouse weight of mice given cisplatin (15 mg/kg) intraperitoneally on the eighth day with (open triangles) or without intraperitoneal administration of modified rice bran (1 mg/day) since the first day (closed triangles). Hundred percent of mouse 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 modified rice bran. Reduced percentage of body weight of mice shows statistically significant differences between the groups with and without intraperitoneal administration of modified rice bran 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.

of polypeptides from NUNC (Mississauga, 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 modified rice bran with (+) or without (-) digestion of modified rice bran 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 modified rice bran on the Covalink microtiter plate.

Immunoreactive compounds of absorbed modified rice bran in the mouse serum treated orally by the bran can be detected by absorption procedure of immunological method by using these polyclonal antibodies against modified rice bran. Thus, immunoreactive substances of modified rice bran in sera of mice must be absorbed by the polyclonal antibodies against modified rice bran 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 administration of modified rice bran (10 mg/kg) and three points of time sequence 1 to 3 hr after oral intake of modified rice bran. Immunoreactive substances of modified rice bran in sera of mice treated by modified rice bran in two different dilution (100× and 1000×) before and after absorption with the polyclonal antibodies against modified rice bran 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 modified rice bran after absorption with

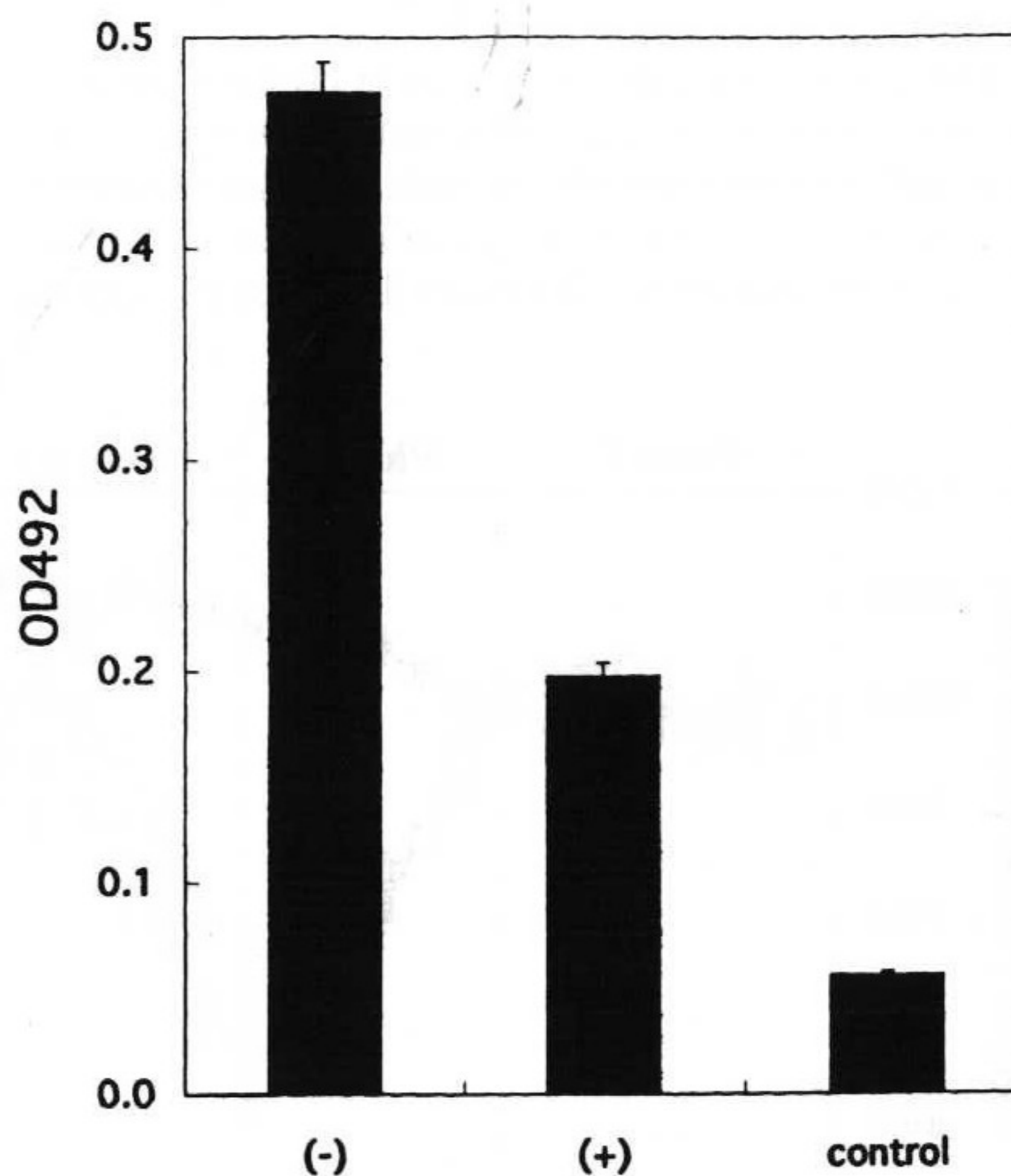


Fig. 3. Polyclonal antibodies against modified rice bran raised by us were tested by ELISA using Covalink microtiter plates. Modified rice bran was digested by proteinase K to eliminate protein antigens. The left histogram (-) shows titer of polyclonal antibodies against whole modified rice bran without proteinase K digestion and indicates that of both protein and polysaccharide antigens in modified rice bran. The middle histogram (+) shows titer of polyclonal antibodies against modified rice bran after proteinase K digestion and indicates that of only polysaccharide antigens in modified rice bran.

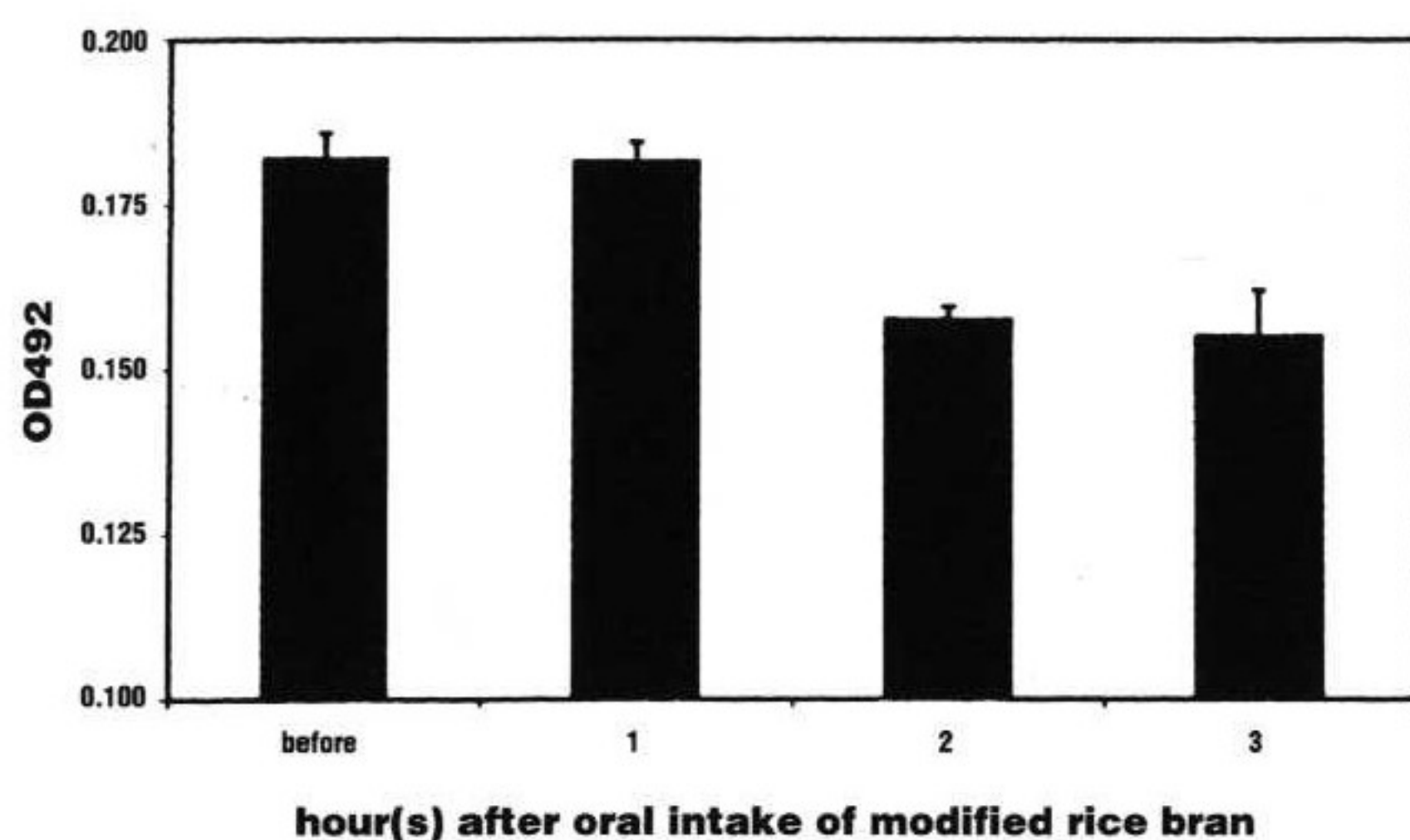


Fig. 4. To summarize absorption data of immunoreactive substances of modified rice bran in the mouse serum after oral intake of modified rice bran, OD values before and 1 hr after oral intake are the same level indicating absence of immunoreactive substances of modified rice bran in the serum followed by decrease of OD value indicating existence of them in the mouse serum at 2 and 3 hr after oral intake of modified rice bran. The decrease of serum titers of polyclonal antibodies against MRB was statistically significant (Wilcoxon test $P < 0.01$).

each serum at 2 and 3 hr after oral intake of modified rice bran. This decrease of titers of polyclonal antibodies against modified rice bran was statistically significant (Wilcoxon test $P < 0.01$) and indicated that immunoreactive substances of modified rice bran must exist in the sera of mice treated orally with modified rice bran. 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 modified rice bran was effective on reduction of weight loss of mice due to cisplatin. The polyclonal antibodies against modified rice bran developed in the present study could identify promising contents of modified rice bran 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 extracted from rice bran has been shown to enhance the activity of natural killer cells after oral intake of modified rice bran (MNG-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 responsible 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 modified rice bran in terms of polypeptides and polysaccharides were definitely absorbed from the gut into the blood after oral intake of modified rice bran.

It has previously been reported that modified rice bran 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 modified rice bran compared to emetic side effects of cisplatin. Despite not knowing how modified rice bran may play a beneficial role in the protection against cisplatin-induced weight loss, our results encourage the performance of a clinical trial of modified rice bran 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 modified rice bran is needed to achieve the best anticancer effect as well as protective effect against anticancer drugs.

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