The Protective effects of Modified Arabinoxylan from Rice Bran (BioBran/MGN-3) against cisplatin

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1. Evaluation of BioBran/MGN-3 on cisplatin-induced emesis in the ferret

(1) Purpose

The objective of this study is to evaluate the potential of using BioBran/MGN-3 for protection against cisplatin induced emesis in the ferret.

(2) Procedure

A group of *Mustela putorius furo*, sable ferrets was received from Marshall Farms. The animals were singly housed, two per cage, in suspended stainless steel caging with mesh floors. Litter paper was placed beneath the cages and was changed at least three times per week. The animal room was temperature controlled and had a 12-hour light/dark cycle. The animals were fed Purina Ferret Lab Diet and filtered tap water was supplied *ad libitum* by an automatic watering system.

Following acclimation to the laboratory for 2 days, 25 healthy male ferrets weighing between 890 and 1130 grams were selected for test and distributed into the following five groups of animals:

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Table 1 : Test Group

Group #	No. of Animals	Test/Control Article	Dose Level of MGN-3 (mg/kg PO)	Dose Level of Cisplatin (mg/kg IP)	Test Suspension Concentration (%)	
					MGN-3	Cisplatin
1	5	Vehicle Control (0.5% CMC in distilled water)	—	—	—	—
2	5	Vehicle Control (0.5% CMC in distilled water)	—	10	—	0.4
3	5	MGN-3	100	10	2.0	0.4
4	5	MGN-3	50	10	1.0	0.4
5	5	MGN-3	20	10	0.4	0.4

PO=oral IP=intraperitoneally

The ferrets were examined for health and weighed. Individual doses for all administrations were calculated based on these bodyweights, taking into account the concentration of the appropriate suspension. Each test group animal was administered the test article or vehicle control daily for 7 days, as described above, by oral intubation using a gavage cannula. The test article was administered as a w/w suspension in a 0.5% w/w solution of carboxymethylcelluose (CMC) in distilled water. All test animals were dosed at a constant volume of 5 ml/kg. The control groups (Group 1 & 2) received 5 ml/kg of a 0.5% w/w solution of CMC in distilled water and were maintained under similar environmental conditions.

Thirty minutes after the last daily dose (Day 7), either the vehicle or 10 mg/kg cisplatin were administered to each animal intraperitoneally. All animals from Groups 2, 3, 4 and 5 were dosed IP cisplatin at a constant volume of 2.5 ml/kg. Group 1 received 2.5 ml/kg of a 0.5% w/w solution of CMC in distilled water. The ferrets were observed for 6 hours after dosing which included at least 1 hour where no signs of emesis were observed (**Table 1**). The number and time of bouts of retching/vomiting (emesis) was noted and the severity of each by noting the number of individual emetic occurrences. Emetic activity was scored by classifying the response of each ferret on the basis of the occurrence of no emesis, mild to moderate emesis (less than 3 occurrences of emesis) and moderate to severe greater than 3 occurrences of emesis.

All animals were observed daily for signs of gross toxicity and behavioral changes. All ferrets were anesthetized using isofluorane following the observation period.

(3) Results and discussion

All of the ferrets in the group receiving vehicle plus cisplatin showed signs of emesis (retching and vomiting) (**Table 2**). This was moderate to severe in four of five ferrets. Surprisingly, the lowest dose of BioBran/MGN-3 (20 mg/kg) showed the most protection against cisplatin with three of five ferrets showing only mild emesis (retching, but no vomiting). This response was statistically different from vehicle + cisplatin. These ferrets appeared to be almost fully protected against the emetic activity of cisplatin. 50 mg/kg BioBran/MGN-3 also appeared to show some protection, with three of five ferrets showing emesis, however in two there was moderate to severe bouts of retching and vomiting. Only two of five ferrets receiving the highest dose (100 mg/kg PO) of BioBran/MGN-3 showed sign of emesis. However, in these two the emetic responses were moderate to severe.

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Overall, BioBran/MGN-3 did protect ferrets from the emetic effects of cisplatin. The most effective dose appeared to be the lowest dose tested. A single dose of BioBran/MGN-3 would be effective and at least for protection form emsis that chronic dosing is not necessary.

BioBran/MGN-3 may be acting as an antagonist to serotonin or neurokinis, both of which are involved with chemotherapy induced emesis. Agents which have this activity are very useful in the treatment of emesis due to chemotherapeutic agents such as cisplatin. It is unknown whether this is a specific activity or wheter BioBran/MGN-3 would also be effective against other emetic agents such as morphine, apomorphine, cholinesterase inhibitors and some phosphodiesterase inhibitors. If it is it may also be useful to counteract the side-effects of agents used in Alzheimer's disease and Perkinson's disease. The antiemetic effect may also be due to an positive effect on the protective mechanisms of the stomach and small intestine. The effect against DSS colitis showed such a protective effect.

Table 2 : Effect of Vehicle and MGN-3 Against Cisplatin Induced Emesis

Treatment	Dose mg/kg PO Daily	Cisplatin 10mg/kg IP N	N	#Ferrets no emesis	#Ferrets Mild-mod Emesis	#Ferrets Mod-Severe emesis
Vehicle	----	–	5	5	0	0
Vehicle	----	+	5	0	1	4
MGN-3	20	+	5	2	3	0*
MGN-3	50	+	5	3	0	2
MGN-3	100	+	5	2	1	2

*Statistically significant difference from Vehicle + Cisplatin group $p < 0.05$ Fisher Exact test

2. Evaluation of the effect of BioBran/MGN-3 on cisplatin-induced toxicity in rats

(1) Purpose

To evaluate the effect of orally administered BioBran/MGN-3 against gastrointestinal pathology produced by a single administration of cisplatin in rats.

(2) Procedure

A group of male Sprague-Dawley rats was received from Ace Animals, Inc., Boyertown, PA. The animals were grouped housed singly in suspended stainless steel wire mesh cages. The animal room was temperature controlled and had a 12-hour light/dark cycle. The animal were fed Purina Rodent Chow #5012 and filtered tap water supplied *ad libitum.*

Following acclimation to the laboratory for 4 days, 50 healthy male rats weighing between 167 and 194 grams were selected for test and distributed into the following five groups of animals:

Individual doses were calculated based on initial body weights. Each test group animal was administered the test article or vehicle control daily for 10 days, as described above, by oral intubation using a gavage cannula. The test article was administered as a w/w suspension in a 0.5% w/w solution of carboxymethylcelluose (CMC) in distilled water. All test animals were dosed at a constant volume of 5 ml/kg. The control groups (Group 1 & 2) received 5 ml/kg of a 0.5% w/w solution of CMC in distilled water and were maintained under similar environmental conditions. Approximately two hours after the Day 7 daily dose, each animal from Group 2 through 5 received a single intraperitoneal injection of 8 mg/kg cisplatin (Sigma Chemical, St. Louis, MO, Lot #014K0993). Group 1 was injected with 5 ml/kg of saline.

All rats were weighed prior to administration (Day 1) and on Days 4, 7, 9 and 11. All animals were

observed for signs of gross toxicity and/or behavioral changes daily (**Table 3**).

Table 3 : Test Group

Group #	No. of Animals	Test/Control Article	Dose Level of MGN-3 (mg/kg PO)	Dose Level of Cisplatin (mg/kg IP)	Test Suspension Concentration (%)	
					MGN-3	Cisplatin
1	10	Vehicle Control (0.5% CMC in distilled water)	—	—	—	—
2	10	Vehicle Control (0.5% CMC in distilled water)	—	8	—	0.16
3	10	MGN-3	100	8	2.0	0.16
4	10	MGN-3	50	8	1.0	0.16
5	10	MGN-3	20	8	0.5	0.16

PO=oral IP=intraperitoneally

On Day 11, all animals were euthanized by CO_2 inhalation. Gross necropsis were performed on all animals. Tissues and organs of the thoracic and abdominal cavities were examined for changes in gross appearance. The entire gastrointestinal tract was evaluated for the presence of damage and the number of lesions found in the stomach, small intestine and colon were counted.

(3) Results and discussion

BioBran/MGN-3 produced significant dose related protection against cisplatin induced gastrointestinal toxicity and pathology (**Table 4**). This did not achieve statistical significance because of the sample size, however, there was a definite dose related protection against cisplatin-induced gastrorintestinal lesions and diarrhea. There were no effects on weigh loss due to cisplatin at any dose of BioBran/MGN-3. BioBran/MGN-3 was an effective protective agent against cisplatin-induced adverse effects in the 50 and 100 mg/kg PO dose groups. However, once a day dosing may not provide sufficient levels for protection against this chemotherapeutic agent, and therefore, the recommendation is to try twice a day dosing starting on the day cisplatin is administered to provide around the clock protection.

Table 4 : Protection of MGN-3 against GI Lesions and Diarrhea

Treatment Daily	Dose mg/kg PO	Cisplatin 8 mg/kg IP	N	#Norm. GI	% Pro	#GI Lesions	% Pro	#Diarrhea	% Pro
0.5%CMC	----	—	10	10	----	0	----	0	----
0.5%CMC	----	+	10	1	----	7	----	7	----
MGN-3	100	+	10	5	56	5	29	1	86
MGN-3	50	+	10	3	33	7	0	4	43
MGN-3	25	+	10	1	0	9	0	4	43

Norm – Normal appearing organs % Pro-percent protection as compared to Group 2

The mechanism of the protective activity may be due to interaction and inhibition of pathological factors released into the gastrointestinal tract by cisplatin and other chemotherapeutic agents. This may be due to some intrinsic effect on the immune system which is adversely effected by most chemotherapeutic agents. The protective effect is not due to interactions with levels of prostaglandins since BioBran/MGN-3 was not

effective in protecting against gastric mucosal lesions produced by a non-steroidal anti-inflammatory agent which inhibits mucosal synthesis of prostaglandins. The gastrointestinal mucosal effect combined with the positive effect against cisplatin-induced emesis suggests its usefulness as a supplement to be given concomitantly with chemotherapeutic agents to relieve some of the disturbing side effects of this therapy.