The Effect of MGN-3 on Cisplatin and Doxorubicin Induced Toxicity in the Rat

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ABSTRACT. MGN-3 (BioBran®) is derived from rice bran and is produced by the partial hydrolysis of the water soluble hemicellulose fraction of rice bran by carbohydrases derived from *Lentius edodes* mycelia. It is a biological response modifier producing an increase in natural killer cell activity in immunocompromised patient. The aim of the study was to evaluate orally administered MGN-3 against gross pathological changes and weight loss produced by a single intraperitoneal dose of cisplatin or doxorubicin by daily oral dosing of 5 or 50 mg/kg MGN-3. Male Sprague-Dawley rats received either vehicle or MGN-3 prior to and after a single dose of cis-platinum or doxorubicin. Rats were observed for clinical signs daily for 11 days and body weights were recorded every other day. All animals were euthanized and necropsied on Day 11. Lethality was observed only in rats receiving cisplatin (50% with cisplatin alone reduced to 10% in rats receiving MGN-3 5 mg/kg, and 40% after MGN-3 50 mg/kg). Rats receiving MGN-3 at 5 or 50 mg plus cis-platinum or doxorubicin had a statistically significant greater weight gain than that observed with the chemotherapeutic agent alone. Rats receiving MGN-3 appeared healthier; gained weight and had a lower incidence of diarrhea and gross intestinal pathology. MGN-3 was effective at maintaining body weight after a toxic dose of either chemotherapeutic agent and protected against gross gastrointestinal pathological changes and diarrhea. MGN-3 may have potential for improving "quality of life" of patients receiving chemotherapy. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: <getinfo@haworthpressinc.com> Website: <http://www.HaworthPress.com>*

KEYWORDS. MGN-3, chemotherapy, chemoprotectants, rats, cisplatin, doxorubicin

INTRODUCTION

Chemotherapeutic therapy for cancer is often associated with adverse effects.[1] Historically, chemotherapeutic agents have been selected on the basis of established toxicity to cancer cell lines and rapidly growing tumors in rodents and not on the basis of sophisticated intervention in tumor specific biology. This strategy usually leads to agents, which have toxicity toward normal cells and tissues that share characteristics with tumor cells, such as high cell turnover. Gastrointestinal mucosal cells are among the most sensitive cells and chemotherapeutic agents often have debilitating effects due to lesions throughout the gastrointestinal tract. This may be manifested as gastrointestinal mucosal lesions and hemorrhage. These often lead to potentially life-threatening hemorrhage and perforation. Because of these, and pathological effects on

other organ systems, there has been great interest in developing concomitant therapy which will protect normal tissue from the effects of chemotherapeutic agents without interfering with their anti-cancer activity.

MGN-3 (BioBran, Lentin Plus) is a hemi-cellulose complex containing arabinoxylane as a major component [US Pat. 5560914].[2] It is a water-soluble product of the hydrolyzing hydrolysis of rice bran using multiple enzymes from mycelia of edible mushrooms. Arabinoxylane is obtained from the enzyme reaction under constant conditions and is highly active and stable. The enzymatic digestion of rice bran to hemicelluloses such as arabinoxylan compounds appears to enhances the intrinsic immune stimulating activity due to their greater water solubility and bioavailability. This product is made by a unique and patented process in which Shitake mushrooms enzymes (hypomycetes mycelia extract) produce a unique and natural blend of hemicelluloses. There is no measurable mushroom content in the end product. The blend of hemicellulose compounds is generically called MGN-3. It is categorized as a food supplement or functional food and is available as a powder, tablet a or capsule in the United States.

MATERIALS AND METHODS

Sprague-Dawley derived, albino rats (Ace Animals, Inc., Boyertown, PA) were singly housed in suspended stainless steel caging with mesh floors. The animal room was temperature controlled and had a 12-hour light/dark cycle. Rats were fed Purina Rodent Chow #5012 and filtered tap water was supplied *ad libitum* by an automatic water dispensing system. Following an acclimation period of 13 days, eighty healthy male rats were selected for test based on body weights and randomly assigned (10 rats/group) to each of the following eight test groups:

GROUP NO.	TEST SUBSTANCE (mg/kg)	CHEMOTHERAPEUTIC AGENT (mg/kg)
1	MGN-3 5 PO	Vehicle
2	MGN-3 50 PO	Vehicle
3	MGN-3 Control	Cisplatin 9 IP
4	MGN-3 5 PO	Cisplatin 9 IP
5	MGN-3 50 PO	Cisplatin 9 IP
6	MGN-3 Control	Doxorubicin 10 IP
7	MGN-3 5 PO	Doxorubicin 10 IP
8	MGN-3 50 PO	Doxorubicin 10 IP

PO–oral, IP–Intraperitoneal

Individual doses were calculated based on the initial bodyweights. The animals from all groups received oral administration of MGN-3 or vehicle control

(water) daily for 11 days beginning on Day 0, using a stainless steel ball-tipped gavage needle attached to an appropriate syringe. The test substance was administered as a 0.1% (5 mg/kg dose) or 1% (50 mg/kg dose) w/w suspension in distilled water. The chemotherapeutic agent (cisplatin or doxorubicin) or vehicle was administered to each animal by a single intraperitoneal injection on day 3 only. Doses of chemotherapeutic agents were chosen based on a pilot study using several different doses of cisplatin and doxorubicin.

All groups of rats were observed for signs of gross toxicity and/or behavioral changes daily for 11 days. Consistency of feces was monitored throughout the study. Body weights were recorded on days 0 (pre-dose of MGN-3), 3, 5, 7, 9 and 11. On day 11, all animals were euthanized by CO_2 inhalation and necropsied. Gross appearances of major organs of the thoracic and abdominal cavities were evaluated and the presence of gastrointestinal damage was noted. The livers from each animal as well as any gross lesions noted during necropsy examination were excised and preserved in 10% neutral buffered formalin.

Histological examinations were performed on the livers of all animals. Eighty slides of rat livers were submitted to Pathco, Inc. for histopathologic examination. The slides were read in a "blind fashion." Following examination, the slides were put into their respective dose groups. All statistical analyses were done using Graph Pad In Stat® (Version 3.00 for Win 95). The statistical significance of body weight changes was analyzed using a one-way Analysis of Variance (ANOVA) and the Bonferroni Multiple Comparison Test for determining group differences with a $p < 0.05$ considered statistically significant. Quantal data such as lethality, lesions and diarrhea was analyzed using a 2×2 contingency table and the chi-square test with a $p < 0.05$ considered significant.

RESULTS

Table 1 shows the effects of treatments on body weight over the duration of the study. Rats receiving either MGN-3 at a dose level of 5 or 50 mg/kg PO for 11 daily doses showed a typical increase in body weight (approximately +72%) while rats receiving cisplatin 8 mg/kg IP or doxorubicin 10 mg/kg IP on Day 3 showed a significantly smaller increase in body weight (−1.5% for cisplatin and +30% for doxorubicin). Rats receiving MGN-3 at 5 or 50 mg/kg PO plus cisplatin or doxorubicin had a significantly greater weight gain than that observed with the chemotherapeutic agent alone. MGN-3 5 g/kg produced a +11% increase in body weight in cisplatin treated rats and a +46% increase in doxorubicin treated rats. MGN-3 50 mg/kg produced a +44% increase in body weight in cisplatin treated rats and a +43% increase in doxorubicin treated rats.

TABLE 1. Effect of MGN-3 on Loss of Body Weight Induced by Cisplatin and Doxorubicin

Treatment	N	Day 0	Day 3	%	Day 5	%	Day 7	%	Day 9	%	Day 11	%
MGN-3 5 mg/kg PO + Vehicle IP	10	156.4 ±1.7	198.8 ±1.8	127.2 ±0.02	216.9 ±2.7	138.8 ±0.02	226.3 ±2.9	144.8 ±0.02	243.6 ±2.9	153.2 ±0.04	269.1 ±3.7	172.2 ±0.03
MGN-3 50 mg/kg PO + Vehicle IP	10	156.5 ±4.2	194.9 ±3.8	124.8 ±0.02	208.8 ±4.2	133.8 ±0.02	219.7 ±4.7	140.8 ±0.03	235.1 ±4.7	148.3 ±0.04	259.7 ±4.7	166.5 ±0.03
MGN-3 Control PO + Cisplatin 9 mg/kg IP	6	161.3 ±0.8	207.0 ±1.3	128.3 ±0.01	184.3 ±2.0	114.3 ±0.01	162.0 ±4.7	100.4 ±0.03	143.2 ±5.9	84.9 ±0.04	159.2 ±10.5	98.5 ±0.06
MGN-3 5 mg/kg PO + Cisplatin 9 mg/kg IP	9	154.0 ±2.9	194.9 ±3.8	126.6 ±0.01	180.4 ±6.7	117.3 ±0.03	169.1 ±12.1	109.8 ±0.08	163.6 ±17.4	104.2 ±0.11	172.2 ±20.0	111.5* 0.13
MGN-3 50 mg/kg PO + Cisplatin 9 mg/kg IP	7	157.4 ±3.2	197.9 ±4.1	125.7 ±0.02	201.0 ±6.6	128.0 ±0.05	204.1 ±12.6	130.2 ±0.09	211.4 ±17.5	130.2 ±0.11	225.3 ±22.4	144.0* 0.15
MGN-3 Control PO + Doxorub. 10 mg/kg IP	10	161.1 ±3.3	197.1 ±6.8	122.1 ±0.03	197.6 ±6.6	122.5 ±0.02	191.0 ±8.5	118.3 ±0.04	196.4 ±10.2	118.2 ±0.03	210.4 ±10.7	130.2 ±0.05
MGN-3 5 mg/kg PO + Doxorub. 10 mg/kg IP	10	158.1 ±2.1	200.1 ±1.7	126.7 ±0.01	203.0 ±4.1	128.5 ±0.02	204.7 ±8.4	129.5 ±0.05	213.8 ±10.1	131.8 ±10.1	231.6 ±12.0	146.6* ±0.08
MGN-3 50 mg/kg PO + Doxorub. 10 mg/kg IP	10	156.8 ±2.4	201.2 ±2.9	128.4 ±0.01	200.0 ±5.3	127.6 ±0.03	197.0 ±8.2	125.5 ±0.04	207.1 ±8.53	130.0 ±0.06	225.3 ±10.7	143.5* ±0.06

* Statistically different from Control + Chemotherapeutic treatment group–$p < 0.05$ ANOVA + Bonferroni Multiple Comparison Test

TABLE 2. Effect of MGN-3 on the Occurrence of Spontaneous Deaths, Gross Intestinal Lesions and Diarrhea

Treatment	D %	GI Path %	Dia.%
MGN-3 5 PO + Vehicle	0	0	0
MGN-3 50 PO + Vehicle	0	0	0
MGN-3 Control + Cisplatin 9 IP	50	70	100
MGN-3 5 PO + Cisplatin 9 IP	10#	40	50#
MGN-3 50 PO + Cisplatin 9 IP	40	50	40#
MGN-3 Control + Doxorubicin 10 IP	0	50	20
MGN-3 5 PO + Doxorubicin 10 IP	0	10#	0
MGN-3 50 PO + Doxorubicin 10 IP	0	30	10

Statistically significant $p < 0.05$ chi-square test
D–Spontaneous deaths
GI Path–Gross damage to gastrointestinal tract indicated by perforations, hemorrhagic spots, mucosal abnormalities, or excess fluid accumulation
Dia.–Incidence of diarrhea or soft stools

Cisplatin caused deaths in 50% of rats treated (Table 2). Lethality of cisplatin was decreased to 10% in rats treated with 5 mg/kg MGN-3 and 40% in groups treated with 50 mg/kg. Doxorubicin did not cause death after a single dose.

One hundred percent of rats receiving a single dose of 9 mg/kg cisplatin IP showed signs of diarrhea on days 7 to 11 (Table 2). MGN-3, 5 mg/kg decreased the incidence to 50%, and 50 mg/kg decreased the incidence to 40%. Cisplatin produced gross gastrointestinal mucosal pathology in 70% of treated rats. This was decreased to 40% after 5 mg/kg MGN-3 and 50% after 50 mg/kg MGN-3. Doxorubicin produced diarrhea in only 20% of rats and the incidence was decreased to 0% after MGN-3 5 mg/kg and 10% after 50 mg/kg. Doxorubicin produced gross gastrointestinal mucosal pathology in 50% of rats. Incidence was decreased to 10% after 5 mg/kg MGN-3 and 30% after 50 mg/kg MGN-3.

There were few changes in the liver parenchyma in any dose group. MGN-3 5 and 50 mg/kg groups were very similar with very fine vacuolization within the cytoplasm of hepatocytes that were slightly more prominent in the periportal area. This was all within the range of normal. All other groups receiving either cisplatin or doxorubicin had varying degrees of chronic/active inflammation of the liver capsule. This was characterized by a thickened capsule due to a proliferation of young fibroblasts, and an infiltrate of acute and chronic inflammatory cells. This change is consistent with an intra-peritoneal injection of an irritating material. The inflammation of the capsule was slightly more severe in rats injected with doxorubicin than cisplatin. There was no necrosis or degenerative changes observed in the liver parenchyma of any dose group. The centrilobular hypertrophy seen in several livers was quite subtle, and may well

be due to secondary changes associated with peritonitis rather than a direct compound effect. Because liver parenchymal changes were so subtle with the administration of chemotherapeutic agents, no attempt was made to evaluate the benefits of administering MGN-3 on histologic examination

DISCUSSION AND CONCLUSIONS

MGN-3 was shown in this study to protect rats given an acutely toxic dose of cisplatin or doxorubicin. The endpoints chosen for evaluation were prevention of the effects of these agents on body weight, stool consistency, gross pathology of the gastrointestinal tract and mortality. Liver histology was also studied, but consistent severe pathology was not observed with the single dose of cisplatin or doxorubicin administered. Significant protection was observed on body weight. It is not clear from this study whether this was due to increased food intake or prevention of the catabolic effects of the chemotherapeutic agent. Protection against gastrointestinal mucosal lesions and diarrhea were also observed, as was a decreased mortality due to cisplatin. MGN-3 administered at 5 mg/kg PO appeared to be more effective than at 50 mg/kg PO. No obvious explanations are apparent to explain the greater efficacy of the lower dose of MGN-3. The peak efficacious dose may actually be lower than the lowest dose tested and would require further testing. The effects on weight gain, lesion formation and diarrhea appear to be plateauing. It is only the lack of protection of lethality from cisplatin that the 50 mg/kg dose is significantly less than the 5 mg/kg dose.

Cisplatin and doxorubicin produce well-documented pathology when used as chemotherapy for malignant diseases.[3,4] Severe cardiac and renal pathology are common. This study was not designed to evaluate these adverse effects since a single dose of cisplatin or doxorubicin was not sufficient to produce significant pathologic changes in the liver. However, there were signs of gross pathology when gastrointestinal mucosa was evaluated. In this study, cisplatin produced more severe adverse effects than did doxorubicin. A study using a multiple dosing protocol with somewhat lower doses of chemotherapeutic agents would be required to evaluate potential protection against pathologic changes in the liver, kidneys and heart.

The mechanism by which MGN-3 protects against the toxicity of doxorubicin and cisplatin is unknown. MGN-3 has been reported to increase NK cell activity in immunocompromised patients and possess scavenging activities against superoxide anion radicals and hydroxyl radicals.[2] Reactive oxygen species produced during metabolism of doxorubicin are purported to play an important role in the pathogenesis of experimental doxorubicin nephropathy in rats.[3] Ghoneum and Jewitt [8] have shown that MGN-3 in an *in vitro* system pro-

duced an increased production of tumor necrosis factor$_\alpha$ and interferon$_\alpha$ from peripheral blood lymphocytes as well as augmentation of natural killer cell cytotoxic function. This activity has been proposed to explain the effectiveness of MGN-3 as a novel biological response modifier. It also suggests its use as a safe alternative or as an adjuvant to existing immunotherapeutic therapy. However, it does not explain its protective effect on gastrointestinal mucosa. There is no indication that the cytoprotective activity is due to an increase in prostaglandin levels in the gastrointestinal mucosa.

Recent advances in chemotherapy have focused on the benefit of high dose regimens, increasing the dose intensity of conventional chemotherapy and using intensified chemotherapy with or without autologous bone marrow rescue.[1,4] Dose intensity usually increases objective response rates of antineoplastic drugs and might, in some circumstances, improves survival. However, unacceptable acute and/or cumulative toxicity often impairs the proper management of patients, leading to dose reduction or treatment delay, thus reducing the efficacy and potentially the quality of life of patients. Therefore, considerable efforts have been made to manage, to prevent, and to delay many acute, and cumulative treatment-related toxicities. Several chemoprotective compounds have now been extensively investigated, including dexrazoxane, amifostine, glutathione, mesna and ORG 2766.[5] Dexrazoxane appears to complex with metal co-factors including iron, to reduce the incidence of anthracycline-induced cardiotoxicity, allowing the delivery of higher cumulative doses of anthracyclines without the expected consequence of cardiomyopathy. Numerous studies have demonstrated that sulfur-containing nucleophiles; including amifostine.[6,7] These studies have not revealed any evidence of reduction in antitumor efficacy.

There is still a great need for non-toxic agents that have significant protective activity against chemotherapy induced adverse effects.[1] MGN-3 appears to be effective in protection against some of the disturbing side effects produced by cisplatin and doxorubicin and may be valuable in improving "quality of life" in-patients receiving chemotherapy. MGN-3 is a natural food supplement and now used for general health-promotion benefits. It is generally regarded as non-toxic at doses used in humans (0.5-3 grams per day). MGN-3 may prove to be useful as an adjunct to cancer chemotherapy.

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