Special article

MGBG: Teaching an old drug new tricks*

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Summary

Methylglyoxalbisguanylylhydrazone or MGBG is an agent with a unique mechanism of action (polyamine biosynthesis inhibition). MGBG was discarded in the 1960s because of severe mucositis and other toxicities. New clinical trials in the late 1970s and early 1980s utilized weekly administration and indicated MGBG had significant activity in patients with chemotherapy-refractory Hodgkin's and non-Hodgkin's lymphoma. In addition, some activity was noted in patients with head and neck, prostate, esophageal, and endometrial cancer. The toxicities on the weekly schedule were minimal and no myelosuppression was noted. Based on MGBG's spectrum of antitumor activity and its activity in severely debilitated patients, we hypothesize that MGBG may have greater antitumor activity in patients who are malnourished (possibly based on polyamine depletion). MGBG is a good candidate for treatment of AIDS-associated NHL because it has proven activity in patients with NHL which is not associated with AIDS, crosses the blood brain barrier, is non-myelosuppressive, and appears to work in patients with inanition (no polyamines available to reverse MGBG's antitumor effects). Clinical trials are ongoing to determine the activity of MGBG in AIDS-associated NHL and other diseases. Based on encouraging initial results, it appears MGBG may become part of our therapeutic armamentarium.

Key words: MGBG, polyamine, AIDS, Hodgkin's lymphoma, non-Hodgkin's lymphoma

Introduction/Methods

Life-cycle of development of an anticancer agent

Figure 1 describes the life-cycle of a new anticancer agent. The agent is discovered (birth), it has a period of clinical testing and if that testing shows either no efficacy or unmanageable toxicity, the drug undergoes programmed drug death, which I like to call 'pharmacoptosis'. Occasionally, when more information is gained about the agent (e.g., pharmacology of the agent or a way to prevent the side effects of the agent), the drug undergoes a rediscovery (or a rebirth) and is found to be a useful agent for the treatment of patients with a particular type of malignancy. Figure 2 details a few of the anticancer agents which have been rescued from 'pharmacoptosis'. To date, the agent which appears to have had the longest period of 'pharmacoptosis' is MGBG.

MGBG and its mechanism of action

Methylglyoxal bis(guanylylhydrazone), also known as mitoguazone, NSC-32946, methyl-GAG, or MGBG was synthesized by Thiele and Dralle in 1898 [1]. The compound has a molecular weight of 257 and the chemical structure is shown in Fig. 3. The compound's structure is similar to the polyamine spermidine. MGBG is one of those agents which had a flurry of clinical trials in the early 1960s but trials were discontinued secondary to severe toxicities noted with the agent (e.g., MGBG underwent a period of 'pharmacoptosis'). Prior reviews of these old anticancer agents have indicated that many of them are worth another ex-

* This manuscript is based on a lecture given in honor of Professor Michel Clavel who fought a courageous battle against the disease he was trying to conquer. May he rest in peace knowing we carry on.
Anticancer agents which have been rescued from 'pharmacoptosis'. Note the reason for induction of pharmacoptosis for each agent and the time it took for the agent to make it back into clinical trial. To date, MGBG appears to hold the record for the longest period of 'pharmacoptosis'.

Fig. 2. Anticancer agents which have been rescued from 'pharmacoptosis'.

Fig. 3. Structure of MGBG.

Amination in a time when we have new technologies to measure blood levels and/or prevent toxicities associated with their administration [2].

MGBG appears to have a variety of mechanisms of action. One mechanism of action appears to be as an inhibitor of polyamine biosynthesis. It does this by acting as a competitive inhibitor of S-adenosylmethionine decarboxylase (SAM-DC) [3]. Figure 4 presents an outline of the polyamine biosynthetic pathway and the various enzymes involved in that pathway.

In addition to inhibition of polyamine biosynthesis, MGBG also causes vacuolization of mitochondria which might result from uncoupling of oxidative phosphorylation [4, 5].

Prior clinical experience with MGBG

Clinical trials with MGBG were initiated in the early 1960s, after Freedlander and French [6–9] described the anticancer activity of polycarbonyl compounds. In addition, MGBG was also found to have antiviral activity and activity against trypanosomiasis [10–12], which made it an even more attractive agent. There have been several excellent review articles written about the agent [13–15].

Regelson and Holland [16, 17], Freirech et al. [18], and Levin and colleagues [19] conducted the early clinical trials with MGBG. Using daily administration schedules they noted marked myelosuppression and mucositis. However, in those trials and in an excellent overview of those trials [14], responses were noted in patients with acute leukemia, chronic myelogenous leukemia, lymphoma, multiple myeloma, head and neck cancer, esophageal cancer and other types of malignancies in patients. After this initial experience, most of the work with MGBG was discontinued, largely based on the feeling that the drug was too toxic. In addition, during the late 1960s agents such as daunorubicin, cytosine arabinoside and others were just being introduced and were more the focus of attention.

In the mid 1960s and early 1970s there were several observations which regenerated interest in MGBG (e.g., took it out of 'pharmacoptosis'). First of all, there was finally information on the pharmacology of MGBG (albeit using radiolabeled MGBG) which indicated that the agent probably had an extremely long half-life [20]. These studies were later confirmed using HPLC methods by Hart et al. [21], Marsh and colleagues [22], and Stewart et al. [23]. The plasma decay after a rapid infusion of the drug was triphasic and the gamma half-life was in the range of 100 hours. In addition, the concentration of MGBG was found to be high in the brain [22, 24]. Based on the long half-life of MGBG, schedules other than intermittent schedules were deemed more appropriate. Another important observation which was to shape the new future for MGBG was the finding by Russell [25] that patients with a variety of malignancies had increased concentrations of polyamines in their urine and the finding by Williams-Ashman et al. [26] that MGBG was an inhibitor of polyamine synthesis through inhibition of the enzyme S-adenosylmethionine decarboxylase (SAM-DC).

As a result of the above information, MGBG enjoyed a rebirth in 1976 when Knight and colleagues conducted phase I and early phase II trials in San Antonio and in the Southwest Oncology Group using a weekly schedule of administration [27]. In those studies they noted MGBG had rather minimal toxicity and some activity in patients with lymphoma (Hodgkin’s and non-Hodgkin’s lymphoma), esophageal cancer, prostate cancer, and other types of tumors [27–30]. Additional phase II trials were pursued by a large number of investigators (see Warrell and Burchenal [14] and Dorr and Von Hoff [31] for review of response rates in various histologic types). Once again these studies confirmed activity for MGBG in patients with refractory Hodgkin’s and non-Hodgkin’s lymphoma, head and
In addition to single-agent efficacy, MGBG also was utilized in combination chemotherapy regimens. Particularly active combination regimens included the MIME regimen (containing MGBG + ifosfamide + methotrexate + etoposide) and other regimens for patients with refractory lymphomas [48-54]. Response rates in patients with refractory lymphoma were as high as 84% in patients treated with those regimens. In patients with advanced non-small cell lung cancer Weick and colleagues [55] also conducted a trial in which patients were randomized to four different treatment arms. In two of those Southwest Oncology Group study arms, patients were randomized to receive either cisplatin + etoposide or cisplatin + etoposide + MGBG. Interestingly, in that large study, the 3-drug regimen had a response rate of 33% versus a response rate of 16% for the 2-drug regimen. The higher response rate was accompanied by greater toxicity. It was disappointing that, despite a doubling of response rate, MGBG had no impact on survival.

Even after all of the above work, some of which was encouraging, MGBG again underwent a second period of 'pharmacoptosis'. I believe this was largely because the patent on the drug had expired many decades ago and hence there was no commercial value ascribed to the agent. At that point it appeared that something on 'pharmacoptosis'.

The new epidemic

The incidence of NHL in the United States and other countries is rising. Since 1970 there has been a 50% increase in incidence and a 22% increase in death rate [56]. The connection between AIDS and NHL was first reported in 1982. This was accompanied by a sharp increase in the numbers of patients with NHL (men 20–54 years of age) [57-59]. Beral et al. [60] have reported that 3% of 100,000 patients with AIDS had NHL which represented a 60-fold increase relative to the rate expected in a general population. In 101 patients with AIDS who had autopsies, 20 had lymphomas (5 in the brain).

The histologic types of NHL which predominate in AIDS patients include the high-grade B-cell types, including large cell immunoblastic and small non-cleaved cell lymphoma (70% of AIDS-associated lymphomas) and the intermediate-grade diffuse large cell lymphomas, which make up the remaining 30%. A Burkitt's histology is also common.

NHLs in AIDS patients frequently involve extranodal sites (particularly the brain, gastrointestinal tract, and bone marrow). A full 40–60% of patients can have primary CNS involvement [58, 61, 62].

AIDS-associated NHLs have a high frequency of multiclonality. Translocations involving the immunoglobulin gene and c-myc loci are common [57, 63].

Ironically, as AZT and other new agents prolong survival of AIDS patients, there is an increasing incidence of NHL in these patients. Recent projections indicate there will be about 4700 new cases of AIDS-associated lymphomas (range 2900–9800) in 1992 [64, 65]. NHL is a particular problem for patients with CD4-positive lymphocyte counts <50/mm³ [65].

NHL in the AIDS patient is a very difficult disease to treat. The tumor is usually histologically high-grade with multi-organ and CNS (in a sanctuary) involvement. The patients usually have a compromised bone marrow [66]. First line, aggressive NHL regimens in these patients yield remission rates of 20%–50% with survival of <1 year's duration [67].

Results

Use of MGBG for patients with AIDS-associated non-Hodgkin's lymphoma

As outlined above, there is a great deal of clinical evidence that MGBG has activity in patients with non-AIDS-associated, non-Hodgkin's lymphoma (NHL). In addition, MGBG has many other features which make it attractive for use in patients with AIDS-associated NHL, since MGBG (a) is non-myelosuppressive; (b) has very few other toxicities; (c) appears to cross the blood-brain barrier (and the central nervous system is one area frequently involved by lymphoma in patients with AIDS); (d) has good in vitro activity against aggressive types of lymphoma cell lines (see Table 1); (e) appears to work better in patients with inanition (see below); and (f) has a mechanism of action which might interact favorably with some of the other medications taken by patients with AIDS.

Based on the above reasoning, a clinical trial with MGBG was initiated in patients with AIDS-associated NHL. This National Cancer Institute sponsored phase I-II clinical trial is an ongoing joint effort between Dr. Alexandra Levine at the University of California

Table 1. In vitro activity of cisplatin + MGBG against lymphoma cell lines.

<table>
<thead>
<tr>
<th>Drug(s)</th>
<th>Concentration mcg/ml</th>
<th>Exposure time</th>
<th>Percent survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>0.02</td>
<td>1 hr</td>
<td>93, 102, 96</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>0.02</td>
<td>24 hrs</td>
<td>90, 104, 96</td>
</tr>
<tr>
<td>MGBG</td>
<td>1.0</td>
<td>1 hr</td>
<td>104, 107, 100</td>
</tr>
<tr>
<td>MGBG</td>
<td>1.0</td>
<td>24 hrs</td>
<td>56, 60, 9</td>
</tr>
<tr>
<td>DDP + MGBG</td>
<td>1.0</td>
<td>1 hr</td>
<td>57, 65, 11</td>
</tr>
<tr>
<td>DDP + MGBG</td>
<td>1.0</td>
<td>24 hrs</td>
<td>98, 86, 84</td>
</tr>
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Table 1. In vitro activity of cisplatin + MGBG against lymphoma cell lines.
School of Medicine and our drug development group in San Antonio. The dose selected was 600 mg/m² given intravenously on days 1, 8 and then every 2 weeks (Levine AM, Weiss G, Tulpule, A et al. MGBG: A highly active drug in relapsed or refractory AIDS lymphoma (abstract) Proc Am Soc Clin Oncol 1994: submitted). To date, a total of 10 patients have been entered on study. All have had refractory or relapsed AIDS-associated NHL. As far as prognostic factors are concerned, the median CD4 cell count prior to initiation of MGBG was 117/dl (range 4-427) and 80% had CD4s of less than 200/dl. Four of the patients had had AIDS prior to receiving MGBG. All patients had aggressive histologies. Four patients had had 1 prior chemotherapy regimen for their NHL, 4 had had 2 prior treatment regimens, and 1 patient each had had 3 and 4 prior treatment regimens respectively.

Of the 10 patients entered on study, complete responses have been seen in 2 patients and partial responses have been documented in 3 additional patients (Levine et al., 1994). Of special interest in these patients is that toxicity was mild, consisting of only flushing, tingling and occasional somnolence during infusion. Mild nausea, vomiting, and fatigue were also noted. No significant myelosuppression has been noted in the patients studied to date. The duration of responses observed in the patients has been surprisingly good with one patient now maintaining a response lasting more than one year.

In addition to objective criteria for response, the patients entered on this study have experienced resolution of 'B' symptoms, gain in weight, and decrease in requirements for pain medications. Levine and colleagues state that 'quality and quantity of survival have been positively impacted'.

Obviously, additional work with MGBG in patients with AIDS-associated NHL is needed and that work is ongoing. However, based on the information reported to date, MGBG appears promising for treatment of patients with that disease. Given the non-myelosuppressive nature of MGBG, it should also be tried in patients with lymphoma whose disease has relapsed after bone marrow transplantation. They generally have a very brittle marrow which makes additional attempts at treatment very difficult, if not impossible, and MGBG could be an ideal agent to try for those patients.

Potential methods to increase the clinical activity of MGBG

In 1973, Pegg reported that spermidine could block the antiproliferative effects of MGBG [68]. Alhonen-Hongisto and colleagues [69] and Seppänen and colleagues [70] noted that when tumor cells are depleted of putrescine and spermidine, the tumor cells concentrate extracellular polyamines more effectively. When they depleted tumor cells of polyamines using the ornithine decarboxylase inhibitor difluoromethylornithine, there was a major increase in transport of MGBG into tumor cells. That finding supported the opinion that natural polyamines and MGBG share a common transport system [71, 72]. They further found that priming of tumor cells with difluoromethylornithine, followed by exposure to MGBG, resulted in marked cytotoxicity.

One additional point to indicate that exogenous polyamines may be actually rescuing tumor cells from the cytotoxic effects of MGBG is that an analog of MGBG, MGBB 'methylglyoxal bis(butylaminohydrazide), has excellent in vitro activity against human melanoma cells, but only modest growth inhibitory properties in vivo [73]. The authors speculate that polyamines supplied from diet and other tissues could be rescuing the tumor from the effects of MGBB.

It is of great interest that, as noted above, MGBG has clinical antitumor activity against a number of tumors associated with severe cachexia and inanition (e.g., esophageal cancer, head and neck cancer, and patients with lymphoma who have had extensive prior therapy). In addition, this might explain why such impressive activity has been noted in patients with AIDS-associated NHL where inanition is such a common event. However, MGBG has not had activity in patients with pancreatic cancer where inanition is also seen.

Based on our personal clinical observations of patients receiving MGBG and based on the preclinical information presented above, our hypothesis is that the activity of MGBG is enhanced in patients who have low endogenous polyamine levels (patients who are severely cachectic and/or who are on broad spectrum antibiotics to decrease the production of polyamines by bacteria in the gut). The reasons for past failures of treatment with MGBG could be at least in part due to endogenous polyamines rescuing the tumor cells from the cytotoxic effects of MGBG. Based on this reasoning, new clinical trials should be conducted in patients after a strategy has been put in place to maximally reduce their endogenous polyamines and therefore improve their chance for an antitumor effect from MGBG. The rationale for this approach is further strengthened by the finding that patients with malignant lymphoma, leukemia and other tumors do have elevated levels of polyamines in their blood [74, 75].

There is one other recently reported observation which is of note and which might also help explain the dramatic antitumor activity noted so far for MGBG in patients with AIDS-associated NHL. Balana-Fouce and colleagues [76] and Bitonti et al. [77] reported that pentamidine (frequently utilized in patients with AIDS to treat their pneumocystis carinii pneumonia) inhibits the same enzyme that MGBG inhibits, namely, S-adenosylmethionine decarboxylase. Even more recently, Libbey and Porter [78] reported that pentamidine inhibits the enzymes of polyamine back-conversion. Both of these actions by pentamidine could lead to a decrease in the amount of serum spermidine available to the tumor cells and thus enhance the effects of MGBG. This is only a theoretical possibility but it will
be explored in depth as we consider drug interactions in the ongoing clinical trial.

**Interesting combination regimens containing MGBG**

A recent trial conducted by the Southwest Oncology Group randomized patients with non-small cell lung cancer to treatment with either cisplatin + etoposide or cisplatin + etoposide + MGBG. Of note is that there was a significantly higher response rate (33% versus 16%) for the 3-drug regimen versus the 2-drug regimen. However, toxicity was greater for the 3-drug regimen and survival was not improved [55]. Based on those findings we have been investigating a number of in vitro and in vivo systems to determine whether or not we could document synergism for the combination of cisplatin + MGBG. Table 1 documents that as a single agent only, MGBG utilized at a concentration of 1.0 mcg/ml for a 24-hour exposure has in vitro activity against the P388 mouse leukemia cell line or against either of the two human Burkitt's lymphoma cell lines. Adding platinum 1 hour before the 24-hour exposure to MGBG does not increase the cytotoxicity of MGBG. In addition, a 24-hour exposure to cisplatin after a 1-hour exposure to MGBG does not increase the cytotoxicity of MGBG. Therefore, based on these in vitro data, it does not appear that the combination of MGBG plus cisplatin has synergistic (or even additive) cytotoxicity.

Our group in San Antonio has also observed the same findings in in vivo P388 and B16 animal tumor models (Dan Dexter, Ph.D., personal communication). In addition, Murata and colleagues [73] have also found that an analog of MGBG, MGBB, can actually reduce the in vivo and in vivo antitumor action of cisplatin against a human malignant melanoma cell line. Murata and colleagues speculate that a polyamine-deficient state caused by inhibition of polyamine synthesis by MGBB causes the structure of DNA to be unstable and the DNA strands to become movable. They further speculate that cisplatin can only poorly cross-link these movable DNA strands which results in decreased antitumor activity for platinum (which depends on interstrand crossing-linking for its mechanism of cytotoxicity). This interpretation is also supported by the work of Tofilon et al. [79, 80]. Based on these data it appears unlikely that any clinical evidence of synergism noted for the combination of cisplatin + etoposide + MGBG is due to synergism between cisplatin + MGBG. However, it is still possible that there is indeed synergism between etoposide + MGBG. Our group is currently exploring that possibility with both in vitro and in vivo studies.

**Conclusions**

I hope, that based on the above information, I have convinced the reader that it is indeed important to re-explore the potential applications of MGBG for treatment not only of patients with AIDS-associated NHL but also for treatment of patients with other lymphomas as well as other types of malignancies. Based on the rather sizable amount of new preclinical information on MGBG, it is almost certain that new clinical research strategies with the compound (including strategies to cause depletion of polyamines in patients' serum) could allow us to see more impressive antitumor activity with MGBG. Based on the data outlined above, I hope the reader will agree you can teach an old drug new tricks.

**Acknowledgement**

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