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Commentary

Guanidino-containing drugs in cancer chemotherapy: biochemical and clinical pharmacology

Sara Ekelund[a,*], Peter Nygren[b], Rolf Larsson[a]

[a]*Department of Clinical Pharmacology, University Hospital, S-751 85 Uppsala, Sweden*
[b]*Department of Oncology, University Hospital, S-751 85 Uppsala, Sweden*

Abstract

The pharmacology and clinical application of three guanidino-containing compounds are reviewed in this commentary with special focus on a new member of this group of drugs, CHS 828 [*N*-(6-(4-chlorophenoxy)hexyl)-*N′*-cyano-*N″*-4-pyridylguanidine]. *m*-Iodobenzylguanidine (MIBG) and methylglyoxal bis(guanylhydrazone) (MGBG) have been extensively studied, preclinically as well as clinically, and have established use as anticancer agents. MIBG has structural similarities to the neurotransmitter, norepinephrine, and MGBG is a structural analog of the natural polyamine spermidine. CHS 828 is a pyridyl cyanoguanidine newly recognized as having cytotoxic effects when screening antihypertensive compounds. Apart from having the guanidino groups in common, there are many differences between these drugs in both structure and their mechanisms of action. However, they all inhibit mitochondrial function, a seemingly unique feature among chemotherapeutic drugs. *In vitro* in various cell lines and primary cultures of patient tumor cells and *in vivo* in various tumor models, CHS 828 has cytotoxic properties unlike any of the standard cytotoxic drugs with which it has been compared. Among these are non-cross-resistance to standard drugs and pronounced activity in tumor models acknowledged to be highly drug-resistant. Similar to MIBG, CHS 828 induces an early increase in extracellular acidification, due to stimulation of the glycolytic flux. Furthermore, ATP levels decrease, and the syntheses of DNA and protein are shut off after approximately 30 hr of exposure, indicating active cell death. CHS 828 is now in early clinical trials, the results of which are eagerly awaited.

Keywords: MIBG; MGBG; CHS 828; Guanidine compounds; Pharmacology; Cytotoxic activity

1. Introduction

Guanidino-containing drugs such as MIBG and MGBG were shown several decades ago to have antitumor properties and, during the years, have been subjected to intensive preclinical and clinical evaluation [1–4]. Despite this, their mechanisms of action, as well as their roles in the clinical management of cancer patients, are still debated. Recently, a novel class of guanidino-containing drugs, the pyridyl cyanoguanidines, has demonstrated promising antitumor activity in preclinical tumor models [5]. The purpose of the present commentary is to summarize the accumulated knowledge on the biochemical and clinical pharmacology of cytotoxic guanidino-containing compounds used for the diagnosis and therapy of malignant tumors. The focus is on various mechanistic aspects of the drugs, as well as on a new member of this group, *N*-(6-(4-chlorophenoxy)hexyl)-*N′*-cyano-*N″*-4-pyridylguanidine (CHS 828).

2. MIBG

2.1. Chemistry and cellular metabolism

MIBG, a guanidino-containing compound, with a molecular mass of 324.1, was synthesized 20 years ago by Wieland *et al*. [6] from MIBA. MIBG is an organic cation with a lipophilic benzyl moiety and, at physiological pH, the diamine group, with a pK_a value of 12, is positively charged [1]. The structure of MIBG is shown in Fig. 1.

The guanidino group of MIBG, rather than the iodinated

* Corresponding author. Tel.: +46-18-611-52-50; fax: +46-18-51-92-37.

E-mail address: Sara.Ekelund@medsci.uu.se (S. Ekelund).

Abbreviations: MIBG, *m*-iodobenzylguanidine; MGBG, methylglyoxal bis(guanylhydrazone); MIBA, *m*-iodobenzylamine; BG, benzylguanidine; NE, norepinephrine; GBG, glyoxal bis(guanylhydrazone); EGBG, ethylglyoxal bis(guanylhydrazone); MGBCP, methylglyoxal bis(cyclopentylamidinohydrazone); ODC, ornithine decarboxylase; and SAMDC, *S*-adenosylmethionine decarboxylase.

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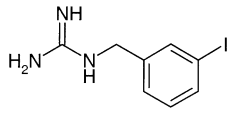
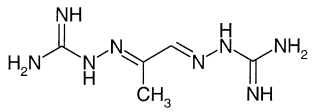
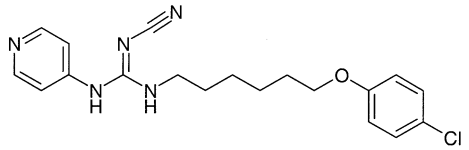


meta-iodobenzylguanidine, MIBG



methylglyoxal bis(guanylhydrazone), MGBG



N-(6-(4-chlorophenoxy)hexyl)-N´-cyano-N´´-4-pyridylguanidine, CHS 828

Fig. 1. Chemical structures of MIBG, MGBG, and CHS 828. The three substances all have in common one or two guanidino groups. MIBG is a structural analog of NE, and MGBG is an analog of the natural polyamine spermidine. MIBG and MGBG have long been known to exert cytotoxic effects. CHS 828 is a relatively new compound with antitumor activity both *in vitro* and *in vivo*, and the drug is currently in early clinical trials.

benzyl moiety, is essential for the cytotoxic effect of the drug, as shown in studies in a leukemia cell line comparing the cytotoxic effects of MIBG to those of the monoamine precursor MIBA [7]. The guanidino group is also the part of the MIBG structure that resembles CHS 828 [8]. Another analog of MIBG is BG, which is almost equipotent in terms of mitochondrial inhibition but does not affect renal clearance to the same extent [9].

MIBG is a structural and functional analog of the natural neurotransmitter NE, but does not act like a false hormone [7], although MIBG is capable of competing with NE for uptake in chromaffin tissues [6]. Like NE, MIBG is predominantly recognized by an active uptake-1 mechanism [10–12]. The uptake-1 mechanism is sodium-dependent, saturable and of high affinity, and has a low capacity for both NE and MIBG, but with a higher uptake of MIBG than of NE [10]. There is also a sodium-independent uptake system, which is probably passive diffusion, used by both NE and MIBG, but MIBG is predominantly accumulated via the neuron-specific uptake-1 mechanism [10]. MIBG also accumulates in intact metabolically active cells [13]. The mechanisms involved in MIBG uptake by non-neuronal cells are not known, but they are not blocked by an excess of natural catecholamine [13].

The intracellular fate of MIBG following its uptake has not been clearly defined. Wieland *et al*. first showed that MIBG was retained in the adrenal medulla and speculated that MIBG, mimicking NE, would be sequestrated within chromaffin storage granules [6]. Storage of MIBG in chromaffin granules in pheochromocytoma has been demonstrated by Smets *et al*.; they also showed an extensive extravesicular storage of MIBG in an *in vitro* model of human neuroblastoma cells [11]. In contrast to these findings, electron spectroscopic imaging *in situ* of MIBG-bound iodine revealed a strong, rapid, and almost exclusive accumulation of MIBG in the mitochondria [14].

2.2. *Effects* in vitro

In micromolar concentrations, unlabeled MIBG is cytotoxic in several cell lines, including neuroblastoma, melanoma, and leukemia cells [7]. Thus, the drug shows no preference for cells derived from neural tissue, and cytotoxicity was not interpreted as related to the tissue-specific effects of catecholamines [7]. Inhibition of proliferation has also been shown in several other studies in MIBG concentrations up to 100 μM [1,7,15–17]. The most commonly used concentrations were in the range of 10–32 μM (32 μM equals 10 μg/mL).

A concentration of 10 μg/mL of MIBG inhibited mitochondrial respiration in L1210 leukemia cells [18,19]. In studies using the human neuroblastoma cell line SK-N-BE(2c), optimal arrest of proliferation was seen at MIBG concentrations of 25 μM, but the mitochondrial respiratory chain was inhibited almost completely at 10 μM [15]. Similar results were also described in a leukemic cell line, Molt-4 [17]. This suggests that MIBG can induce proliferation arrest in ways other than by effects on mitochondrial oxidative phosphorylation [15,17].

A local high H^+ concentration significantly potentiates the cytotoxic activity of several conventional anti-cancer drugs, e.g., mitomycin C, melphalan, chlorambucil, cisplatin, and camptothecin [20–23]. These drugs are potential candidates for combined therapy involving tumor selective pH modulation to improve the therapeutic index. MIBG-induced acidification *in vitro* (see below), was shown to potentiate the effects of cisplatin, melphalan, mitomycin C, and chlorambucil [18].

2.3. *Effects* in vivo

MIBG has antitumor effects in animal models. In mice, the drug is acutely lethal at intraperitoneal doses above 40 mg/kg [7], and stress-related sympathomimetic side-effects have been observed at doses below 40 mg/kg, plausibly explained by the release of bioamines from their storage granules [24].

Manipulation of intra-tumoral pH has been used to modulate the cytotoxic effect of various conventional as well as experimental drugs. Glucose is a commonly used non-toxic agent to lower pH selectively in tumor tissue [25]. Other substances used to decrease extracellular pH include lactate, insulin, inorganic phosphate, and MIBG [18,21,22,26]. MIBG alone causes hyperglycemia and induces a homogeneous lowering of tumor extracellular pH [24]. There is a

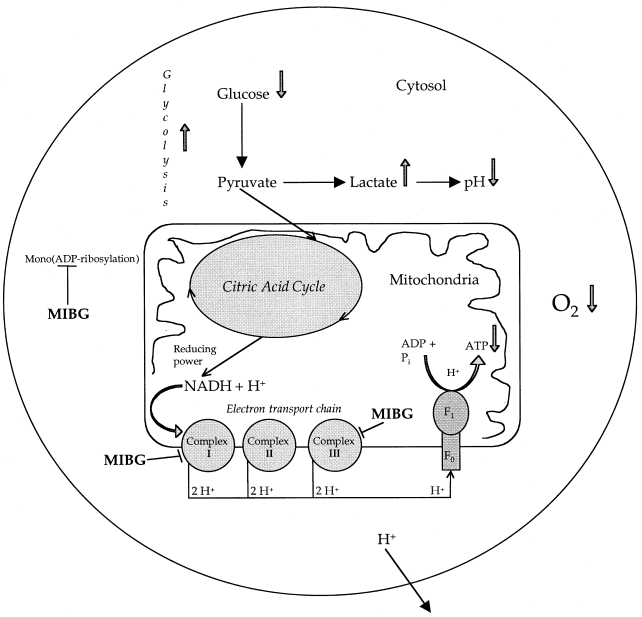


Fig. 2. Outline of the major mechanisms of cytotoxic action of MIBG discussed in the text. The drug inhibits oxidative phosphorylation in cells with a subsequent increase in glycolytic flux, leading to a lowering of extracellular pH. This also includes a lowering of intracellular ATP and O_2. The inhibition is considered to involve Complex I and possibly even Complex III in mitochondrial respiration. Probably other mechanisms are also affected, e.g., inhibition of mono(ADP-ribosylation).

stimulatory effect on tumor cell glycolysis *in vivo* indicated by a downshift of tumor pH and an increase in plasma lactate levels in tumor-bearing animals [26]. In animals exposed to MIBG, the decrease in pH was limited to malignant tissue [26]. MIBG, in combination with glucose infusion, has been shown to decrease tumor pH and to increase the antitumor activity of mitomycin C [27,28].

In rat tumors and human tumor xenografts, the compensatory induction of glycolysis following exposure to MIBG is limited [26] by an insufficient supply of glucose [29]. Simultaneous administration of glucose, lowers pH for up to 10 hr in several human tumor xenografts [24,26]. Kuin *et al*. [18] developed a non-toxic intervention, using MIBG and glucose in combination with amiloride and 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) for a sustained and selective reduction of extracellular tumor pH to 6.2 and intracellular pH to 6.5.

2.4. Mechanisms of action

The exact mechanisms by which MIBG induces cytotoxicity and cell death are not fully known, but there are several proposals (Fig. 2).

Progressive acidification of the culture medium, by lactate and other metabolic by-products of cells incubated with MIBG, has suggested that MIBG primarily affects mitochondrial respiration with subsequent compensation in glycolytic flux [1,15,19,30]. The respiratory chain is localized in the inner mitochondrial membrane, and it is associated with the outward transport of protons [31]. The stimulation of glycolysis occurs, since most of the cellular ATP is produced by the electron transport chain in the mitochondria. When the mitochondria cannot produce ATP, there is extra pressure on glycolysis to satisfy the energy demand [32]. pH measurements may be used as an indicator of changes in tumor glycolysis. In studies measuring extracellular pH following exposure of cells to MIBG, there was an almost instant increase in acidification that was sustained for 10–15 hr [30]. pH is important in interpreting the effects of MIBG on cellular electron transfer reactions since inhibition by MIBG results in the stimulation of glycolysis and the production of lactate and protons [1,17,19].

Inhibitors of oxygen consumption block electron transfer in the mitochondria. Inhibition of mitochondrial oxygen uptake by MIBG has been reported for neuroblastoma, pheochromocytoma, glioma cells, and mammary carcinoma cells [7,17,33].

The increased glycolytic flux following MIBG exposure does not seem to be cell specific but rather a general phenomenon. MIBG-induced stimulation of glucose consumption was completely accounted for by the production of two molar equivalents of lactic acid-reduced oxygen consumption and decreased intracellular levels of ATP [19]. High concentrations of lactate can be cytotoxic to cells [34] but are unlikely to be the explanation in the case of MIBG since the level of lactate in the most sensitive cell line was quite low [19].

MIBG has been shown to selectively inhibit complex I in the respiratory chain [19]. Complex I is the part of the respiratory chain that catalyses the transfer of electrons from NADH to ubiquinone [19,32]. MIBG inhibits complex I without affecting F_1-ATPase since there was a lack of effect on state 3 oxidation of succinate in intact mitochondria [19]. In studies by Cornelissen *et al*. [15], succinate-driven ATP synthesis was shown to be affected, but to a lesser extent, and this would indicate that MIBG inhibits at least one additional complex of the respiratory chain in addition to complex I. Measurements of the individual complexes in the respiratory chain revealed inhibition of complex III as well [17]. The difference in results was explained by Cornelissen *et al*. by the fact that ATP synthesis suppressed by malate inhibits the activity of both complex I and III, whereas succinate inhibits only the activity of complex III [15].

The mechanism of cytotoxicity of MIBG may also be related to its previously described capacity to inhibit mono(ADP-ribosylation) [35], a process with an unknown biological role. *N*-linked, mono(ADP-ribosylation) is mainly confined to arginine residues of cytosolic and membrane-associated proteins, and the diamine group of MIBG has structural similarities to L-arginine. MIBG has been shown to be a high-affinity substrate for cellular mono(ADP)ribosyltransferases [36] and to affect levels of endogenus acceptors of these enzymes [13].

Further, MIBG can cause oxidative stress to neuroblastoma cells leading to cellular damage, but over longer periods this does not result in inhibition of cell proliferation since the cells adapt to the increase in oxidative stress and up-regulate detoxifying enzymes [16]. Other results suggests that the formation of superoxide and lipid peroxidation may contribute to the cytotoxicity of ^{131}I-MIBG, as these processes can be enhanced by inhibitors of complex I in the respiratory chain [37].

2.5. Toxicology and pharmacokinetics

Doses of >40 mg/kg of MIBG in mice have been reported to be associated with reversible nephrotoxicity, including changes in renal clearance, reduced renal perfusion, and histological changes [9]. Histological damage caused by BG, an MIBG analog, given at twice the molar dose of MIBG, was less pronounced, and the renal clearance was not decreased. BG is equipotent to MIBG, as confirmed by high lactate levels after BG treatment. Hence, mitochondrial inhibition alone does not seem to be responsible for MIBG-induced renal impairment [9]. Since MIBG is primarily excreted by the kidneys, high local concentrations could plausibly exert a cytotoxic effect [38].

Intravenous administration of therapeutic doses of ^{131}I-MIBG indicated that glomerular filtration is only partially responsible for excretion of MIBG in adult patients [38]. The elimination half-time was determined to be 11.5 hr, with a linear relationship between excretion rate and dose up to 80 mg [38].

2.6. Clinical use

In its radio-iodinated form, MIBG is used clinically as a tumor-seeking radiopharmaceutical agent for the diagnosis and treatment of neuro-endocrine tumors [39]. The efficacy of ^{131}I-MIBG scintigraphy for the detection of pheochromocytoma is well established, and the same diagnostic accuracy can be achieved in neuroblastoma [39].

^{124}I-MIBG has been used for positron emission tomography (PET) imaging, while ^{123}I- and ^{131}I-MIBG are used for scintigraphic localization of tumors [40–42]. The imaging characteristics of ^{123}I are superior to those of ^{131}I, with better physical properties, but the most extensive experience has been acquired with ^{131}I [40,41]. MIBG scintigraphy is considered to have an established role in diagnostic nuclear medicine in adults as well as in children [40,41].

^{131}I-MIBG therapy is based on the underlying hypothesis that the administration of large doses of ^{131}I-MIBG would deliver therapeutic doses of radiation to tumors, due to the relatively intense uptake and prolonged retention of the tracer in tumor cells. ^{131}I-MIBG therapy has been recognized to be effective in a wide range of neural crest tumors, e.g. malignant pheochromocytoma, neuroblastoma, medullary thyroid carcinoma, and carcinoids [2,43]. Both ^{131}I-MIBG and unlabelled MIBG are effective in the palliation of symptoms, but duration of the response tends to be much longer with the radioactive compound in patients with metastatic carcinoids [44].

MIBG therapy has low toxicity and yet has therapeutic value as a systemic treatment for all disease sites [45]. The therapy is well tolerated and side-effects are mild and transient even when high doses are given repeatedly [2,43,44].

3. MGBG

3.1. Chemistry

MGBG is a guanidino-containing compound with a molecular mass of 257.1 (Fig. 1). The basic molecule consists of two polar aminoguanidino groups separated by an aliphatic skeleton. The drug has many different names, the most commonly used being MGBG, methyl-GAG, and mitoguazone. Several analogs of MGBG have been investigated for anti-tumor activity, e.g. its precursor GBG, its ethyl-derivative EGBG, MGBCP, and others [46–48].

There are structural similarities between MGBG and the natural polyamines, especially with spermidine. With four N atoms in the middle, MGBG has the same number of atoms in the main chain as spermidine.

3.2. Polyamines

The natural polyamines spermidine, spermine, and their precursor putrescine are present in all mammalian cells [49,50]. Their physiological function is not well understood. They are small polycationic peptides that are positively charged at physiological pH [3]. The polyamines are critically involved in many biologic processes [51], in particular the regulation of cell proliferation and macromolecular synthesis through stabilization of DNA and methylation of tRNA [49,52,53]. Furthermore, an increased level of polyamines has been reported in several malignant diseases [53]. Therefore, polyamine metabolism offers several possibilities for intervention [49].

The primary precursors of polyamines, in both microorganisms and animal tissues, are the amino acids L-ornithine and L-methionine [53]. The first step in the synthesis of the polyamines is the conversion of ornithine to putrescine in a reaction catalysed by ODC. Putrescine serves as a precursor for spermidine synthesis. Another decarboxylase, SAMDC, is required for the synthesis of spermidine. SAMDC decarboxylates *S*-adenosylmethionine, which then acts as a source of propylamino groups. The coupling of putrescine to the propylamine group to yield spermidine is catalysed by spermidine synthase. A similar coupling of spermidine to the same propylamine moiety is made to yield spermine [3,50,53,54]. All these reactions are, in practice, irreversible [3].

ODC and SAMDC have very rapid turnover rates [50].

The short half-lives of these enzymes permit a rapid change in their activity in response to stimuli to adjust the polyamine level to that needed for cell growth and development [50,55]. These enzymes are highly regulated by growth factors and other stimuli to increase their levels and by polyamines themselves to reduce the enzyme activity [55].

Intracellular polyamine pools are in exchange with the extracellular environment via active transport systems. Polyamines are absorbed from the gastrointestinal tract and excreted in urine [56]. Putrescine can be synthesized *de novo* in rapidly dividing cells in contrast to non-proliferating cells where the formation of putrescine from spermidine is considerable [56].

3.3. Characteristics of MGBG uptake and accumulation

Normal and malignant cells possess a high affinity and saturable transport system that promote intracellular accumulation of putrescine, spermidine, and spermine. It appears that MGBG is transported by the same membrane transport system [57]. This transport system is regulated both negatively, by the intracellular polyamine content, and positively, by growth factors and oncogenes [55]. The negative regulation may be of great importance in preventing the accumulation of polyamines to levels that have deleterious effects on the cell [55].

Cultured mammalian cells concentrate MGBG in a remarkably effective manner as millimolar drug concentrations were easily achieved in cells grown in the presence of micromolar concentrations of the drug [57,58]. In a study comparing the biological properties of spermidine derivatives, it was concluded that a positive charge plays an important role in uptake specificity [59].

Cellular accumulation of the bis(guanylhydrazones) is determined by their different efflux rates. GBG and MGBG are effectively retained, whereas EGBG is rapidly excreted by tumor cells [60]. GBG and MGBG rapidly produce extensive morphological damage to mitochondria, in contrast to EGBG [60]. EGBG appears to possess the highest affinity for SAMDC [60]. Thus, EGBG inhibits SAMDC to at least the same extent as MGBG, whereas GBG is considerably less potent [57,61].

3.4. Effects in vitro

In cultured cells, MGBG induces a reduction in the concentrations of spermidine and spermine [3,62–64]. In parallel with depletion of intracellular polyamines, DNA synthesis and cell proliferation are inhibited [50]. MGBG also exerts other effects not associated with inhibition of SAMDC, e.g., antimitochondrial action, inhibition of carnitine-dependent oxidation of long chain fatty acids, and blocking of intestinal diamine oxidase [3]. These antiproliferative effects are exerted in most cells by MGBG at concentrations of 1–10 μM [3]. MGBG induced apoptosis in different human cancer cell lines in a concentration- and time-dependent manner, and triggered p53-independent cell death in a breast cancer cell line [65].

In several human and murine cell types, MGBG produces ultrastructural damage to the mitochondria. These changes occur prior to any significant alteration in intracellular pools of spermidine or spermine and, therefore, the antimitochondrial effect is probably not related to polyamine depletion [66,67]. Rapidly dividing cells seem to take up MGBG more effectively than do slowly dividing cells [58]. The onset of the mitochondrial damage correlates with the generation time of the cell lines, suggesting a relationship between proliferative activity and drug-induced damage [66]. Other evidence for mitochondrial damage of MGBG has been reported [68]. Pleshkewych *et al*. demonstrated changes in mitochondrial function and structure, and inhibition of polyamine biosynthesis in a leukemia cell line. The changes appeared independent of one another, indicating that MGBG interference with mitochondrial function may be responsible for the early antiproliferative action of the drug [69].

On the other hand, MGBG was without effect on mitochondrial respiration, as there was no effect on oxygen consumption, ATP content, or lactate production in a study with human and mouse neuroblastoma and lymphosarcoma cell lines. It was concluded that the cytotoxicity of MGBG was not associated with mitochondrial dysfunction [1].

Furthermore, Toninello *et al*. found two effects of MGBG on mitochondria: protection of mitochondria at low concentrations and aggregation at higher concentrations. This could be a common consequence of an electrostatic interaction between the drug cation and the negatively charged surface of the inner membrane, as reported previously [70]. MGBG has been found to rapidly bind to isolated rat liver mitochondria in a study of Diwan *et al*. The binding was assumed to involve adsorption of the drug to anionic surface groups [71]. This is in line with earlier findings that at millimolar concentrations, MGBG significantly inhibits state 4 respiration and acts by displacing potassium ions from the inner membrane of rat liver mitochondria. Accordingly, the drug was thought to be attracted by the negative charge of this structure, resulting from the proton gradient generated across the membrane during state 4 respiration [72].

3.5. Effects in vivo

MGBG was reported in 1958 to increase the life span of mice with L1210 leukemia even when given in micromolar concentrations [73]. MGBG inhibited pyruvate utilization (used as a measure of mitochondrial function) by L1210 cells, *in vivo*, in mouse ascites fluid. In these cells there was ultrastructural damage to mitochondria and reduced levels of ATP, both compatible with apoptosis. Spermidine and spermine pools were decreased and the level of SAMDC was increased as a result of MGBG-induced enzyme stabilization [69]. An analog of MGBG, MGBCP, dose-depen-

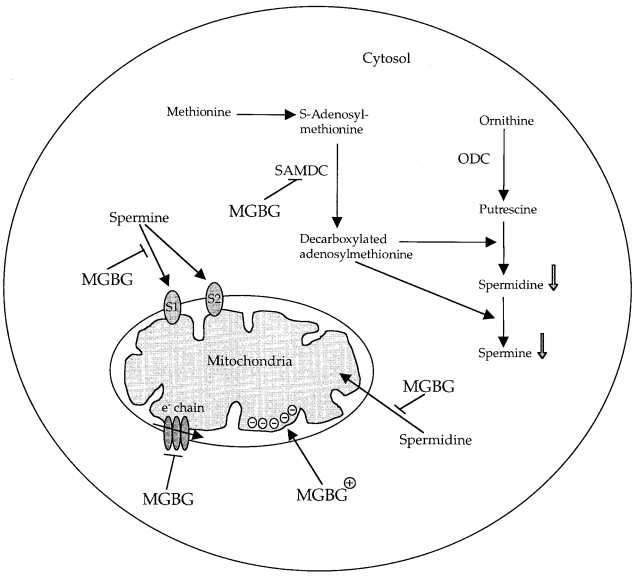


Fig. 3. Outlines of the major mechanisms of cytotoxic action of MGBG discussed in the text. These include depletion of intracellular levels of the polyamines, with subsequent inhibition of DNA synthesis and proliferation. MGBG inhibits one of the key enzymes in the biosynthesis of polyamines, SAMDC. Apart from this, damage to the ultrastructure of mitochondria has been described.

dently inhibited the growth of human osteosarcoma cells, both *in vitro* and *in vivo*, with concomitantly decreased levels of spermidine and spermine. The exposed cells exhibited morphological changes characteristic of apoptosis [47]. Similar results were also described in a hepatocellular carcinoma cell line [48].

In experimental animals receiving the drug, MGBG was only moderately effective in decreasing polyamine levels in tissues [60]. Host toxicity of MGBG included toxicity related to antiproliferation, cardiotoxicity, hepatotoxicity, and irreversible hypoglycemia in rodents and rabbits [3].

3.6. Mechanism of action

While the exact mechanisms whereby MGBG exerts its cytotoxic effects are not fully understood, there appear to be a variety of mechanisms involved (Fig. 3). For a long time, MGBG has been known to be a powerful inhibitor of the key polyamine biosynthetic enzyme, SAMDC, from many different tumor types, leading to depletion of intracellular polyamines [46,61,67,74]. The inhibition is competitive with *S*-adenosylmethionine and apparently uncompetitive with putrescine, the activator of the reaction [57,60]. The inhibition of ODC and SAMDC triggers a series of compensatory reactions, all aimed at circumventing the inhibition, e.g., enhanced accumulation of extracellular polyamines and overproduction of ODC and SAMDC [49,60, 75].

Both ODC and SAMDC are known to be degraded much more slowly when combined with a competitive inhibitor such as MGBG [49]. This is because MGBG induces stabilization of the enzymes against intracellular degradation, and this action results in a considerable increase in the total amount of enzymes present [50].

MGBG effectively reduces the intracellular concentrations of spermidine and spermine at micromolar concentrations, and its antiproliferative action is fully reversible upon the addition of exogenous polyamines [53,76]. Most of the effects of MGBG on cultured cells can be prevented by the concurrent administration of spermidine at equimolar or higher concentrations [76]. This phenomenon is unlikely to be limited to a few cell types, since several examples have been described in the literature [53,64,76]. The antagonism between MGBG and spermidine suggests competition for a common binding site [76].

MGBG decreases the rapid binding and uptake of spermidine [71]. In addition, MGBG inhibits both the adsorption of spermidine to mitochondrial membranes and its transport into the mitochondrial matrix [71]. The outer mitochondrial membrane seems to limit access to the inner membrane and, thus, acts as a barrier to penetration by MGBG and spermidine.

In a recent study, MGBG was also found to inhibit both the binding of spermine to mitochondrial membranes and its transport into the matrix space [77]. MGBG blocked spermine efflux after its accumulation in the matrix, probably due to the main action of the drug, namely the inhibition of the primary step of polyamine transport. The cytotoxicity of this drug is probably partially due to its ability to prevent mitochondrial spermine flux. Spermine uptake is determined by the sum of two processes: electrophoretic matrix transport and membrane binding [78]. There are two spermine binding sites (S_1 and S_2) on mitochondrial membranes, and MGBG may competitively block spermine binding to the S_1 site. An almost complete inhibition of spermine transport is induced by a high concentration (6 mM) of MGBG [78].

3.7. Pharmacokinetics

There seems to be little or no metabolism of MGBG, as the radiolabelled drug is excreted in urine unchanged [51, 57]. After intravenous infusion, MGBG is rapidly distributed in total body water [51] with a large volume of distribution at steady-state, indicating tissue sequestration [79]. The elimination of the drug from the plasma is triphasic with a prolonged terminal elimination half-life [51,79,80]. The long half-life of the drug can result in its accumulation and lead to toxicity in cases of overdosing.

3.8. Clinical use

Clinical trials were initiated in the early 1960s after the finding of Freedlander and French demonstrating anticancer activity of MGBG [73]. The drug is now known to be an antileukemic agent and has also been reported to have activity against some solid tumors [49,81].

In most of the studies performed during the 1960s and early 1970s, MGBG was given as an intravenous daily dose (bolus or rapid infusion). Severe side-effects including mucositis, skin ulcerations, hypoglycemia, diarrhea, mild to moderate myelosuppression, nausea, and vomiting were observed [3,39,51,80]. In the late 1970s, a weekly dosing schedule showed therapeutic activity in solid tumors and lymphomas, with little toxicity [51,80]. Intermittent administration every 10–14 days was also well tolerated and produced considerably less toxicity than many standard chemotherapeutic agents [51].

In a broad phase II trial, MGBG was found to have substantial antitumor activity in Hodgkin's and non-Hodgkin's lymphomas, with an overall response rate of 40% [80]. MGBG also demonstrated activity, although more modest, in patients with non-small cell lung cancer, esophageal carcinoma, and head and neck cancer [80].

MGBG is now in use in several combination chemotherapeutic protocols for patients with relapsed lymphoma [51, 82] and AIDS-related lymphoma [79,83]. The drug has been used in combination therapy with, for example, daunorubicin and cytosine arabinoside [51], but the best results have been obtained against Hodgkin's and non-Hodgkin's lymphoma using a drug combination denoted MIME (MGBG, ifosfamide, methotrexate, etoposide) [84–86].

4. The pyridyl cyanoguanidine CHS 828

4.1. Chemistry

The synthesis of pyridylguanidines was reported in 1978 [8]. At this time there was an interest in the hypotensive effect of a structural prototype, the potassium channel opener, pinacidil and its analogs [8]. Later it was discovered that a number of related pyridyl cyanoguanidines demonstrated antitumor activity in a rat model after oral administration in a routine screening program [87]. This finding inspired further investigation of the guanidines in order to find the structure-activity relationship for this class of substances. Crucial for the antiproliferative effect was the cyanoguanidine moiety, notably the length of the carbon chain [87]. Optimization and testing of the antitumor activity of series of cyanoguanidines resulted in the selection of a drug candidate, CHS 828 (Fig. 1), without hypotensive effects.

4.2. Effects in vitro

CHS 828 has demonstrated interesting properties as a potential anticancer agent and has been found to be active against many tumor cell lines *in vitro* [5,87]. When the cytotoxic effect of CHS 828 was evaluated in ten human tumor cell lines representing different forms of drug resistance, the drug showed potent cytotoxic activity in several of the cell lines, including those with resistance associated with the expression of P-glycoprotein, altered topoisomerase II, increased GSH levels, and tubulin defects [5]. CHS 828 was also cytotoxic to NYH lung cancer and MCF-7 breast cancer cell lines but was significantly less cytotoxic to normal fibroblast and endothelial cells tested in parallel [5]. The ratio of IC_{50} values obtained in normal cells over those in the malignant cells was significantly higher than for the reference compounds paclitaxel and daunorubicin [5].

CHS 828 has also been tested against primary cultures of tumor cells from patients with hematological or solid tumors as well as against normal lymphocytes. A total of 79 samples from various diagnoses were tested with 72-hr continuous drug exposure. CHS 828 showed high relative activity against tumor cells from chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), and acute myelocytic leukemia (AML). Activity was also observed in several solid tumor cell samples although this group appeared less responsive than the hematological tumors. CHS 828 was also significantly more active against hematological malignancies compared with normal lymphocytes. Correlation analysis with standard drugs indicated non-cross-resistance. Furthermore, the activity of CHS 828 did not require active proliferation.[1]

4.3. Effects in vivo

CHS 828 demonstrated significant anti-tumor activity in several tumor models *in vivo* at doses causing little or no general toxicity [5,87]. This was especially pronounced in a nude mouse model of small cell lung cancer (NYH) and in the xenograft model of MCF-7 breast cancer cells, two tumor model systems known to be resistant to standard drugs [5]. Total eradiction of these tumors could be achieved in the virtual absence of host toxicity. In mice with NYH tumors, long-term survival was observed after treatment with CHS 828 was stopped. In this study, CHS 828 was shown to compare favorably with established chemotherapeutic agents such as cyclophosphamide, etoposide, methotrexate, and paclitaxel [5]. *In vivo* activity was also observed in Yoshida hepato-sarcoma and Walker 256 breast carcinoma as well as in chemically induced breast tumors in the rat [5,87]. No effect was observed in the non-small cell lung cancer xenograft model H-460 [5].

4.4. Mechanism of action

The mechanisms of the cytotoxic action of CHS 828 are not yet fully understood, and antitumor activity has not been shown for other pyridyl cyanoguanidines. To investigate the

[1] Åleskog A, Bashir-Hassan S, Hovstadius P, Kristensen J, Höglund M, Tholander B, Binderup L, Larsson R, Jonsson E. Manuscript submitted for publication.

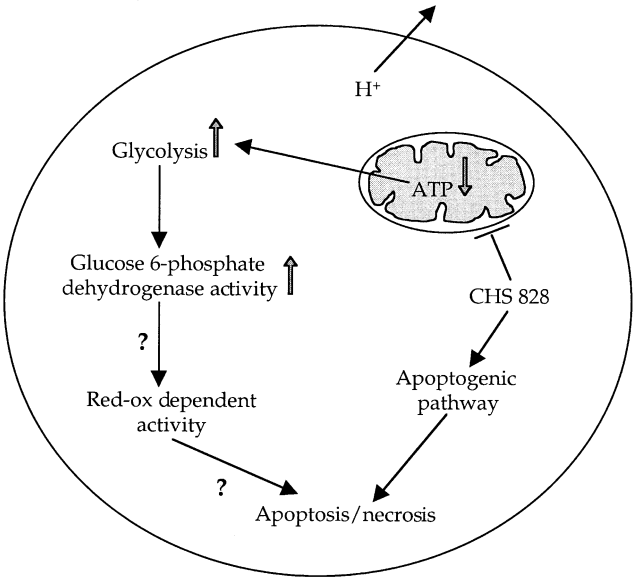


Fig. 4. Outlines of the putative mechanisms for cytotoxicity induced by CHS 828, as discussed in the text. CHS 828 is a new cyanoguanidine with interesting properties and anti-tumor activity both *in vitro* and *in vivo*. CHS 828 produces an early increase in extracellular acidification, an increase similar to that induced by the mitochondrial inhibitor MIBG. ATP levels decrease, which is accompanied by inhibition of DNA and protein synthesis, indicating active cell death.

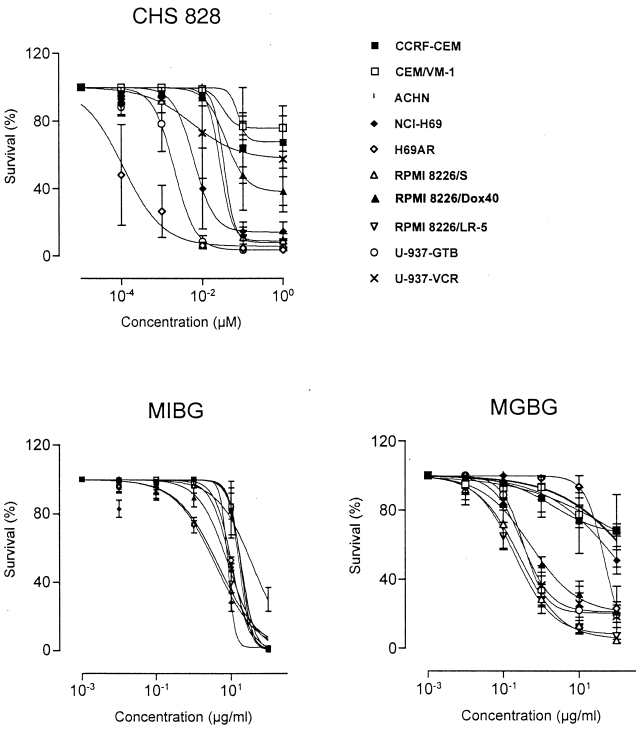


Fig. 5. Cytotoxic effects of the three guanidino-containing compounds, as evaluated in a cell line panel, consisting of ten human cell lines of different origins. Four of the cell lines are parental, five are drug-resistant sublines, and one is primary drug-resistant. The cell lines included were: the leukemia cell line (CCRF-CEM, ■) and its subline (CEM/VM-1, □), the renal adenocarcinoma cell line (ACHN, |), the small cell lung cancer cell line (NCI-H69, ◆) and its subline (H69AR, ◇), the myeloma cell line (RPMI 8226/S, △) and its sublines (8226/Dox40, ▲) and (8226/LR5, ▽), and finally the lymphoma cell line (U-937-GTB, ○) and the subline (U-937-VCR, ×). The cells were exposed to the indicated concentrations of the drugs for 72 hr followed by fluorescein diacetate based measurement of overall survival [88,89]. Cell survival is expressed in percent of untreated control cells. One micromolar CHS 828 equals 0.32 μg/mL of MIBG and 0.26 μg/mL of MGBG.

mechanism of action of CHS 828, the activity pattern of the drug was investigated in a human cell line panel consisting of ten cell lines. The activity pattern generated by CHS 828 did not correlate with activity patterns of standard chemotherapeutic agents, indicating a potentially novel mechanism of action [5]. Cell death induced by CHS 828 was characterized by an initial 15- to 24-hr period of increased metabolic activity, measured as the extracellular acidification rate [30], followed by a sharp inhibition of DNA and protein synthesis at 24–30 hr. After 24 hr of exposure, glucose consumption was inhibited completely. Measurements of ATP and ADP levels demonstrated decreasing levels of ATP after 20 hr of drug exposure, declining to below 30% of control values after 30 hr and with a parallel increase of ADP levels (Fig. 4).

The first signs of cell death were observed after 40 hr of continuous exposure to CHS 828. At this point, cell death could be blocked by cycloheximide, an inhibitor of protein synthesis. However, many cells appeared to lose their membrane integrity in the presence of a relatively well preserved nuclear structure. Morphologically, the mode of cell death could be described as a combination of apoptosis and necrosis. Only a slight increase in caspase-3 activity, an effector of classical apoptosis, was observed at 40 hr,[2] and the kinetics and magnitude of cell death induced by CHS 828 could not be blocked by caspase inhibition (unpublished data).

CHS 828 has also been extensively compared to the structurally related benzylguanidines, MIBG and MGBG. In the correlation analysis using the cell line panel comparing CHS 828 with standard cytotoxic drugs, these compounds were included as references [5]. The correlations were fairly weak for MIBG and MGBG, and the shapes of the concentration-response curves were clearly dissimilar (Fig. 5). However, CHS 828 and several of its analogs produced an early increase in extracellular acidification in cells from cell lines and in fresh patient cells, similar to the increase induced by the mitochondrial inhibitor MIBG [30]. The increase in extracellular acidification rate could be explained by inhibition of mitochondrial respiration causing a compensatory increase in glycolytic activity and lactate production as for MIBG. The increase in extracellular acidification induced by CHS 828 was blocked by removal of glucose from the medium, supporting this notion.

Nevertheless, this mechanism alone does not explain the

[2] Martinsson P, de la Torre M, Binderup L, Nygren P, Larsson R. Manuscript submitted for publication.

cytotoxic action of CHS 828 since it induced cytotoxicity at concentrations well below those producing extracellular acidification [30]. Paradoxically, the substitution of glucose for pyruvate in the medium protected the cells from the cytotoxicity induced by CHS 828 in sharp contrast to the potentiation of cytotoxicity observed with MIBG. This protection could not be mimicked by addition of the glycolytic inhibitor 2-deoxy-glucose. These results may implicate a dependence on the hexose-monophosphate shunt for CHS 828-induced cytotoxicity, since this pathway may still be operative in the presence of 2-deoxy-glucose [90,91].

Thus, based on the data available, one might speculate that CHS 828 stimulates a yet unidentified apoptogenic pathway after a lag time requiring *de novo* protein synthesis. This stimulation may be dependent on intact hexose-monophoshate shunt activity (Fig. 4). The necrotic features of CHS 828-induced cell death may be due, in part, to the early and rapid decrease of ATP levels secondary to inhibition of mitochondrial respiration leading to inhibition of energy-dependent activation of caspase-mediated DNA fragmentation, as described by others [92]. However, the exact molecular mechanisms of cell death induced by CHS 828 remain to be clarified.

4.5. Pharmacokinetics and clinical use

In rats, the disposition of CHS 828 administered orally is characterized by a one-compartment model with the dissolution and absorption of drug by a consecutive zero order rate and a first order rate input with a calculated half-life of approximately 4 hr [93]. CHS 828 is presently in early clinical phase I/II trials, and the pharmacokinetics of CHS 828 in humans as well as the clinical outcome of these trials are not yet available.

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