CHAPTER SIX

Goralatide (AcSDKP) Selectively Protects Murine Hematopoietic Progenitors and Stem Cells Against Hyperthermic Damage

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From: Exp. Hematol. (1996) 24:246-252

ABSTRACT

Hyperthermic purging procedures may be improved by methods that selectively inhibits the proliferative activity of normal hematopoietic progenitors and stem cells since active proliferation of these subsets is accompanied by an increased heat sensitivity. For this reason, bone marrow cells from CBA/H mice were incubated with Goralatide (tetrapeptide Acetyl-N-Ser-Asp-Lys-Pro), a well known inhibitor of normal hematopoietic progenitor cells to enter the S-phase of the cell cycle. Subsequently, the cell suspensions were heat-treated at 43°C up to 90 minutes. After an exposure of 8 hours to 10^{-9} M Goralatide, the number of CFU-GM cells in S-phase decreased from ±30% to ±10%, resulting in an almost tenfold increase in survival after 90 minutes at 43°C. No effect on the primitive subsets could be detected because of their quiescent cell cycle state in normal bone marrow. In order to investigate the potential vulnerability of these subsets for Goralatide, bone marrow cells from 5-fluorouracil (5-FU) pretreated mice were used. The 5-FU induced increase in proliferative activity of the CFU-S-12 (colony-forming unit in the spleen at day 12) and the stem cell with marrow repopulating ability (MRA) could be abolished by an incubation period with 10^{-9} M Goralatide for 16 and 24 hours, respectively. Hence, this decrease in proliferative activity confers a decrease in hyperthermic sensitivity for the primitive hematopoietic subsets. The cytotoxic effect of the incubation on the absolute number of the hematopoietic progenitors and stem cells was <10%. A Goralatide (10^{-8}, 10^{-9} and 10^{-10} M) treatment up to 24 hours had no effect on the growth kinetics and cell cycle distribution and consequently on the hyperthermic sensitivity of L1210 cells. Based on these results, it can be concluded that Goralatide will have a positive effect on the survival of hematopoietic progenitors and stem cells after hyperthermia and may lead to a gain in the therapeutic window of this purging modality.

INTRODUCTION

Autologous bone marrow transplantation is widely used as treatment for hematopoietic malignancies in patients for whom no HLA-matched donor is available. A major problem in autologous bone marrow transplantation is the occurrence of relapse. It is unknown whether relapses result from residual disease in the patient or from malignant cells contaminating the autologous transplant; hence the use of purging techniques remained somewhat controversial. Recently, however, evidence was presented showing the necessity to purge remission bone marrow before transplantation (Rill *et al*., 1992; Brenner *et al*., 1993, 1994). An increasing set of data indicate the feasibility of hyperthermia as a purging modality (Moriyama *et al*., 1986; Da *et al*., 1989; Murphy and Richman, 1989; Gidali *et al*., 1990; Iwasawa *et al*., 1991; Moriyama *et al*., 1991; Herrmann *et al*., 1992; Moriyama *et al*., 1992, 1993). With respect to the hyperthermic sensitivity of the subsets within the hematopoietic stem cell compartment the primitive and quiescent hematopoietic stem cells with long-term repopulating ability (LTRA) and marrow repopulating ability (MRA) are less sensitive to hyperthermia compared to the committed and active proliferating subsets CFU-in the spleen at day 8 (CFU-S-8), CFU-granulocyte/macrophage (CFU-GM), burst-forming unit-erythroid (BFU-E) and CFU-erythroid (CFU-E) (Wierenga and Konings, 1993; Wierenga *et al*., 1995). The apparent relationship between the stem cell hierarchy and heat sensitivity is predominantly based on the differences in proliferative activity. Even within the same subset an increase in proliferative activity confers an increase in heat sensitivity (Baeza *et al*., 1989; Wierenga and Konings, 1990, 1991). It will be clear that the possibility of arresting active proliferating hematopoietic cells can be used to protect these cells in a hyperthermic purging protocol.

Goralatide (tetrapeptide Acetyl-N-Ser-Asp-Lys-Pro) is known to act as a selective inhibitor of normal hematopoietic progenitors to enter the S-phase (Monpezat and Frindel, 1989; Guigon *et al*., 1990; Bonnet *et al*., 1992; Cashman *et al*., 1994) and can therefore be considered as a potent protector of these cells in purging protocols. The introduction of a protected stem cell compartment without affecting leukemic cells will enhance hematopoietic recovery by conserving a larger population of progenitors and hence help to alleviate the spectre of bone marrow aplasia as a dose-limiting factor in cancer treatment. Apart from the effects of Goralatide on progenitors responsible for a transient hematopoietic repopulation, knowledge of the sensitivity of the primitive stem cells for Goralatide is relevant because these cells have the capacity for stable long-term hematopoietic reconstitution. The drug 5-fluorouracil (5-FU) drastically reduces the number of progenitors and nucleated cells in the bone marrow (Van Zant, 1984) and due to this myeloablative effect the proliferative activity

of the primitive stem cells is enhanced after a treatment with 5-FU (Harrison and Lerner, 1991). Hence, the sensitivity of the primitive subsets for Goralatide can be studied under these regenerative circumstances.

Therefore the effect of Goralatide on the proliferative activity and hyperthermic sensitivity of the different subsets in the hematopoietic stem cell compartment in normal and 5-FU pretreated mice was investigated and compared with the effect on leukemic L1210 cells to offer a possibility for its future use towards improvement of a hyperthermic purging protocol.

MATERIALS AND METHODS

Mice. Bone marrow from normal and 5-FU pretreated female CBA/H mice was used as the source of the hematopoietic progenitors and stem cells. The mice were bred at the Netherlands Energy Research Foundation, Petten, The Netherlands and maintained under clean conventional conditions in the animal facilities of the Department of Radiobiology, University of Groningen, The Netherlands. The mice were fed ad libitum with food pellets and acidified tap water (pH=2.8)

Hematopoietic cells. Normal bone marrow cells were obtained by flushing the femoral cell content with alpha-medium (Gibco BRL, Paisley, Scotland) buffered with 20 mM morpholinopropane sulphonic acid (Janssen Chimica, Beerse, Belgium) and in the presence of 5% fetal calf serum (Gibco). Regenerating bone marrow cells were harvested at the same way from the femurs of mice four days after an injection with 5-FU (150 mg/kg body weight) (Sigma, St. Louis, MO, USA) intravenously. The cell suspensions were dispersed through a 21 gauge needle. Nucleated cell count was performed on a Coulter Counter Model ZF (Coulter Electronics, Hialeah, FL, USA). The cell suspensions were kept on ice until use.

The CFU-S subset was determined using the spleen colony assay (Till and McCulloch, 1961). Starting one week before the irradiation, the recipients were put on acidified water supplemented with neomycin sulphate (3.5 g/l). The recipients were irradiated with a dose of 9.5 Gy by an X-ray source (Philips MG 300, 200 kVp, 15 mA, HVL 1.05 mm Cu at a dose rate of 0.5 Gy/min). The mortality of the recipients at day 12 did not exceed 10%. Control and heated cells were injected via the orbital sinusoid into 5-7 mice per time and temperature point. Spleen colonies were counted 12 days (CFU-S-12) after transplantation. Spleens were fixed in Bouin's solution before counting. No endogenous colonies could be detected in irradiated mice without bone marrow transplantation. The stem cells with marrow repopulating ability (MRA) were assayed as described by Ploemacher and Brons (1989). Donor cells were injected into

lethally irradiated mice and after 13 days aliquots of their femoral cells were assayed for the presence of cells capable of granulocyte-macrophage colony formation (CFU-GM) in vitro. In control irradiated mice no endogenous regeneration could be detected as assessed by the CFU-GM content of their femora at comparable aliquots to the experimental groups. The progenitor cells (CFU-GM) were assayed according to the technique of Iscove and Sieber (1975) in alpha-medium containing 0.8% methylcellulose (Fluka, Bachs S.G., Switzerland), 30% fetal calf serum, 1% deionized bovine serum albumin (Sigma, St.Louis, MO, USA) and 10^{-4} mol/l 2-mercaptoethanol (Merck, Darmstadt, Germany). The medium was buffered with bicarbonate and HEPES. The cultures were plated in 35-mm polystyrene culture dishes (Falcon, Becton and Dickinson, Etten-Leur, The Netherlands) and grown at 37°C in a 5% CO_2 humidified atmosphere. Colony growth was stimulated by the addition of pokeweed mitogen-stimulated spleen-conditioned medium (10 µl/ml). CFU-GM colonies (>50 cells) were scored after 8 days of culture.

Leukemic cells. Murine leukemic cells (L1210) were grown in suspension culture in RPMI-1640 medium (Gibco) supplemented with 10% fetal calf serum. Cultures were maintained in exponential phase at a density of 0.5-2.0 x 10^6 cells/ml. The colony forming ability was tested by applying 0.1 ml of an appropriately diluted sample to soft-agar plates made in RPMI-1640 medium supplemented with 20% horse serum (Gibco). The agar plates were incubated in a humidified CO_2-incubator at 37°C. Colonies containing more than 50 cells were counted after 7 days. The plating efficiency was always >90%.

Goralatide treatments. Goralatide (kindly provided by IPSEN-BIOTECH, Paris, France) was dissolved in alpha-medium as a stock solution of 10^{-7} M. Normal and regenerating bone marrow cells (1 ml) were plated out at a concentration of 2-3 x 10^6/ml in a 35 mm petri-dish and incubated up to 24 hours with and without Goralatide in alpha-medium and in the presence of 30% fetal calf serum and 1 µM Captopril (Sigma). Leukemic cells were incubated in RPMI-1640 medium supplemented with 10% FCS and 1 µM Captopril. After the incubation the cells were washed twice and plated out for the clonogenic assays.

Proliferative activity. The proliferative activity of the hematopoietic progenitor cells was determined by the hydroxyurea-kill assay as described before (Wierenga and Konings, 1990). Hydroxyurea (Sigma) was added (200 µg/ml) to the cell suspensions ± Goralatide after 0, 7, 15 or 23 hours, respectively and incubated for another 60 minutes at 37°C. Then the cells were washed and plated out for the clonogenic assays.

Hyperthermic treatments. The cell suspensions were placed in culture tubes at a concentration of 2-3 x 10^7/ml in alpha-medium (bone marrow) or RPMI-1640 medium (L1210) supplemented with 5% fetal calf serum. The hyperthermic treatments were performed at 43 ± 0.1°C and interrupted by chilling. Cells were diluted to the appropriate concentration and immediately used for the clonogenic assays.

FACS analysis. To determine the cell cycle distribution of the L1210 cells, leukemic cell suspensions were centrifuged after the treatment and washed with phosphate buffered saline plus 5 mM $MgCl_2$. The cell pellets were resuspended in ethanol/acetone (3:1) and kept at 4°C for minimal 18 hours. After this, 0.3 ml of a propidium-iodide solution (10µg/ml) and 10 µl of a RNAse solution (10 µg/ml) was added followed by an incubation of 30 minutes at 37°C. The cells were analyzed on a FACStar 550 (Becton Dickinson, Sunnyvale, CA, USA). The cell cycle distribution was calculated using the DNA Cell-Cycle Analysis Software-Ver C 12/86 (Becton Dickinson).

RESULTS

In Figure 6.1, the survival curves at 43°C of two different murine hematopoietic subsets and the murine leukemic cell line L1210 are shown. This figure clearly demonstrates the feasibility of hyperthermia as a purging modality. After 90 minutes at 43°C a four log cell kill of the leukemic cells is observed while at the same time the normal hematopoietic subsets show only a one to two log cell kill. The most primitive clonogenic subset MRA is less sensitive than the committed progenitor CFU-GM as was observed by us before (Wierenga and Konings, 1993).

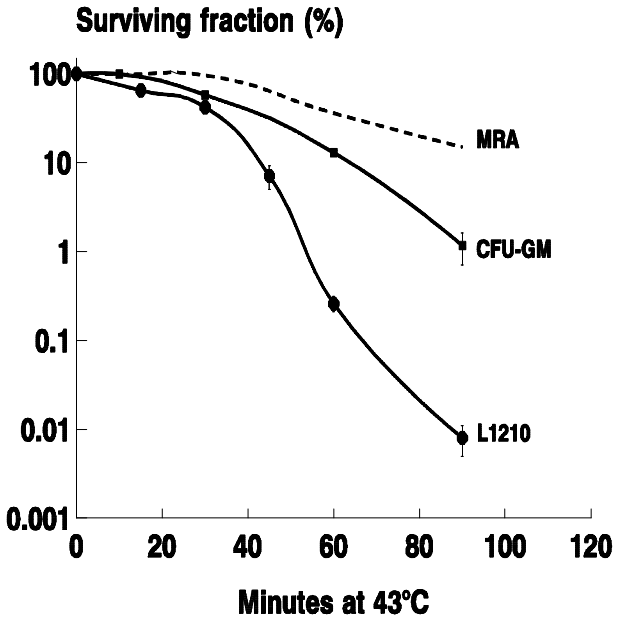

Figure 6.1. *Survival curves for the MRA and CFU-GM and leukemic L1210 cells at 43°C.* Bone marrow cells and L1210 cells are heat treated in culture medium as described in Materials and Methods. At the indicated time points the cell suspensions are put on ice and used for the colony forming assays. MRA data are adapted from a previous study (13). Data points for the CFU-GM and the L1210 cells are derived from 3 different experiments. Bars=SEM.

The effect of an 8 hour-incubation with increasing concentrations of Goralatide on both the percentage S-phase cells and the total number of CFU-GM is depicted in Figure 6.2. In normal bone marrow, about 30% of the CFU-GM appears to be in the S-phase of the cell cycle. This value decreases to about 10% after the incubation and reaches a nadir at 10^{-9} M Goralatide (Figure 6.2a). This concentration was used in the rest of the experiments. The total number of CFU-GM did not significantly change during the incubation period with and without Goralatide (Figure 6.2b).

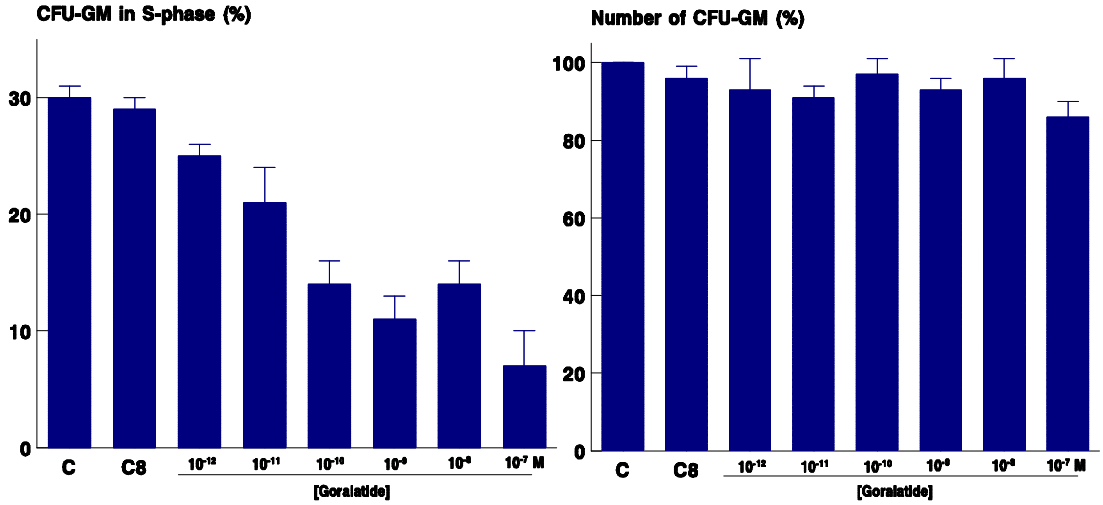

Figure 6.2. *Effect of Goralatide (10^{-12}-10^{-7} M) on the proliferative activity and the number of CFU-GM in murine bone marrow.* Bone marrow cells are incubated with and without Goralatide for 8 hours at 37°C. After 7 hours, hydroxyurea (200μg/ml) was added to duplicate cultures. After the incubation period, the cells were harvested, counted, and plated out for CFU-GM. N=3, bars = SEM.
C= control at t=0, C8 = control at t=8 hours.

Based on these results it can be concluded that an 8 hour-incubation with 10^{-9} M Goralatide is suitable for the determination of the inhibiting effect on the CFU-GM. We could not demonstrate an inhibiting effect of Goralatide on the primitive stem cells in normal bone marrow (data not shown), most probably because of the dormant proliferation state of these subsets. In bone marrow from 5-FU pretreated mice the proliferative activity of the primitive subsets is increased (Table 6.1) and hence the vulnerability for Goralatide can be studied under these circumstances. In Table 6.1, the effect of an incubation period up to 24 hours with 10^{-9} M Goralatide on the MRA, CFU-S-12 and CFU-GM in this regenerating bone marrow cell suspension is presented. It is demonstrated that also the primitive stem cell MRA, when forced into

cycle, can be inhibited by Goralatide. In contrast to the other two subsets an incubation period of 24 hours is needed. For the CFU-S-12 the inhibiting effect is already detectable after 16 hours. The number of S-phase cells in the CFU-GM population could be decreased comparable to the normal bone marrow situation where also 8 hours turned out to be the optimal incubation period.

Table 6.1. *Effect of Goralatide on the percentage S-phase cells of MRA, CFU-S-12, and CFU-GM in regenerating bone marrow.*

	Percent S-phase cells				
	Normal b.m.	Regenerating b.m.	t=8	t=16	t=24
MRA	0.3 ± 0.5	43 ± 9	37 ± 5	50 ± 14	7 ± 5
CFU-S-12	11 ± 4	41 ± 5	41 ± 14	16 ± 5	5 ± 2
CFU-GM	29 ± 1	58 ± 2	9 ± 3	18 ± 3	15 ± 2

Regenerating bone marrow cells (2-3 x 10^6/ml) were incubated at 37°C with 10^{-9} M Goralatide for 8, 16, or 24 hours. At 7, 15, or 23 hours, hydroxyurea (200 μg/ml) was added to duplicate cultures and incubated for another 60 minutes at 37°C. At the end of the incubation period, the cells were washed and used for the different clonogenic assays (n=3). For comparison, the values for normal bone marrow are included (Wierenga and Konings, 1993).

The effect of Goralatide on leukemic cells is demonstrated in Figure 6.3 where the relative increase in the number of L1210 cells during a 24 hour-incubation in the absence and presence of different Goralatide concentrations is shown. So, over two doubling times in control cells this number of cells did not significantly change after incubation with the tetrapeptide, indicative for the lack of inhibiting effect on the proliferation of these leukemic cells. In Table 6.2, the effect of 10^{-9} M Goralatide on the cell cycle distribution of L1210 cells is presented. The absence of an effect on the leukemic cells could be confirmed by these FACS analysis. The percentage of S-phase cells in the L1210 cell population is not significantly changed in contrast to the active cycling normal hematopoietic subsets (Table 6.1).

Figure 6.4a illustrates the ultimate effect of an 8 hour-incubation with 10^{-9} M Goralatide on the survival of the hematopoietic subset CFU-GM in normal bone marrow and L1210 cells as is demonstrated by the survival curves at 43°C. For the progenitors and stem cells in regenerating bone marrow a comparable effect could be observed (Figure 6.4b) although in this case cells were incubated for 24 hours with 10^{-9} M Goralatide. The hyperthermic sensitivity of leukemic L1210 cells remains unchanged in the presence of the tetrapeptide. From this figure it is clear that the

Goralatide treatment results in a 1-log gain of the therapeutic window of the hyperthermic purging protocol.

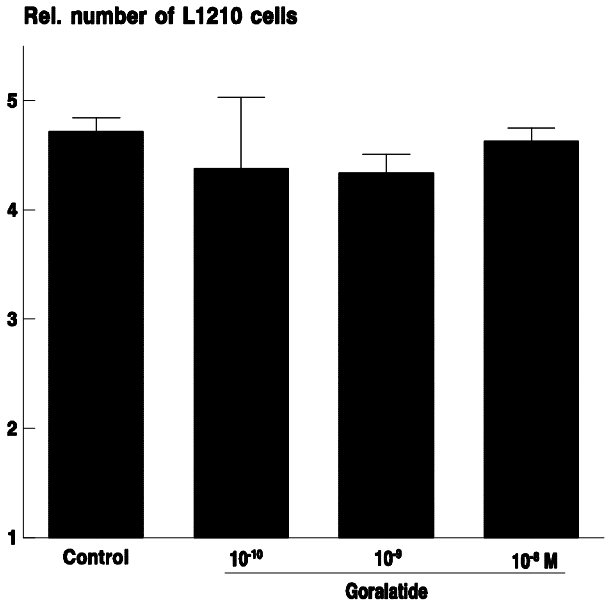

Figure 6.3. *The effect of Goralatide (10^{-10}-10^{-8} M) on the number of L1210 cells.* L1210 cells were incubated in the presence and absence of Goralatide for 24 hours in RPMI 1640 supplemented with 10% fetal calf serum and 1 μM Captopril. The total cycle time of L1210 cells is about 11 hours. The increase in cell number is related to the cell number at the start of the incubation period. Bars = SEM.

DISCUSSION

Hyperthermia can be considered as a potent purging modality, either alone (Moriyama *et al*., 1986; Gidali *et al*., 1990; Iwasawa *et al*., 1991 Moriyama *et al*., 1991; Herrmann *et al*., 1992; Moriyama *et al*., 1992, 1993) or in combination with cytostatic drugs (Okamoto *et al*., 1988; Higuchi *et al*., 1989; Gidali and Feher, 1992). To our knowledge there is only one study in which the absence of a selective effect between normal and leukemic cells was reported (Marie *et al*., 1989). These data were however error prone because of a probable technical artifact (Moriyama *et al*., 1990). In most published studies the hyperthermic effect on leukemic progenitor cells or leukemic cell lines was compared with that on the hematopoietic progenitor CFU-GM. The selective effect is also clearly demonstrated in the present experiments. It is important however to notice that the primitive subsets (MRA and LTRA), responsible for permanent long-term engraftment, are less heat sensitive than the committed progenitor CFU-GM (Wierenga and Konings, 1993; Wierenga *et al*., 1995) as is shown in Figure 6.1.

Although the survival curve for the MRA is adapted from a previous study (Wierenga and Konings, 1993) in which C57Bl/B6JIco mice were used, a direct comparison can be made because the CFU-GM from the two mice strains exhibited the same hyperthermic sensitivity.

Table 6.2. *Effect of Goralatide on the cell cycle distribution of L1210 cells.*

	G1	S	G2/M
Control	46 ± 12	41 ± 3	13 ± 20
t=3 without Goralatide	39 ± 5	43 ± 1	17 ± 13
t=3 with Goralatide	41 ± 9	38 ± 4	21 ± 31
t=8 without Goralatide	49 ± 5	40 ± 2	11 ± 23
t=8 with Goralatide	45 ± 4	46 ± 1	10 ± 27
t=24 without Goralatide	45 ± 3	54 ± 1	2 ± 38
t=24 with Goralatide	40 ± 11	50 ± 2	11 ± 27

L1210 cells were incubated up to 24 hours in the presence or absence of 10^{-9} M Goralatide. At the indicated time points, 1 ml was removed from the cell culture, counted, and pretreated for FACS analysis. Data are expressed as percent ± SD.

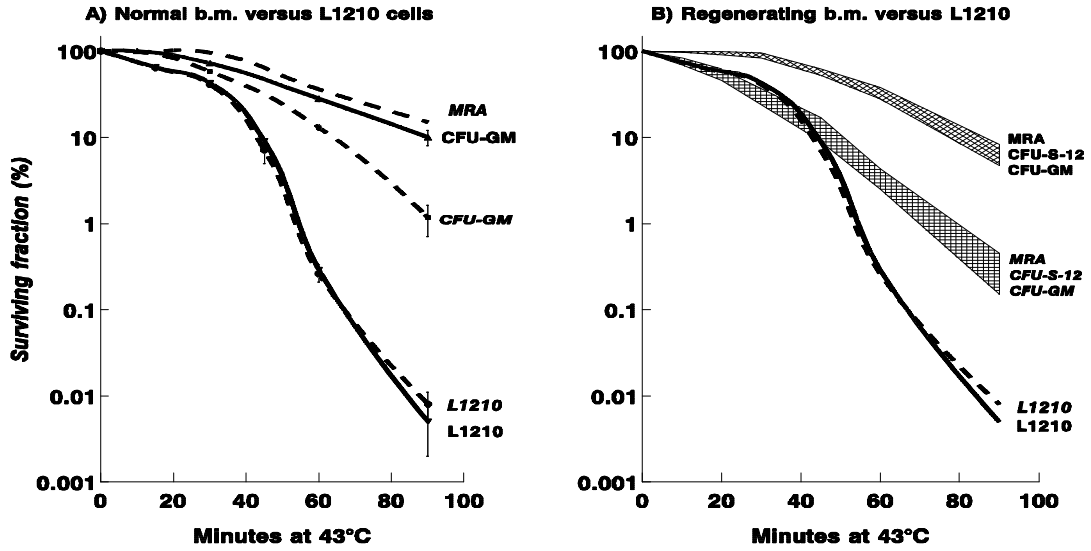

Figure 6.4. *Effect of Goralatide on the hyperthermic sensitivity of hematopoietic subsets in normal and regenerating bone marrow and leukemic cells at 43°C.* Normal/regenerating bone marrow cells were heat-treated. A) solid= with Goralatide; dashed= without Goralatide; n=3 for L1210 and n=5 for CFU-GM. B) For reasons of clarity only areas of the survival curves of the normal subsets in regenerating bone marrow are presented (n=3) and compared with those of L1210 cells.

Because of the observed differences in heat sensitivity between MRA and CFU-GM, a hyperthermic purging regimen could be determined from the effects on these primitive stem cells. However, the hyperthermic effect on CFU-GM is still relevant because a depletion of the progenitor pool prohibits the short-term reconstitution and will cause aplasia. This necessitates the protection of the progenitors without reducing the anti-tumor efficacy of the purging modality. As previously demonstrated, the heat sensitivity within the stem cell hierarchy is predominantly based on the differences in proliferative activity (Wierenga and Konings, 1993). A possible approach to increase the selectivity of a hyperthermic purging protocol towards killing of leukemic cells is decreasing the proliferative activity of the normal hematopoietic progenitors.

An inhibiting factor for CFU-S proliferation could be demonstrated in fetal calf bone marrow (Frindel and Guigon, 1977) which was characterized as a tetrapeptide Acetyl-N-Ser-Asp-Lys-Pro (Lenfant *et al*., 1989). This tetrapeptide is now chemically synthesized and called Goralatide. The protective effect of Goralatide is explained by the inhibition of entry of the cells into the S-phase (Guigon *et al*., 1990; Lombard *et al*., 1990). With respect to the *in vitro* effect of Goralatide on CFU-GM, the reported results are contradictory. Monpezat and Frindel (1989) and Robinson *et al*. (1993) could not detect an inhibiting effect on the proliferative activity of the murine CFU-GM. In these studies the cells were incubated for maximal 4 hours with the tetrapeptide. However, the total cycle time of the murine hematopoietic progenitors is 8 hours (Loeffler and Wichmann, 1985; Novak, 1994) and with a random distribution of the cells over the cell cycle, an incubation period of at least 8 hours should be used to determine an effect. In our hands the number of CFU-GM in S-phase decreased from 30% in the control situation to about 10% after an 8 hour-incubation (Figure 6.2). In other studies (Guigon and Bonnet, 1990; Bonnet *et al*., 1992, 1993) using the same range of Goralatide concentration, a decrease in the percentage progenitors in S-phase was reported but unfortunately this decrease was accompanied by a decrease in total number of progenitors after incubation periods varying from 1.5 to 24 hours. In these studies cells were incubated at low serum concentration because the tetrapeptide degrades in serum (Grillon *et al*., 1993; Rieger *et al*., 1993). These circumstances, however, might be considered as suboptimal culture conditions for the hematopoietic stem cells because certain viability factors may be insufficient present. Indeed, at low serum concentration (5%) the total number of CFU-GM was decreased in our experiments by almost 50% after a 24 hours incubation period (data not shown). The process of apoptosis may have led to the observed decrease in total number of progenitors (Sachs and Lotem, 1993). We therefore increased the serum concentration concomitantly with the addition of Captopril which prevents the degradation of Goralatide (Grillon *et al*., 1993).

Until now only data on the effect of Goralatide on the primitive hematopoietic stem cells in normal bone marrow have been published (Bonnet *et al*., 1992; Cashman *et al*., 1994). In normal bone marrow these primitive subsets are quiescent with respect to their cycling state. However, knowledge of the sensitivity of these cells when they are active proliferating is relevant. Especially in the clinical practice where bone marrow cells are usually collected after repeated courses of cytotoxic chemotherapy and an increased cycling rate for these primitive stem cells can be postulated. We could demonstrate an inhibiting effect of Goralatide on the proliferative activity of the primitive hematopoietic stem cells when 5-FU pretreated bone marrow was used (Table 6.1). The inhibitive effect was comparable to the effect on progenitor cells, however, a longer incubation time was necessary corresponding with the longer total cycle time for the primitive stem cells.

The total cycle time of the L1210 cells is about 11 hours. Since all these leukemic cells are in cycle, a block in the cell cycle caused by Goralatide would result in a decrease in the total number of cells after 24 hours incubation. As can be seen (Figure 6.3), the relative number of cells does not change after this incubation period with the tetrapeptide nor does the cell cycle distribution (Table 6.2). Thus, at concentrations which affect the normal hematopoietic stem cell proliferation, Goralatide has no effect on the proliferation of leukemic cells confirming other reports where no effect on leukemic cells (Bonnet *et al*., 1992; Cashman *et al*., 1994) or leukemic cell lines (Lauret *et al*., 1989) could be detected.

In our experiments the L1210 cells are less heat sensitive compared to the data reported by Symonds *et al*. (1981) and by Flentje *et al*. (1984). In those reports an exponential decrease in the survival curve at 43°C of the leukemic cells was shown without a shoulder region. At least two possible explanations can be given for this difference. Firstly, our cells were grown in vitro while the other cells grew as ascites in the mouse. Secondly, cell kill by hyperthermia was indirectly assessed by scoring animal kill by the tumor cells instead of a clonogenic assay. Bhuyan *et al*. (1977) demonstrated a difference in heat sensitivity between L1210 cells that grew as ascites and cells growing in culture both assayed in vitro by cloning in soft agar comparable to our experimental set-up. It appeared that ascites growing cells were more heat sensitive compared to cells grown in culture. Furthermore, the survival data in the current report resemble those of Bhuyan *et al*. (1977) where the survival curve was sigmoidal; i.e., a shoulder region followed by an exponential slope.

Our data indicate possibilities to improve hyperthermic purging protocols. This is illustrated in Figure 6.4a where the differences in heat sensitivity at 43°C between

normal bone marrow and leukemic cells are shown. The decrease in the proliferative activity of the CFU-GM leads to an increase in survival after the hyperthermic treatment almost reaching the level of the heat sensitivity of the MRA stem cells. In regenerating bone marrow (Figure 6.4b) the effect is even more pronounced because here the MRA and CFU-S-12 revealed a higher hyperthermic sensitivity than in normal bone marrow due to the increased proliferative activity. The Goralatide treatment, however, reduced the heat effects to levels comparable with normal bone marrow. The hyperthermic sensitivity of the L1210 cells is unaffected under the chosen conditions. It is clear that the therapeutic window will be increased by one log after a treatment of 90 minutes at 43°C. The presented data suggest the suitability of hyperthermia as purging modality of leukemic cells from autologous bone marrow transplants especially when combined with a negative regulator of hematopoiesis like Goralatide.