Protection From Arabinofuranosylcytosine and n-Mustard-Induced Myelotoxicity Using Hemoregulatory Peptide pGlu-Glu-Asp-Cys-Lys Monomer and Dimer

By Walter R. Paukovits, Marie-Hélène Moser, Konrad A. Binder, and Johanna B. Paukovits

We have previously shown that the synthetic peptide pGlu-Glu-Asp-Cys-Lys (pEEDCK monomer) inhibits the cytostatic drug-induced proliferation of hematopoietic stem cells (CFU-S). Keeping CFU-S quiescent by pEEDCK treatment renders them insensitive to cycle-specific cytostatic drugs and leads to reduced toxicity. Here we show that pEEDCK application during repeated (twice) administration of clinically relevant (nonlethal) 1-β-D-arabinofuranosylcytosine (Ara-C) doses reduced the percentage of CFU-S in S-phase from 60%–70% to 25%–30% and led to a sustained stem cell number in the bone marrow (BM), whereas unprotected mice had lost about 75% of their CFU-S population. Owing to its cysteine content, the pEEDCK monomer is easily oxidized. The resulting dimer (pEEDCK), is a potent stimulator of hematopoiesis.

As we show, it can be used for postchemotherapy acceleration of hematologic recovery, similar to the use of recombinant hematopoietic growth factors. A single injection of 30 μg/kg pEEDCK monomer to mice 2 hours before the second Ara-C injection retarded onset of neutropenia (by 2 to 3 days) and improved recovery after depression. The quantitative degree of neutropenia was not changed. Postchemotherapy (Ara-C administered twice, followed by N-mustard) infusion of the stimulatory (pEEDCK), dimer (1.4 μg/kg/d) produced a 4.6-fold increase of progenitor levels (6.7 CFU-GM/1,000 BM cells × 1.45 CFU-GM/1,000 in normal mice) 2 days after the end of the cytostatic treatment when CFU-GM were not detectable in unprotected mice. This increase was followed after several days by strongly elevated granulocyte counts, which remained high for ~1 week. Up to 75% of the peripheral leukocytes were mature polymorphonuclear leukocytes (PMN) during this phase. Ara-C (twice) and monomer treatment as above followed by dimer infusion resulted in the complete protection of hematopoiesis. Mice treated with the protective pEEDCK monomer plus stimulatory dimer did not develop the leukocyte depression noted in unprotected animals. The inhibitory monomer appears to keep the stem cell population numerically and qualitatively intact, thus providing optimum target cell conditions for the subsequent stimulator (dimer) treatment. Our results show that the hemoregulatory peptide monomer and dimer can be used for improving the hematologic status of mice treated with clinically relevant doses of cytostatic drugs (antimetabolite and alkylating, alone and in combination). Combining both peptides can prevent occurrence of neutropenia completely. Both peptides can be obtained easily by chemical synthesis and are also active on human cells. They are thus highly promising candidates for application as multilevel hemoprotectors in cancer chemotherapy.

© 1991 by The American Society of Hematology.

Hematologic Damage is the prime cause of complications associated with use of cytostatic drugs in cancer therapy. Of 24 most frequently used cytostatics, 22 (92%) lead to hematologic problems; 18 of the 22 (75%) cause severe damage. Such damage to the hematopoietic system occurs at two cellular levels: (a) the stem cell population, and (b) later hematopoietic compartments. Of these, the relatively mature populations (committed progenitors and mitotically competent precursors of the various lineages) are actively proliferating (40% to 50% in S-phase), whereas most stem cells are much less active under normal conditions (5% to 10% in S-phase).

Application of cycle-specific cytostatic drugs initially eliminates most of the proliferating cells in the production line of the hematopoietic system, resulting in a reduced output of mature cells and leukopenia (neutropenia) within a few days. The stem cell population is usually not a major primary target of the first drug application because most of these cells normally are not proliferating. After elimination of most of the more mature populations, these quiescent stem cells begin to proliferate, repopulating the hematopoietic system. In time, normal leukocyte levels are restored.

A single application of a cytostatic drug or drug combination usually will not eliminate a tumor, however. To reduce the tumor population significantly, several consecutive drug applications are often administered in intervals roughly comparable to the duration of a cell cycle.

At a second drug application and at any further drug applications, increased numbers of stem cells will be in a sensitive phase, however. Depending on the particular type of drug(s) applied, the stem cell population will not only be quantitatively reduced but the individual surviving stem cells also may be nonlethally damaged. Extremely dangerous situations may arise in two ways. First, the regenerative capacity of the hematopoietic system will be severely compromised after several consecutive drug applications. Reconstitution may occur very slowly or, in extreme cases, never. Second, nonlethally damaged stem cells will begin to repair their DNA, but at the same time will be under extreme proliferation pressure. They may replicate their DNA and divide before all types of repairable damage have actually been corrected. Fixation of genetic damage in this way may lead to development of secondary leukemia after cancer chemotherapy.

Numerous ways of protecting hematopoiesis and improving the hematologic condition after chemotherapy have been suggested. Of these, application of recombinant hematopoietic colony-stimulating factors (CSF) has recently gained considerable interest. In two situations, how-
ever, stimulator treatment may be problematic: (a) when not enough stem cells have survived the cytostatic treatment, and (b) when the stimulator forces also those surviving stem cells that have not yet completed their repair activities into proliferation.

Instead of forcing potentially damaged survivors into proliferation, we propose a different strategy for preventing stem cell damage: ie, keeping the stem cells in their initial quiescent state during drug applications and releasing them into proliferation only after completion of the cytotoxic treatment. We previously showed that the pentapeptide pyroGlu-Glu-Asp-Cys-Lys (pEEDCK), which we have isolated from normal human leukocytes,\(^14\) prevents the post-chemotherapy proliferation of hematopoietic stem cells CFU-S.\(^5\) pEEDCK appears to have a physiologic role in maintenance of low levels of CFU-S proliferation. We showed\(^17\) that removal of normal pEEDCK from the organism by immunization leads to increased CFU-GM numbers in the femora of mice. pEEDCK-like sequences have been identified in the G\(_{in}\) chain of GTP-binding proteins\(^8\) at positions 63 through 67, proximal to the major phosphorylation site. Free pEEDCK may compete with G\(_{in}\) regulatory sites on the effector, but this model would give pEEDCK and other similar peptides (eg, epidermal pEEDSG\(^9\)) a central role in proliferation regulation, a role that remains completely speculative at present. Recently,\(^11\) mutations in the G\(_{in}\) (gip2) gene were shown to result in oncogenic activation. Whether free biosynthetic pEEDCK originates from processing of the G\(_{in}\) protein or if a precursor protein different from G\(_{in}\) exists is not known.

This pEEDCK peptide, which is now easily available by improved methods of chemical synthesis,\(^12\) is equally active on human and murine hematopoietic cells.\(^13\) We showed that prevention of CFU-S proliferation by in vivo application of pEEDCK leads to good tolerance of otherwise lethal multiple Ara-C doses and an increased survival of peptide-treated mice.\(^3\) Oxidation of the cysteine thiol groups of pEEDCK leads to formation of a disulfide bridged homodimer (pEEDCK\(_2\)) which is a strong stimulator of hematopoiesis in vitro\(^14\) and in vivo.\(^3\) Because many hematologic demand situations (eg, infections) lead to leukocyte activation and production of oxidative molecules, a rapid conversion of inhibitory monomer to stimulatory dimer may occur. Formation of a stem cell stimulator within minutes may constitute an emergency mechanism for increasing blood cell production preceding the more slowly reacting synthesis of hematopoietic growth factors by gene activation and protein synthesis. This dimer may play a role similar to that of the recombinant hematopoietic growth factors for accelerating hematologic recovery after cytostatic drug treatment.

We present preliminary results from further animal experiments showing that (a) stem cell protection by the pEEDCK monomer can be achieved under clinically relevant conditions, (b) stem cell protection by the pEEDCK monomer leads to an improved neutropenia situation after cytostatic damage, (c) the (pEEDCK)\(_2\) dimer can be used to accelerate hematopoietic regeneration after chemotherapy, and (d) a monomer/dimer combination prevents development of neutropenia after repeated Ara-C applications.

**MATERIALS AND METHODS**

**Animals.** All in vivo experiments were performed with female BALB-c mice obtained from the breeding unit of the University of Vienna and kept under strict SPF conditions. The animals were age-matched to 8 to 12 weeks and had free access to food (autoclaved) and water (acidified). The experiments we describe were performed in accordance with Austrian legislation on animal experiments under permits GZ68 205/7-12/88 and GZ68 205/259-12/88.

**Chemicals.** All chemicals and solvents were of reagent grade and were obtained from Merck (Darmstadt, Germany). Culture media and sera were from Boehringer (Vienna, Austria). Ara-C was a gift from Upjohn (Vienna, Austria). N-Mustard (N-methylbis-(2-chloroethyl)-amine hydrochloride) was purchased from Aldrich Chemie (Steinheim, Germany).

**Hemoregulatory peptide monomer and dimer.** The hemoregulatory peptide monomer and dimer were synthesized, purified, and stored as described in detail previously.\(^12\) The monomer, which according to this technique was obtained as the oxidation-resistant, biologically inactive, mixed disulfide of pEEDCK and tert.butyl mercaptane (S-tert.butyl-sulfenyl-pEEDCK) was activated immediately before use in the following way: 10 \(\mu\)g S-tert.butyl-sulfenyl-pEEDCK was dissolved in 10 \(\mu\)L water and added to a solution of 100 \(\mu\)g dithioerythritol (DTE) in 10 \(\mu\)L 0.5 mol/L triethylamine in 20% aqueous methanol. After incubation at 37\(^\circ\)C for 30 minutes, the solvent and the tert.butyl mercaptane were removed in vacuo. The residue was dissolved in isotonic salt solution, sterile filtered, and diluted appropriately. We showed previously\(^17\) that this procedure completely prevents formation of stimulatory dimer. The pEEDCK was applied by intraperitoneal (IP) injection of 30 \(\mu\)g/kg in a 0.2 mL vol.

The dimer (pEEDCK)\(_2\) of the hemoregulatory peptide was synthesized as described previously\(^15\) by oxidation of S-acetamidomethyl-pEEDCK with iodine. It was applied by continuous infusion using Alzet osmotic minipumps (Alza, Palo Alto, CA; no. 2001 for 6-day infusion or no. 1003 for 3-day infusion). The pumps were filled and assembled under sterile conditions according to the instructions of the manufacturer and implanted under the dorsal skin of the mice under light ether anesthesia. The skin incision was closed with a standard surgical wound clip.

**Determination of CFU-S numbers and percentage in S-phase.** Pluripotent hematopoietic stem cells (CFU-S) were determined by the spleen colony-forming assay of Till and McCulloch.\(^16\) Six to 10 recipient mice in each group were irradiated with 8.5 Gy (1 Gy/min) and within 6 hours received 5.10\(^8\) to 1.10\(^9\) donor bone marrow (BM) cells (in 0.2 mL) into their lateral tail vein. After 9 days, the spleens were excised and fixed in Carnoy’s solution and the visible splenic nodules were counted under a stereomicroscope. The number of transplanted BM cells was adjusted to give 15 to 20 colonies per spleen from undamaged donor marrow.

Proliferating CFU-S were assayed by the suicide method with Ara-C as S-phase killing agent.\(^17\) The marrow cell suspension was divided into two aliquots. One was incubated for 1 hour at 37\(^\circ\)C with 10\(^{-3}\) mol/L Ara-C in RPMI 1640 medium, and the other was incubated with medium alone. After being washed twice with medium, the cells of both aliquots were suspended in equal volumes and 5 \(\times\) 10\(^4\) to 1 \(\times\) 10\(^5\) cells (contained in 0.2 mL) were injected into irradiated recipients for CFU-S determination. The percentage of CFU-S in S-phase was calculated as 100n\(_{\text{S}}\)/n\(_{\text{S}}\) where n\(_{\text{S}}\) and n\(_{\text{S}}\) are the node numbers in the suicided and control group, respectively.
Myeloid progenitors CFU-GM. Myeloid progenitors CFU-GM were cultured as described previously. In vitro colony formation of CFU-GM was induced with GM-CSF in media composed of α-MEM supplemented with 20% horse serum and containing methylcellulose (0.8%, 4,000 cps). Mouse lung conditioned medium was used as source of GM-CSF at a dilution yielding optimum colony growth. A limiting dilution method was used for quantitation of CFU-GM. The cultures were set up in microwell plates. Groups of 60 wells each contained 100, 300, 600, 1,000, 1,500 or 2,100 BM cells. After 7-day incubation, the percentage of wells containing no CFU-GM colony was determined by microscopic inspection. The number of CFU-GM was calculated from the slope of the regression line of In [percentage of empty wells] versus the number of cells plated per well.

RESULTS

CFU-S activation after Ara-C treatment of mice. Mice received two successive IP injections of up to 300 mg/kg Ara-C, 12 hours apart. At selected times after the second Ara-C injection, BM was obtained and the cell cycle status of the CFU-S was determined by the spleen colony and suicide techniques already described. The results are shown in Fig 1. The non–S-phase CFU-S that survive the second Ara-C injection continue active cycling and begin to repopulate the empty S-phase. Immediately after injection, the Ara-C concentration is apparently still too high to permit successful DNA synthesis, but after ~4 hours (ie, 12 half-life times) the first CFU-S begin to enter S-phase. In ~4 to 5 hours, a plateau is reached at which ~60% of the entire CFU-S population is synthesizing DNA; this high level is maintained until at least 15 hours after the second Ara-C injection.

pEEDCK (30 μg/kg) was applied between and after the two Ara-C injections. Independent of the time of application of the peptide, reduction in S-phase percentages of approximately 50% was observed (Fig 1). Our data show, however, that the kinetics of S-phase influx was not changed by the peptide.

We also determined the number of CFU-S surviving the double Ara-C treatment. In mice treated only with 2× Ara-C, the second drug application killed the S-phase cohort of the recruited CFU-S, leaving only 27% of them alive. When CFU-S cycling was inhibited with pEEDCK, no CFU-S were lost, as shown in Fig 2.

Effect of pEEDCK monomer on neutropenia and recovery after 2× Ara-C. The partial prevention of Ara-C-induced CFU-S cycling by pEEDCK should result in better leukocyte counts after several days as compared with controls not receiving the peptide. In an experiment designed to investigate this possibility, the mice were treated as before with 2× Ara-C, the second drug application killed the S-phase cohort of the recruited CFU-S, leaving only 27% of them alive. When CFU-S cycling was inhibited with pEEDCK, no CFU-S were lost, as shown in Fig 2.
leukocyte counts at nadir were about the same in both groups. Both treatment groups showed a considerable overshoot after their respective neutropenic phase. Whereas unprotected animals reached their maximum WBC levels rather slowly on days 14 through 17, the monomer-protected animals had maximum leukocyte counts on day 10 and already approached normal levels when the unprotected animals were still at their maximum (Fig 3).

Recovery acceleration by pEEDCK dimer. Recombinant hematopoietic CSFs have been used for improving hematopoietic recovery rates after cytostatic chemotherapy.\(^1\) The hematopoiesis-stimulating activity of the (pEEDCK)\(_2\) dimer led us to investigate possible uses of this synthetically available peptide in a similar setting. Mice received two injections of 300 mg/kg Ara-C at 0 and 12 hours as before as well as 2 mg/kg N-mustard at 24 hours. Our previous results showed (Fig 1) that at this time the CFU-S were actively proliferating, and N-mustard treatment should therefore cause severe hematopoietic damage. The (pEEDCK)\(_2\) dimer was applied as a 6-day continuous infusion of 1.4 μg/kg/d starting at 12 hours after the N-mustard injection. At various times after the start of the dimer infusion, the BM cellularity, the CFU-GM content in the femoral marrow, leukocyte counts, and the percentage of mature PMN in the peripheral blood were determined.

The results show that on day 2, when control CFU-GM were undetectable by limiting-dilution assay (Fig 4), the dimer-treated animals already showed an increase to levels 4.6-fold higher than normal (calculated per 10\(^3\) BM cells). Table 1 shows that the BM cellularity, although strongly reduced below normal levels, was higher by a factor of 1.8 in the dimer-treated mice as compared with mice not receiving dimer. Because CFU-GM levels were below the detection limit, quantifying the improvement achieved by dimer treatment was difficult. Table 1 shows, however, that the femoral CFU-GM content was at least 200-fold higher than that in mice not receiving dimer. Absolute CFU-GM numbers (per femur) were even higher than those of undamaged control mice.

This early increase of progenitor numbers was followed on day 6 by greatly increased leukocyte counts (Fig 5). Dimer-treated mice showed about threefold to fourfold increases in granulocyte counts as compared with mice not receiving the dimer infusion. As compared with normal untreated control animals, these dimer-treated mice had 10-fold higher granulocyte counts. Most notably (as shown in Fig 5), in the dimer-treated animals up to 60% to 70% (average) of the peripheral leukocytes were mature PMN on day 6, and some mice reached peak values of 75% to 80%. The increase in the total leukocyte count thus resulted almost exclusively from extremely elevated PMN values. After 9 days, these extreme values began to decrease but were still elevated on day 15 when animals not

![Graph showing the effects of pEEDCK monomer on development of neutropenia after Ara-C treatment.](image)

![Graph showing the augmentation by (pEEDCK)\(_2\) dimer of myeloid progenitors CFU-GM after combination treatment with Ara-C(2×) and N-mustard (HN2).](image)

![Graph showing the augmentation by (pEEDCK)\(_2\) dimer of myeloid progenitors CFU-GM after combination treatment with Ara-C(2×) and N-mustard (HN2).](image)

---

**Table 1. Femoral CFU-GM Content After Treatment With Ara-Ara-HN2 ± Dimer**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>BM Cells/Femur (Millions)</th>
<th>CFU-GM/1,000 BM Cells</th>
<th>CFU-GM/Femur</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent controls</td>
<td>12.2 ± 1.7</td>
<td>1.46 ± 0.02</td>
<td>17,800 ± 2,500</td>
</tr>
<tr>
<td>Ara-Ara-HN2</td>
<td>1.95 ± 0.25</td>
<td>&lt;0.05*</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Ara-Ara-HN2-dimer</td>
<td>3.50 ± 0.50</td>
<td>6.70 ± 0.07</td>
<td>23,500 ± 3,400</td>
</tr>
</tbody>
</table>

The treatment protocol was the same as that described in the legend to Fig 5: 48 hours after the start of dimer infusion, the BM cells were counted and the relative (per 1,000) and absolute (per femur) CFU-GM content was determined by limiting-dilution assay. Values are means ± SEM from five mice.

*Detection limit of the limiting-dilution assay.
HEMOPROTECTION BY STEM CELL INHIBITION

1317

treated with dimer had returned to normal levels. Between 2 and 4 weeks after treatment, all parameters had returned to approximately normal levels in both groups (data not shown).

Combination treatment with monomer and dimer. As we showed (Fig 3), application of pEEDCK monomer improved the hematologic situation of mice treated with 2 × 300 mg/kg Ara-C. In view of the beneficial results obtained with postchemotherapy dimer treatment (Table 1 and Fig 5), we investigated the effects of a combined treatment of monomer plus dimer in the 2× Ara-C protocol. Mice received 300 mg/kg Ara-C at 0 and 12 hours. Stem cell protection was achieved by injecting the pEEDCK monomer (30 μg/kg) at 10 hours, 2 hours before the second Ara-C application, as described. In addition, these mice also received a continuous dimer infusion (1.4 μg/kg/d) for 3 days, starting at 16 hours, 4 hours after the second Ara-C injection. Leukocyte counts were measured several times after the start of the dimer infusion. The results shown in Fig 6 indicate that during the entire observation period (≤ 21 days; data not shown) animals treated with monomer + dimer did not develop even a mild neutropenia.

DISCUSSION

Recent developments have shown the utility of stem cell inhibitors for protecting the hematopoietic system against damage by cytostatic drugs. Guigon et al showed that semipurified extracts containing a stem cell inhibitor could reduce the lethality of multiple Ara-C injections in mice. This inhibitor, a tetrapeptide, was later isolated and synthesized. Bogden recently showed that the synthetic tetrapeptide had a protective effect against the lethal effects of high Ara-C and cyclophosphamide doses. At lower doses, however, more relevant for the human clinical situation, no significant protective effects were observed. Because other data have shown that the CFU-S inhibitory effect of this tetrapeptide depends strongly on the application time, further data are needed to specify the conditions for protective effects.

The hemoregulatory peptide used in our studies was isolated from normal human leukocytes. Its sequence was determined as pyroGlu-Glu-Asp-Cys-Lys (pEEDCK), and improved methods were developed for its synthesis, providing protection against oxidation of the thiol group. We showed earlier that pEEDCK could protect mice against multiple lethal doses of Ara-C. We now wish to investigate the protective effects of pEEDCK under more clinically relevant conditions, using nonlethal doses of cytostatic drugs. Thus, we have studied the effects of cytostatics and the hemoregulatory peptide on proliferating stem cells (CFU-S) and possible consequences on parameters frequently used clinically to assess the hematologic status of cancer patients (eg, WBC counts). For this purpose, mice were treated twice with Ara-C (300 mg/kg). Elimination of most of the dividing BM cells by the first Ara-C injection led to initiation of CFU-S proliferation, as we showed previously. The purpose of the second Ara-C application was to eliminate CFU-S then in S-phase. This induced a considerable hematopoietic demand, and allowed investigation of the influx of CFU-S into the empty S-phase. The plasma half-life (1/2) of Ara-C in mice is ~20 min. Our data (Fig 1) show that CFU-S started to reenter S-phase ~4 hours after the second Ara-C injection and reach plateau levels between 8 and 9 hours. This level was maintained for several hours. The existence of a plateau may indicate that at 8 to 9 hours the first CFU-S were about to leave S-phase after completion of DNA synthesis, giving minimal S-phase

Fig 5. Leukocyte kinetics in mice receiving a (pEEDCK), dimer infusion after cytostatic treatment with an Ara-C(2×)/N-mustard combination. The treatment protocol was the same as that shown in Fig 4. At the indicated times, the total leukocyte number and the granulocyte (PMN) content were determined from Giemsa-stained peripheral blood smears. The black sector in each data point indicates the percentage of PMN in the total WBC count. Untreated mice had about 10% granulocytes. Each data point is the mean PMN count from five mice; SD deviation in the WBC counts was less than 15%.

Fig 6. Effects of pEEDCK monomer plus dimer on development of neutropenia after Ara-C treatment. Ara-C 300 mg/kg and pEEDCK monomer (30 μg/kg) were applied as described in the legend to Fig 3. The (pEEDCK), dimer was given as a continuous infusion with osmotic minipumps (1.4 μg/kg/day for 3 days) starting at 12 hours after the second Ara-C injection. The WBC count was determined at the indicated times. Each point represents the mean ± SEM of five mice.
length of ~4 to 5 hours. Our data also indicate that application of Ara-C at 12-hour intervals is a highly efficient regimen for numerical depletion of the CFU-S compartment. This also appears to be true in humans, in whom such highly efficient antitumor protocols led to severe hematologic problems in almost all patients.

Application of pEEDCK monomer leads to a reduction of the percentage of CFU-S in S-phase (Fig 1); consequently, further drug applications should cause less severe hematopoietic damage, as evidenced by our results (Figs 3 and 5 and Table 1). Figure 1 also shows that the kinetics of S-phase repopulation was not changed by application of pEEDCK monomer, indicating that the S-phase duration was the same in peptide-treated and in untreated CFU-S. Thus, the pEEDCK monomer may have a reducing effect on the G1-S transition frequency.

As we showed previously, the monomer (pEEDCK) and the dimer (pEEDCK) of the hemoregulatory peptide have opposite biologic activities on hematopoietic stem cells, the monomer acting as an inhibitor and the dimer as a stimulator. Our data suggest their use as hematologic protectors in cytostatic cancer chemotherapy. We have shown that stem cell inhibition with the pEEDCK monomer can maintain a sufficient number of stem cells in a quiescent state (where they are insensitive to cycle-specific cytostatic drugs) to enable the hematopoietic system to begin its regeneration after cytostatic treatment with a normal number (Fig 2) of undamaged, fully potent stem cells. This leads, as our data indicate, to improved recovery of functionally mature compartments after termination of cytostatic treatment. Our data (Fig 3) show that pEEDCK-treated mice experience a less severe neutropenia. The overall pattern appears to be consistent with the general properties of pEEDCK. Although the peptide has its strongest effect at the level of CFU-S and CFU-GM, it also exerts a moderate inhibitory effect on later populations (eg, myelocytes), which constitute most of the Ara-C-sensitive proliferating myeloid BM population. Thus, with pEEDCK treatment, early cells should be protected more efficiently than later cells, leading to a numerically pronounced Ara-C killing effect on the total BM cellularity and consequently leading to neutropenia. The protection and enhanced survival of CFU-S (Fig 2) and CFU-GM will then lead to an improved regenerative capacity, as shown in Fig 3.

Significant improvement of the neutropenia situation has also been achieved by a completely different strategy, using hematopoietic stimulators (CSFs) to accelerate proliferation of the myelopoietic progenitors that survive the cytostatic treatment. We have shown that the stimulatory dimer [(pEEDCK)] of the hemoregulatory peptide has a similar accelerating effect on hematopoietic regeneration after cytostatic combination treatment with Ara-C(2x) and N-mustard. Posttreatment dimer infusion led to an early increase in CFU-GM numbers (Table 1 and Fig 4) when these cells were below the detection limit in mice not treated with dimer. This was followed several days later by highly elevated leukocyte numbers (Fig 5) that remained at high levels for ~1 week. Up to 70% of the leukocytes were mature PMN during this period; normal BALB/c mice have ~10% PMN among their peripheral leukocytes. Thus, the highly hemospecific (pEEDCK), dimer, which is easily synthesized by chemical methods, appears capable of becoming a useful alternative to use of recombinant hematopoietic growth factors for improving the postchemotherapy recovery of WBC counts.

Keeping the neutropenic phase short is only one aspect of hematopoietic protection, however. We want to stress the importance of keeping the stem cell compartment qualitatively and quantitatively intact by specific stem cell protective measures. Depletion of the (unprotected) stem cell compartment of its most mature (ie, most ready to proliferate and differentiate) members will lead to a shifting of the age structure toward the later types and eventually to a situation in which the few “real stem cells” at the roots of the system also will be required to proliferate more intensely than they normally would. When that exceeds a certain limit, the result will be a reduction in the repopulation capacity of the system, leading to long-term deficiencies, although superficially everything appeared to be in order. Our preliminary evidence indicates that hemoprotection by stem cell inhibition (with the pEEDCK monomer) is effective, especially against such long-term damage that cannot be avoided by postchemotherapy stimulator treatment. On the basis of our results, we expect that recovery stimulation (by CSF or pEEDCK dimer) will lead to long-term benefits only when excessive stem cell killing can be prevented by inhibitor protection during the cytostatic treatment phase. Preliminary data (Paukovits et al, manuscript in preparation) indicate that this is indeed true.

Thus, the hemoregulatory peptide monomer may be a highly valuable adjunct to cytostatic cancer chemotherapy, leading to quantitative and qualitative improvements at the stem cell level. In particular, the dangers of stem cell exhaustion and secondary leukemia may be reduced by inhibiting stem cell proliferation during the most critical phases. The stimulatory (pEEDCK), dimer may then be used to accelerate hematopoietic recovery after completion of the cytostatic treatment, allowing nearly complete prevention of hematopoietic damage, (Fig 6).

Both peptides are easily available by chemical synthesis, they are highly specific for cells of the murine and human hematopoietic system, and they act reversibly without detectable side effects on other organs. Their combined use (monomer plus dimer) may allow prevention of short- and long-term hematopoietic damage.

ACKNOWLEDGMENT

We thank Ch. Balcarek for technical help, and M. Sachs for typing major parts of the manuscript. We thank U. Fuchs and B. Krenn of the Department of Radiology, Gynecological Clinic, University of Vienna, for providing irradiation facilities. The protection experiments we describe were performed by M. H. Moser in partial fulfillment of requirements for obtaining a degree as Doctor of Veterinary Medicine from the Veterinary University of Vienna.
22. Bogden AE: Amelioration of chemotherapy induced toxicity by co-treatment with a hematopoiesis inhibiting tetrapeptide acSDKP. Ann NY Acad Sci (in press)
Protection from arabinofuranosylcytosine and n-mustard-induced myelotoxicity using hemoregulatory peptide pGlu-Glu-Asp-Cys-Lys monomer and dimer

WR Paukovits, MH Moser, KA Binder and JB Paukovits