Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 111–115

BIOORGANIC & MEDICINAL CHEMISTRY

Sensitization of Hyperthermic Treatment of Leukemic Cell Lines by a Synthetic Peptide

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Received 14 May 2001; accepted 7 July 2001

Abstract—A compound named HPH–Pep, a peptide constructed from pyridine and histidine units, showed sensitizing effect on the hyperthermic treatment of L-1210, Molt-4, and HL60 cells. The survival rate of these cells was greatly reduced by combined treatment with heating at 44 °C and HPH–pep. The treatment of L-1210 and Molt-4 cells with HPH–Pep resulted in a significant breakdown of the survival rate at 44 °C. The cell death induced by HPH–Pep under hyperthermic condition seemed to involve iron and peroxide.

Introduction

The significance of hyperthermia, the elevation of body or tissue temperature in cancer therapy, has recently been recognized in addition to the conventional therapeutic means including surgery, radiotherapy, chemotherapy, and immunotherapy.[1,2] However, the hyperthermic treatment of leukemia has been considered to be still difficult and the development of effective sensitizers is strongly desired, especially for the whole body hyperthermia. Previously tetraplatin, carboplatin, and thymidine have been reported to be useful sensitizers for the hyperthermic treatment of cancer.[3–5] We also reported the combined effects of hyperthermic treatment and doxorubicin on killing mouse leukemic cells.[6] We report herein a new candidate for sensitizing agent that shows remarkable effects on the basal model on the cultured cells, namely L-1210, Molt-4, and HL60.

Previously, we reported a peptide named HPH–Pep (Fig. 1), constructed from pyridine and histidine units, based on the simplification and symmetrization of the metal binding site of an antitumor antibiotic bleomycin.[7] The ferrous complex of HPH–Pep did not generate active oxygen species whereas the bleomycin ferrous complex is known to activate molecular oxygen to produce ferric peroxo species.[7] Instead, the copper complex of HPH–Pep was shown to have oxygen radical quenching activity with an intriguing physico-chemical profile.[8,9] We have now found that HPH–Pep shows cytotoxic effect on the above cancer cells with heat treatment.

Materials and Methods

Cells

Mouse leukemia L-1210 cells and human leukemia Molt-4 and HL60 cells were cultured with RPMI-1640 medium that includes 10% fetal calf serum, 10 μg streptomycin, 100 U/mL penicillin and 10 mM HEPES.

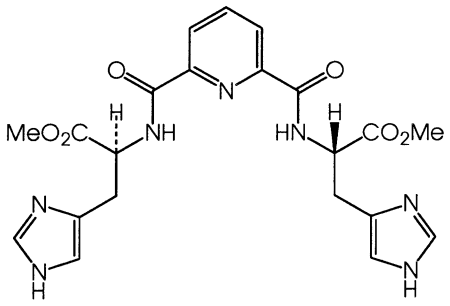

Figure 1. Structure of HPH–Pep.

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0968-0896/02/$ - see front matter
PII: S0968-0896(01)00246-2

Treatment with hyperthermia

Heat treatment was carried out by soaking the cultured flask for 180 min in a water bath adjusted to the designed temperature for the experiment.

Chemicals

HPH–Pep was prepared as described,[8] dissolved in DMSO (10 μM concentration), and kept at −80 °C at freezer.

Survival rate

The survival rate were determined by dye exclusion tests with 0.4% trypan blue solution under phase contrast microscope, and were calculated on the average out of quadripartite measurements.[6]

DNA fragmentation

L-1210 cells or Molt-4 cells were collected and suspended in a DNA extraction SDS buffer containing 100 μg/mL protein kinase K. The mixture was incubated at 55 °C for 120 min. After the addition of phenol solution saturated with TE, the whole was centrifuged at 7500 rpm for 12 min. The supernatant collected was treated with RNase (1 μg/mL) and incubated at 37 °C for 60 min. DNA was precipitated with ethanol and dissolved in TE buffer. The DNA samples were subjected to electrophoresis in 2% agarose gel and visualized by ethidiun bromide staining.

Effect of catalase

L-1210 cells ($10^4 \sim 1.5 \times 10^4$) were mainly used for the experiment. To exclude the hydrogen peroxide, catalase (500 U/mL) was added to the experimental system.

Results and Discussion

Hyperthermic effect of HPH–Pep

Figure 2 shows the survival rate for the effects of hyperthermic treatment with the various concentrations of HPH–Pep on killing L-1210 cells (A), Molt-4 cells (B), and HL60 cells (C). Whereas HPH–Pep did not show any cytotoxic effect without heating, the survival rate was greatly reduced by combined treatment with heating at 44 °C and HPH–pep. L-1210 cells were particularly sensitive and marked effect of HPH–Pep was observed at 8.4 μM concentration, resulting in the survival rate of 6.4%. As 8.4 μM HPH–Pep was effective on L-1210, we examined the effect of 10 μM DMSO, the solvent used in the experiments. It was found that DMSO (10 μM) showed virtually no effect on the survival rate of L-1210 either at 37 °C (survival rate 75.9% without DMSO, 69.8% with DMSO) or 44 °C (49.7% without DMSO, 45.0% with DMSO). This effect of HPH–Pep seemed to be comparable, or even superior, to doxorubicin since we previously found that the treatment of L-1210 cells with 20 μM of doxorubicin hydrochloride at 44 °C resulted in 19.2% survival rate.[6] It is evident that the killing effect of HPH–Pep on L-1210, Molt-4, and HL60 cells were markedly enhanced by heat treatment.

Figure 3 shows the influence of HPH–Pep on the hyperthermic treatment (37, 41, 42.5, and 44 °C) of L-1210 cells (A) and Molt-4 cells (B). Although the survival rate of both L-1210 cells and Molt-4 cells did not decrease very much upon hyperthermic treatment, the treatment with HPH–Pep resulted in a significant breakdown of the survival rate at 44 °C for both cells. Thus, it was found that the hyperthermic effect was enhanced by the treatment with HPH–Pep.

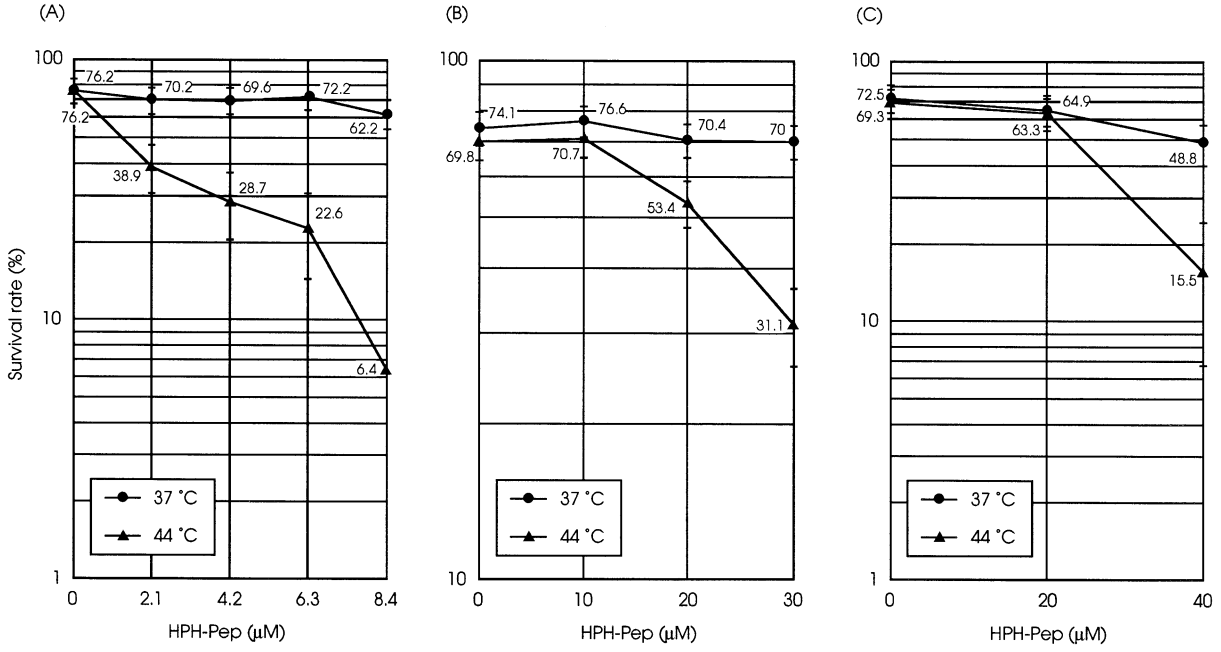

Figure 2. Sensitizing effect of HPH–Pep on the hyperthermic treatment of L-1210 (A), Molt-4 (B), and HL60 (C) cells.

Mechanism study

It seemed possible that the cell death induced by HPH–Pep is apoptosis since our structurally related histidine–pyridine compound induced apoptosis on AsPC-1 cells without heating, as reported previously.[10] Therefore, we examined the internucleosomal DNA fragmentation of L-1210 and Molt-4 cells treated with HPH–Pep under hyperthermic condition. Figure 4 shows internucleosomal fragmentation of DNA at 4 μg/mL

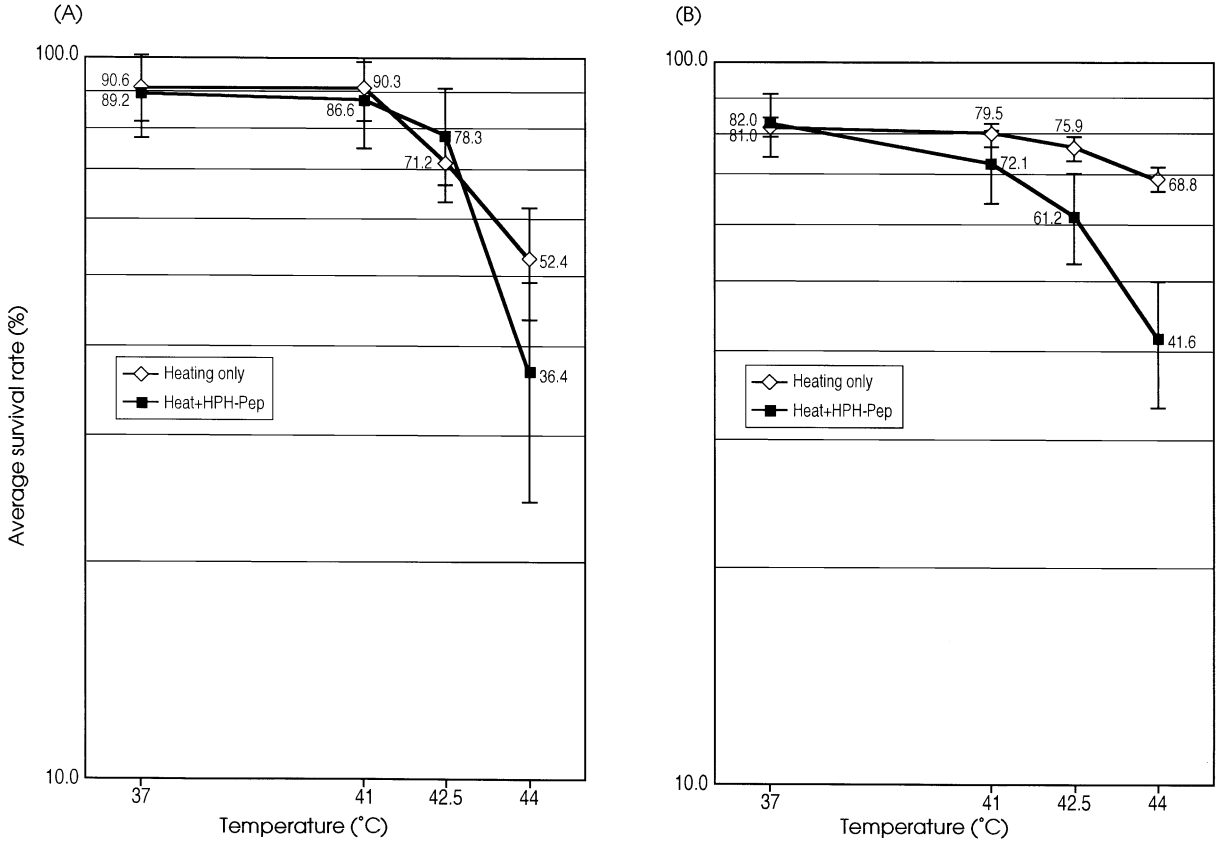

Figure 3. Influence of temperature on the hyperthermic treatment of L-1210 (A) and Molt-4 (B) cells in the presence of HPH–Pep.

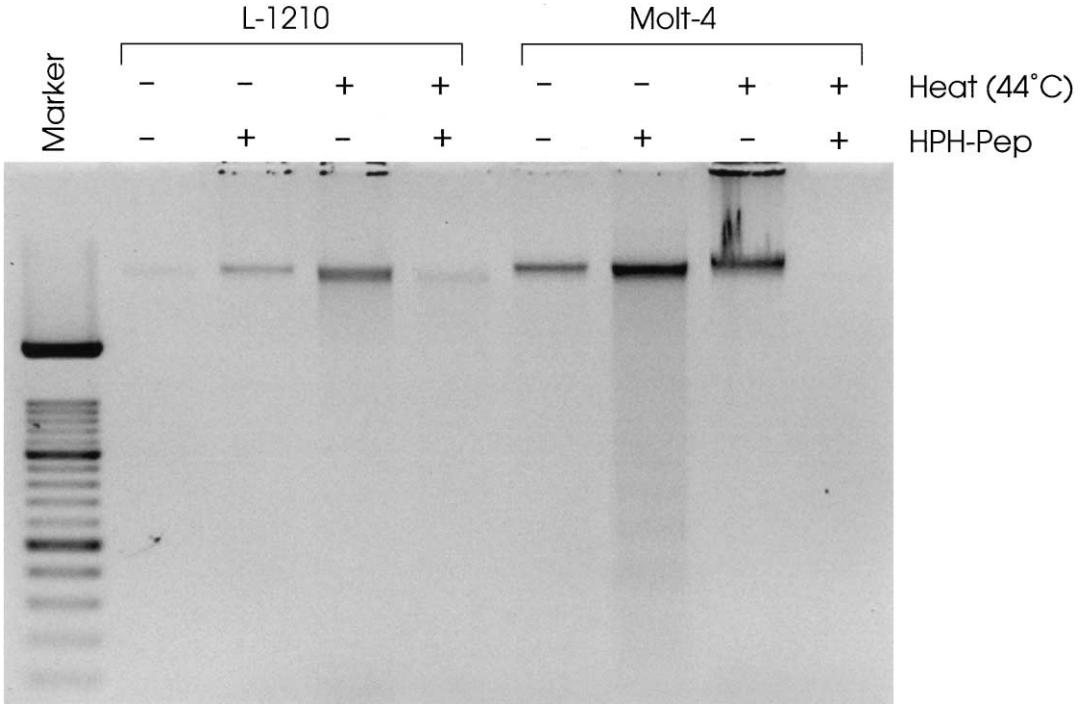

Figure 4. DNA fragmentation of L-1210 and Molt-4 cells treated with HPH–Pep under hyperthermic condition.

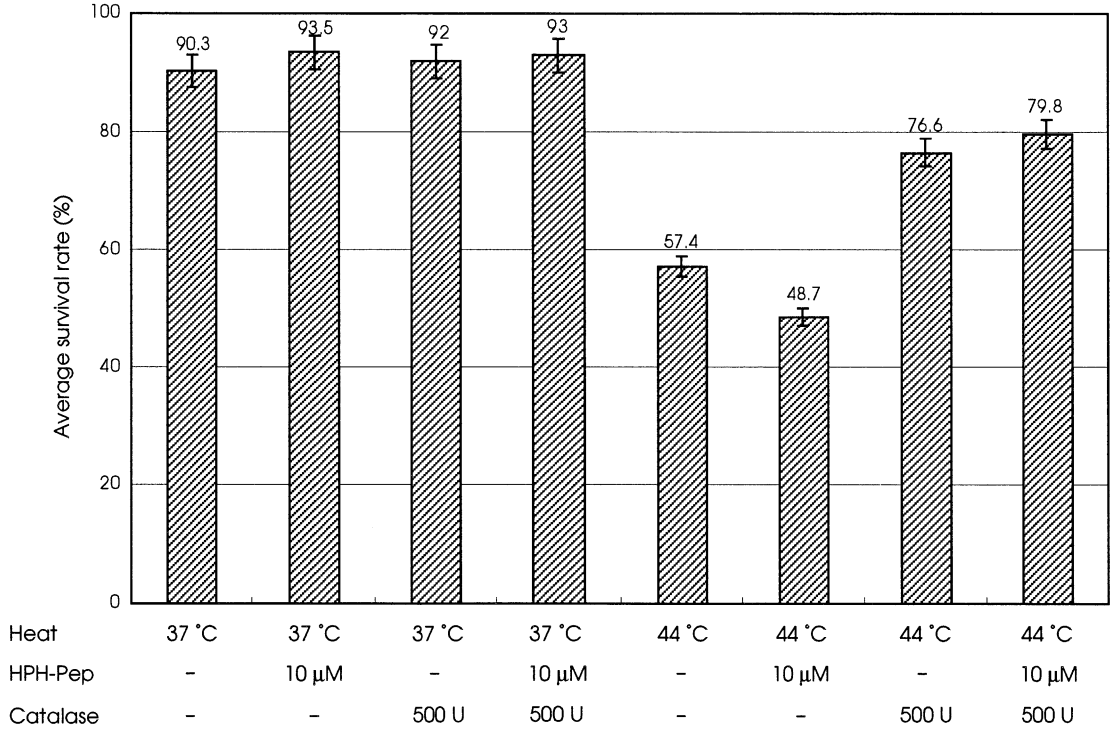

Figure 5. Influence of catalase on the hyperthermic treatment of L-1210 cells in the presence of HPH–Pep.

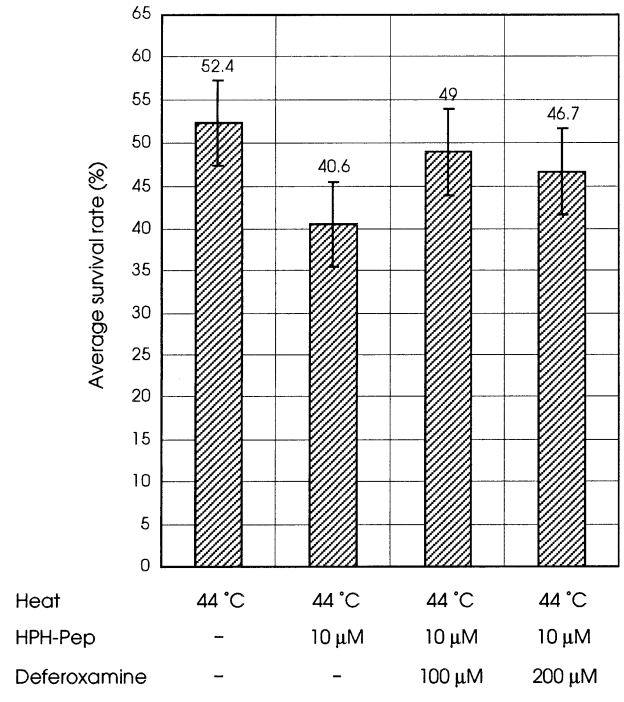

Figure 6. Influence of deferoxamine on the hyperthermic treatment of L-1210 cells in the presence of HPH–Pep.

induced by HPH–Pep. The apoptotic DNA fragmentation of L-1210 and Molt-4 cells was not observed, resulting in the total fragmentation under the hyperthermic condition.

Figure 5 shows the influence of catalase on the hyperthermic effect of HPH–Pep. The presence of catalase recovered the decrease in the survival rate of L-1210 cells induced by heating and HPH–Pep. Although this suggests an involvement of peroxide species in the decrease of the survival rate by heating, participation of peroxide species in the effect of HPH–Pep is not clear.

Figure 6 shows the influence of deferoxamine, a specific iron chelator, on the hyperthermic treatment of L-1210 cells in the presence of HPH–Pep. The hyperthermic effect of HPH–Pep was cancelled by the addition of 100 or 200 μM concentration of deferoxamine, suggesting participation of iron that presumably forms the HPH–Pep–iron complex that may be responsible for the peroxide species. Virtually the same effect of deferoxamine was observed in the case of Molt-4 cells (data not shown).

Conclusion

A compound named HPH–Pep, a peptide constructed from pyridine and histidine units, showed sensitizing effect on the hyperthermic treatment of L-1210, Molt-4, and HL60 cells. The survival rate of these cells was greatly reduced by combined treatment with heating at 44 °C and HPH–pep. The treatment of L-1210 and Molt-4 cells with HPH–Pep resulted in a significant breakdown of the survival rate at 44 °C. This effect seemed comparable to that of doxorubicin. The cell death induced by HPH-Pep under hyperthermic conditions seemed not apoptotic and involvement of iron is suggested. Whereas the mode of the hyperthermic activity remains to be elucidated, we have demonstrated that HPH–Pep is promising as a sensitizer for the hyperthermia therapy of cancer.

Acknowledgements

The authors thank Mrs. Nariko Kureyama for the experimental assistance. The present study was financially supported in part by a grant from Japanese Society of Hyperthermic Oncology.

References and Notes

1. Robins, H. I. *Cancer Res*. **1984**, *44*, 4878s.
2. Robins, H. I.; Hugander, A.; Cohen, J. D. *Radiol. Clin. North Am*. **1989**, *27*, 603.
3. Cohen, J. D.; Robins, H. I. *Cancer Res*. **1987**, *47*, 4335.
4. Cohen, J. D.; Robins, H. I. *Int. J. Hyperthermia* **1990**, *6*, 1013.
5. Cohen, J. D.; Robins, H. I.; Javid, M. J. *J. Neurooncol*. **1990**, *9*, 1.
6. Hori, Y.; Hisaka, Y.; Kawasaki, S.; Tsunoda, S. *Jpn. J. Hyperthemic Oncol*. **1998**, *14*, 251.
7. Otsuka, M.; Satake, H.; Sugiura, Y.; Murakami, S.; Shibasaki, M.; Kobayashi, S. *Tetrahedron Lett*. **1993**, *34*, 8497.
8. Otsuka, M.; Satake, H.; Murakami, S.; Doi, M.; Ishida, T.; Shibasaki, M.; Sugiura, Y. *Bioorg. Med. Chem*. **1996**, *4*, 1703.
9. Kurosaki, H.; Sharma, R. K.; Aoki, S.; Inoue, T.; Okamoto, Y.; Sugiura, Y.; Doi, M.; Ishida, T.; Otsuka, M.; Goto, M. *J. Chem. Soc., Dalton Trans*. **2001**, *2001*, 441.
10. Suginaka, R.; Izui, R.; Inoue, J.; Muraoka, Y.; Yamaguchi, K.; Otsuka, M.; Umezawa, K. *Jpn. J. Cancer Res*. **1998**, *89*, 947.