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Effect of Hyperglycemia on Thermochemotherapy of a Spontaneous Murine Fibrosarcoma[1]

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ABSTRACT

The effect of hyperglycemia on the cytotoxic effect of a chemotherapeutic agent was studied at elevated temperature. The seventh generation of a spontaneous C3Hf/Sed mouse fibrosarcoma, FSa-II, was transplanted into the footpads of the same strain of mice. Hyperthermia was given by immersing animal feet into a water bath where 41.5 ± 0.1° (range) was maintained by a constant temperature circulator. Cyclophosphamide (CY), an alkylating agent, was selected as a test agent. Single i.p. doses of glucose and of CY were given 60 and 30 min before the initiation of hyperthermia. Tumor response was assayed by determining the median tumor growth time (time required for one-half of the treated tumors to reach 1000 cu mm), and the dose-response curve was obtained.

Hyperthermia enhanced tumor response to CY. The enhancement was greater when a glucose dose of 10 mg/g was administered before CY and heat treatments. The enhancement ratio, calculated as a ratio of the tumor growth time following CY (200 mg/kg) with heat or with glucose and heat to that following CY alone, was 1.31 or 2.86, respectively. Glucose given at ambient temperature did not enhance the effect of CY. A dose-response curve obtained for a glucose dose with fixed CY (200 mg/kg) and heat (90 min at 41.5°) doses demonstrated that a significant enhancement was obtained following a glucose dose as low as 2 mg/g, suggesting that the glucose enhancement could be safely achieved for human cancer treatment.

INTRODUCTION

Hyperthermia enhances the response of cells to radiation and chemotherapy (2). It has been disclosed that the thermal sensitivity of cultured mammalian cells and of murine tumor cells increases with a decreasing environmental pH (6, 19, 22). The tumor tissue pH is lower than the blood or normal tissue pH because of anaerobic glycolysis in hypoxic tumor cells which results in an accumulation of lactic acid (7). Direct measurement of tissue pH and of interstitial fluid pH proved the low tumor tissue pH (7, 12, 17). Notably, the thermal enhancement is more predominant with decreasing pH (6). In this situation, additional reduction in the tumor tissue pH could enhance thermal response of the tumor more substantially than that of the normal tissue. Recent studies have demonstrated that the selective reduction in tumor tissue pH could be successfully obtained by artificial hyperglycemia (11, 18).

Hyperthermia enhances the cytotoxic effect of various chemotherapeutic agents, including thioTEPA, CY,[4] bleomycin, and Adriamycin. A variety of mechanisms has been proposed (1, 8, 9, 13). The magnitude of the enhancement apparently increases with increasing heat dose or with increasing thermal damage. Accordingly, hyperglycemia which could selectively reduce tumor tissue pH may induce more thermal damage in the tumor tissue than in the normal tissue with a resultant differential sensitization between the 2 tissues. We have studied the effect of hyperglycemia on the thermochemotherapy of a murine fibrosarcoma. CY, one of the most commonly used alkylating agents for human cancer treatment, was selected as a test agent.

MATERIALS AND METHODS

Animals were 10- to 12-week-old C3Hf/Sed mice derived from our defined-flora mouse colony (21). They were kept in an animal facility where defined flora conditions have been maintained. Wayne Lab Blox and acidified vitamin K-fortified water were provided *ad libitum*. Seventh generation isotransplants of a spontaneous fibrosarcoma in the C3Hf/Sed mouse, FSa-II, were used throughout. Single-cell suspensions were prepared by trypsinization, and the number of viable tumor cells was counted by the trypan blue exclusion method. Approximately 2×10^5 unstained cells in 5 μl of a single-cell suspension were transplanted into the right footpad. Details of these procedures are given elsewhere (23).

Hyperthermia was given in a water bath where a desired temperature ± 0.1° (range) was maintained by a constant temperature circulator (Lauda Model B-1; Lauda, West Germany). Each animal was kept in an individual holder with a short arm where the animal leg was gently taped. Animal holders were placed on a plastic plate which had holes through which animal legs were immersed into the water bath. Tumor temperature in the 4-mm tumor was equilibrated within 90 sec and was no less than 0.1° below the water bath temperature (22).

Tumor response was examined by TG time analysis (22). Tumors were treated when they reached an average diameter of 4 mm. Three diameters of the tumor, a, *b*, and *c*, were measured at least 3 times a week, and the tumor volume was calculated by $\pi abc/6$. The TG time was obtained on a graph, and the median value was calculated by logit analysis for each dose group. All data presented were results of at least 2 experiments. Seven or 8 mice were used in each dose group.

Test agent was CY (Mead Johnson & Co., Evansville, Ind.). The agent was dissolved in 0.9% NaCl solution immediately before use and injected i.p.

Glucose (50% dextrose; Elkins-Sinn, Inc., Cherry Hill, N. J.) was given i.p.

RESULTS

The effect of a single CY injection on the TG time was studied as a function of drug dose. CY alone slightly prolonged the TG time, while hyperthermia for 90 min at 41.5° enhanced tumor

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[4] The abbreviations used are: CY, cyclophosphamide: TG, time for a tumor to reach 1000 cu mm; ER, enhancement ratio; Ti, time interval between treatments.

response to CY (Chart 1). The TG time following a CY dose of 200 mg/kg given alone or with hyperthermia was 14.5 (13.3 to 15.9) or 19.0 (17.6 to 20.6) days, respectively. A substantial enhancement was observed when glucose was administered before CY and hyperthermia. The time interval between glucose and hyperthermia was 60 min, and that between CY and hyperthermia was 30 min. The TG time following the same dose of CY was prolonged to 41.4 (35.2 to 48.8) days. The ER was hardly obtained by routine methods, since the dose-response curve was exponential only at low CY dose and exhibited a downward concave nature. Therefore, the ER was expressed as a ratio of TG times following the same amount of CY. It was 1.31 and 2.86 for CY (200 mg/kg) given with heat alone and for that given with glucose and heat, respectively, as compared with CY alone.

The second experiment investigated if glucose enhanced the response to CY at ambient temperature. A glucose dose of 0, 5, or 10 mg/g was administered 30 min before CY. Small differences observed between the TG times following glucose (5 and 10 mg/g) were not significant, indicating that the glucose enhancement was observed only at elevated temperature (Chart 2).

The glucose effect was investigated as a function of time at 41.5°. CY and glucose doses were 200 mg/kg and 10 mg/g, respectively. The glucose enhancement of heat damage appeared to be maximal following 60 min (Chart 3). A similar effect was seen for combined CY and heat. The *solid line* in the chart indicates that 41.5° hyperthermia with or without glucose did not prolong the TG time.

The next experiment investigated the effect of glucose as a function of dose (Chart 4). A fixed treatment time of 90 min at 41.5° and a fixed CY dose of 200 mg/kg were used. The Ti was the same as in the previous experiments; *i.e.*, Ti between glucose administration and hyperthermia and Ti, between CY injection and hyperthermia were 60 and 30 min, respectively. The TG time was prolonged following injection of glucose (2 mg/g). A slight but definite prolongation of the TG time was observed with increasing glucose dose.

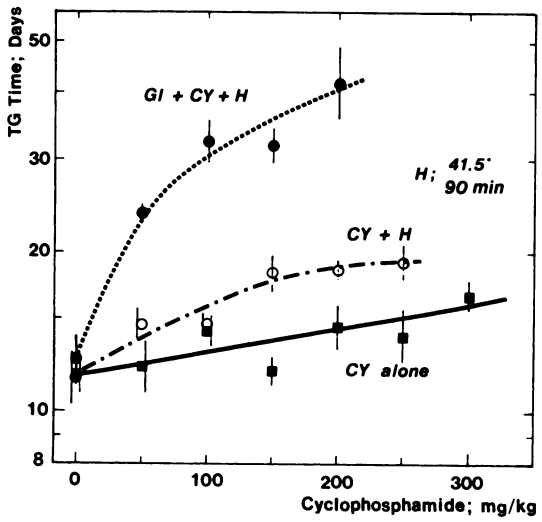

Chart 1. Effect of hyperglycemia on thermochemotherapy. The FSa-II tumors were treated with CY alone, CY and hyperthermia (*H*), or glucose (*Gl*), CY, and heat. Hyperthermia was for 90 min at 41.5°, and the glucose dose was 10 mg/g. The TG time was shown as a function of CY dose. *Bars*, 95% confidence limits.

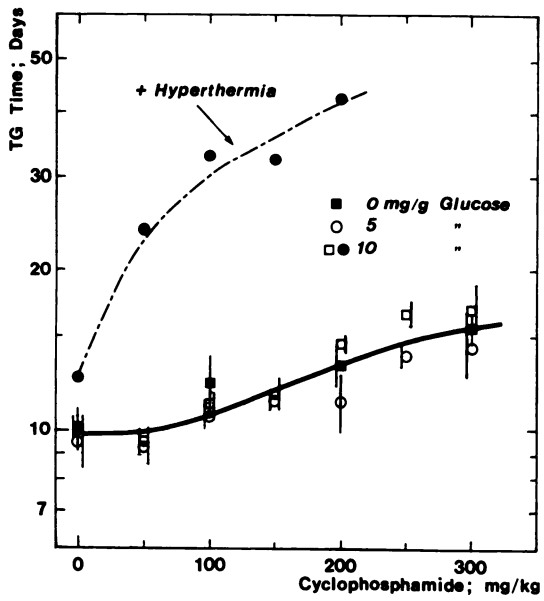

Chart 2. Effect of hyperglycemia on the cytotoxic effect of CY at normal temperature. The FSa-II tumor-bearing animals received single doses of glucose and CY without hyperthermia. The TG time is shown as a function of CY dose. ●---●, taken from previous chart for comparison. *Bars*, 95% confidence limit.

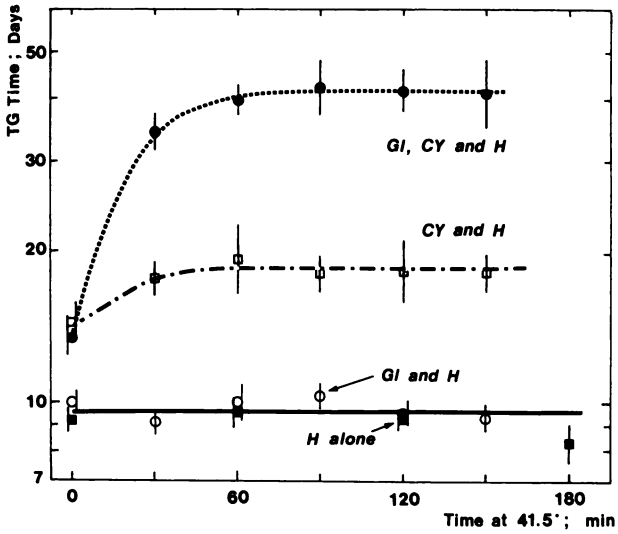

Chart 3. Effect of hyperglycemia on thermochemotherapy as a function of treatment time at 41.5°. A glucose (*Gl*) dose of 10 mg/g and a CY dose of 200 mg/kg were given 60 and 30 min before the initiation of hyperthermia (*H*), respectively. *Bars*, 95% confidence limits.

We studied the glucose effect on the preheated tumors. The 4-mm FSa-II tumors were heated for 90 min at 41.5°, and 24 hr later, when thermotolerance was fully developed, a single dose of CY was given alone, with hyperthermia, or with glucose and hyperthermia (Chart 5). The TG time following CY (200 mg/kg) given alone, with heat, or with glucose and heat was 11.3 (10.9 to 11.7), 12.0 (11.5 to 12.5), or 30.7 (23.0 to 40.9) days, respectively. The ER was 1.06 and 2.72 for CY (200 mg/kg) given with heat and that given with glucose and heat, respectively, indicating that glucose enhanced the cytotoxic effect of CY on preheated cells. However, the comparison of Chart 5 with Chart 1 suggested the ER at low CY doses is smaller for preheated cells.

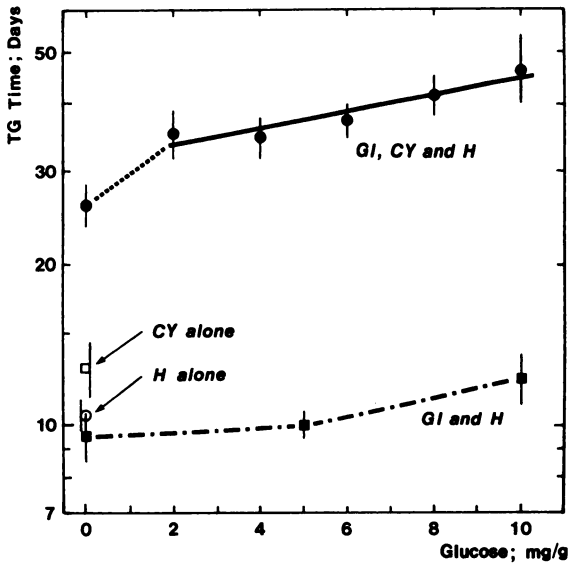

Chart 4. Effect of hyperglycemia on thermochemotherapy as a function of glucose dose. A fixed CY dose of 200 mg/kg was given 30 min before hyperthermia (for 90 min at 41.5°). Glucose (*GI*) was administered i.p. 60 min before heat treatment (*H*). ●, combined glucose, CY, and heat treatments; ■, glucose and heat treatments without CY. *Bars*, 95% confidence limits.

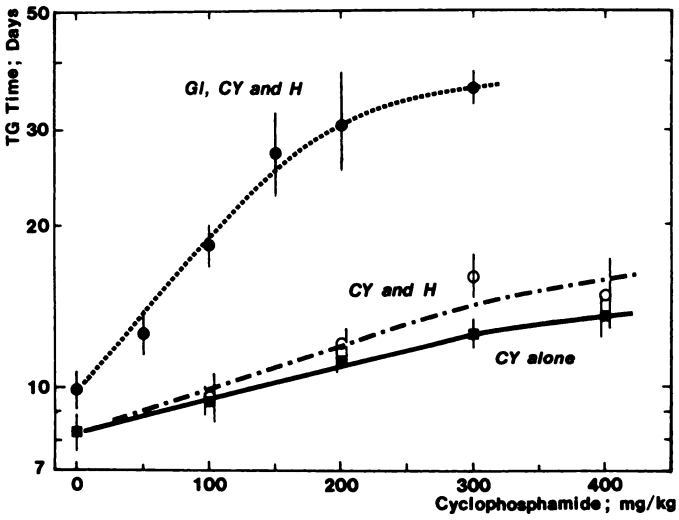

Chart 5. Effect of hyperglycemia on thermochemotherapy given to FSa-II tumors heated previously. The 4-mm tumors were treated for 90 min at 41.5°, and 24 hr later, they received CY given alone, with hyperthermia (*H*), or with glucose (*GI*) and heat treatments. The TG time is plotted as a function of CY dose. *Bars*, 95% confidence limits.

Finally, we conducted a study on the normal tissue damage. Non-tumor-bearing feet were treated for 90 min at 41.5° with or without CY and/or glucose. CY and glucose doses were 200 mg/kg and 10 mg/g, respectively. All animals demonstrated no sign of damage or only slight epilation in the treated foot, regardless of the type of treatment.

DISCUSSION

Hyperglycemia exhibits 2 biological effects on animal tissues; one is to facilitate glucose metabolism, and the other is to increase extracellular fluid osmolarity. It has been demonstrated that hyperglycemia selectively reduces tumor tissue pH (11, 18). Presumably, hyperglycemia stimulates insulin production and release which eventually activates all pathways of glucose metabolism. In the hypoxic cells, anaerobic glycolysis may be facilitated with a resultant accumulation of lactic acid, which undoubtedly reduces tumor tissue pH. Glucose is an important molecule which controls extracellular fluid osmolarity, and an increase in blood glucose is associated with an increase in the osmotic pressure which could lead to intracellular dehydration and osmotic diuresis. Relative increase in the concentration of lactic acid may be the result of intracellular dehydration. Furthermore, the osmotic diuresis may lead to hypovolemia and hypotension with a resultant decrease in oxygen supply to tissues. It has been observed that blood flow in some animal tumors decreases following glucose injection (3, 24). Accordingly, hyperglycemia could reduce tissue pH by 2 different mechanisms.

The present study demonstrated that the hyperglycemia enhanced the cytotoxic effect of CY at elevated temperature. Although no attempt was made to disclose the mechanism, it is likely that the reduced tissue pH is a key event in glucose enhancement. Namely, reduced pH enhanced thermal damage (lethal and sublethal) and eventually enhanced thermal sensitization of a chemotherapeutic agent. However, the present study does not eliminate the possibility that the increased osmotic pressure, although it may contribute to the pH reduction, could be a mechanism. At elevated temperature and low pH, alkylation might have been enhanced, or the repair of CY-induced cross-linking of DNA (14) might have been inhibited. Further studies regarding the mechanism are in progress in our laboratory.

It is more than encouraging that a glucose dose of 2 mg/g substantially enhanced thermochemotherapy. Clinically, the glucose tolerance test utilizes a p.o. dose of 100 g which is slightly less than the 2-mg/g mouse dose in comparison to average adults, indicating that a significant glucose effect could safely be obtained in human cancer treatments. Local infusion together with insulin may be another potential method.

The development of thermotolerance is a critical phenomenon in hyperthermia (5, 10, 15, 20). It has also been reported that the cells heated once become resistant to subsequent drug administration (16). Present study indicated that the preheated cells are slightly resistant to subsequent thermochemotherapy. It is encouraging, however, that hyperglycemia enhanced thermochemotherapy against preheated cells. It may have a significant clinical implication, although further studies on the kinetics of thermotolerance and drug (thermo) tolerance are indicated.

It should be taken into account that the dose-response curve for CY was downward concave (Chart 1). This may be due to the saturation phenomena of the CY or to the presence of a CY-resistant cell population. The CY resistance might be a result of nonuniform pH distribution in the tumor, a result of the presence of tumor cells with decreased drug uptake or with increased repair of CY-induced damage, or a result of thermal vascular damage which limits the drug defusion. The presence of a plateau in the dose-response curve for time at 41.5° (Chart 3) appeared to be due to the rapid exclusion from the animal body of the CY (4).

The mild normal tissue damage observed following combined glucose and thermochemotherapy suggests that a great therapeutic gain could be obtained by this combined modality. The toxicity of chemotherapeutic agents may be systemic, including bone marrow depression and mucositis. Accordingly, studies on systemic effects of the treatment are indicated, since the local hyperthermia may increase whole-body temperature as well.

Furthermore, it is of interest to study glucose enhancement using other types of chemotherapeutic agents.

REFERENCES

1. Braun, J., and Hahn, G. M. Enhanced cell killing by bleomycin and 43° hyperthermia and inhibition of recovery from potentially lethal damage. Cancer Res., *35:* 2921–2927, 1975.
2. Dewey, W. C., Hopwood, L. E., Sapareto, S. A., and Gerweck, L. E. Cellular responses to combinations of hyperthermia and radiation. Radiology, *123:* 463–473, 1977.
3. Dickson, J. A., and Calderwood, S. K. Temperature range and selective sensitivity of tumors to hyperthermia; a critical review. Ann. N. Y. Acad. Sci., *335:* 180–205, 1980.
4. Foley, G. E., Friedman, O. M., and Drolet, B. P. Studies on the mechanism of action of cytoxan, evidence of activation *in vivo* and *in vitro*. Cancer Res., *21:* 57–63, 1961.
5. Gerner, E. W., and Schneider, M. J. Induced thermal resistance in HeLa cells. Nature (Lond.), *236:* 500–502, 1975.
6. Gerweck, L. E. Modification of cell lethality at elevated temperatures. The pH effect. Radiat. Res., *70:* 224–235, 1977.
7. Gullino, P. M., Grantham, F. H., Smith, S. H., and Haggerty, A. C. Modifications of the acid-base status of the internal milieu of tumors. J. Natl. Cancer Inst., *24:* 857–869, 1965.
8. Hahn, G. M. Thermochemotherapy: interactions between hyperthermia and chemotherapeutic agents. *In:* Proceedings of the International Symposium on Cancer Therapy by Hyperthermia and Radiation, Washington, D. C., April 28 to 30, 1975, pp. 61–65. American College of Radiology.
9. Hahn, G. M., and Strande, D. P. Cytotoxic effects of hyperthermia and Adriamycin on Chinese hamster cells. J. Natl. Cancer Inst., *57:* 1063–1067, 1976.
10. Henle, K. J., and Dethlefsen, L. A. Heat fractionation and thermotolerance: a review. Cancer Res., *38:* 1843–1851, 1978.
11. Jähde, E., and Rajewsky, M. F. Tumor-selective modification of cellular microenvironment *in vivo*: effect of glucose infusion on the pH in normal and malignant rat tissues. Cancer Res., *42:* 1505–1512, 1982.
12. Jähde, E., Rajewsky, M. F., and Baumgärtl, H. pH distributions in transplanted neural tumors and normal tissues of BDIX rats as measured with pH microelectrodes. Cancer Res., *42:* 1498–1504, 1982.
13. Johnson, H. A., and Pavelec, M. Thermal enhancement of thio-TEPA cytotoxicity. J. Natl. Cancer Inst., *50:* 903–908, 1973.
14. Loveless, A., Cook, J., and Wheatley, P. Recovery from the 'lethal' effect of cross-linking alkylation. Nature (Lond.), *205:* 980–983, 1965.
15. Maher, J., Urano, M., Rice, L., and Suit, H. D. Thermal resistance in a spontaneous murine tumor. Br. J. Radiol. *54:* 1086–1090, 1981.
16. Morgan, J. E., Hones, D. J., and Bleehen, N. M. The interaction of thermal tolerance with drug cytotoxicity *in vitro*. Br. J. Cancer, *39:* 422–428, 1979.
17. Myer, K. A., Kammerling, E. M., Arntman, L., Killer, M., and Hoffman, S. J. pH studies of malignant tissues in human beings. Cancer Res., *8:* 513–518, 1948.
18. Naeslund, J., and Swenson, K. E. Investigations on the pH of malignant tumors in mice and humans after the administration of glucose. Acta Obstet. Gynecol. Scand., *32:* 359–367, 1953.
19. Overgaard, J., and Bichel, P. The influence of hypoxia and acidity on the hyperthermic response of malignant cells *in vitro*. Radiology, *123:* 511–514, 1977.
20. Rice, L., Urano, M., and Maher, J. The kinetics of thermotolerance in the mouse foot. Radiat. Res., *89:* 291–297, 1982.
21. Sedlacek, R. S., and Mason, K. A. A simple and inexpensive method for maintaining a defined flora mouse colony. Lab. Animal Sci., *27:* 667–670, 1977.
22. Urano, M., Gerweck, L. E., Epstein, R., Cunningham, M., and Suit, H. D. Response of spontaneous murine tumor to hyperthermia: factors which modify the thermal response *in vivo*. Radiat. Res., *83:* 312–322, 1980.
23. Urano, M., Nesumi, N., Ando, K., Koike, S., and Ohnuma, N. Repair of potentially lethal radiation damage in acute and chronically hypoxic tumor cells *in vivo*. Radiology, *118:* 447–451, 1976.
24. Von Ardenne, M., and Kruger, W. The use of hyperthermia within the frame of cancer multistep therapy. Ann. N. Y. Acad. Sci., *335:* 356–361, 1980.