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The Combined Effect of Fast Neutron and Hyperthermia according to the Sequence and Interval in MKN-45 Cells

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Purpose : It has been well established that the response of cells and tissues to low LET radiations (X- or gamma-ray) can be enhanced by combining with hyperthermia. However, there has been relatively little work of hyperthermia on the possible modification of either cellular or tissue responses to other types of radiation. So, we investigated the combined effect of fast neutron irradiation and hyperthermia according to the sequence and time interval of the two.

Materials and Methods : In MKN-45 cells, a human stomach cancer cell line, surviving fractions were measured according to the sequential treatment of 6, 4, 2, 0 hour-interval for fast neutron irradiation (1.5 Gy) combined with hyperthermia (41℃ for 30 min or 43℃ for 30 min).

Results : D_0 and n of MKN-45 for neutron were 0.8 Gy and 2.5, respectively. The surviving fraction by 1.5 Gy of neutron was 0.36±0.34. Interacting powers were mostly ranged between 1 and 2, but they were 3.0 and 2.7, respectively for hyperthermia (41℃ for 30 min) followed by neutron irradiation 6 and 4 hours later.

Conclusion : The combined effect of fast neutron (1.5 Gy) and hyperthermia (41℃ or 43℃ for 30 min) is largely independently additive. Preceding mild hyperthermia (41℃ for 30 min) 4 or 6 hours before neutron may cause decreased sensitivity to subsequent neutron irradiation.

Key Words : MKN-45, Fast neutron, Hyperthermia, Sequence, Interval

INTRODUCTION

It has been well established that the response of cells and tissues to X irradiation can be enhanced by combining the radiation treatment with hyperthermia. Thermal enhancement ratio (TER) is dependent upon the severity of the hyperthermia, the treatment sequence and the interval between the two modalities. Although many data are available for thermal enhancement of x-irradiation damage, there has been relatively little work on the possible modification of either cellular or tissue responses to other types of radiation. There is, however, evidence that the radiosensitizing effects of heat is related to the quality of the radiation, low LET (linear energy transfer) radiations being affected to a greater extent than high LET radiations.[1~4] In most studies of combination of high LET radiations and hyperthermia, the time interval of the two was short; irradiation shortly after hyperthermia, or vice versa. Although, from the data with low LET radiations, it can be hypothesized that as the interval of the two modalities is shorter the combined effect is higher, there is no published data which supports the hypothesis as far as we know. So, as a model of combined effect of high LET radiation and hyperthermia we investigated the change of cell survival according to the sequence and time interval of fast neutron irradiation and hyperthermia.

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MATERIALS AND METHODS

1. Cells

MKN-45, a human stomach adenocarcinoma cell line, which was in the exponentially growing phase, was used for this study. The cells were maintained in T-25 flasks (Costar, USA) which contains RPMI-1640 medium (Gibco, USA) supplemented with 10% heat inactivated fetal bovine serum (Gibco, USA), 100 U/ml of penicillin and 100 μg/ml of streptomycin (Gibco, USA) at 37℃ in a highly humidified atmosphere of 5% CO_2 as described previously.[5]

2. Sequence and interval of neutron irradiation and hyperthermia

Neutron of 1.5 Gy and hyperthermia of two temperatures (41℃ for 30 min and 43℃ for 30 min) were combined with the intervals of -6, -4, -2, -0 (5 min), 2, 4, 6 hours. Negative means hyperthermia before neutron irradiation. During the interval of the two treatments the T-25 flasks were put at 37℃, 5 % CO_2 incubator.

3. Neutron irradiation

Neutron irradiation was undertaken at room temperature with the cyclotron (MC 50, Scanditronix, Sweden) which is installed in Korea Cancer Center Hospital (KCCH).[6] The neutron beam was produced by 50.5 MeV protons bombarding a beryllium target. Irradiation dose was calculated at the depth of 1.5 cm in the field size of 20× 20 cm with a dose rate of 0.3 Gy per minute. During irradiation, the T-25 flasks were placed over the tissue-equivalent material with 10 cm thickness to receive back-scattering effectively, and also the tissue-equivalent material with 1.5 cm thickness was put over the plates to make Dmax point be located on the surface of the culture media.

4. Hyperthermia

For hyperthermia T-25 flasks were immersed into a constant-temperature water bath (±0.01℃) (Techne B-18, Techne, UK) with a digital immersion circulator (Tempette TE-8D, Techne, UK) as described previously.[5] Temperature of cell suspension was measured with a digital thermometer (BAT-8, Baily, USA). Duration of hyperthermia was counted from the time attained to the desired temperature.

5. Measurement of sensitivity to neutron and hyperthermia

Sensitivity to each or combined modalities was assessed by cell surviving fraction. Surviving fraction was measured by colony-forming ability using limiting dilution method as described previously.[5] The radiation survival curve parameters were determined by a least-squares regression analysis of all data points. D_0 is the inverse of the slope of the survival curve, and the extrapolation number (n) is the back extrapolation of the slope to the ordinate. All experiments were repeated 4 times independently. Interacting power was calculated by Streffer and Müller[7] as below.

$$Sc = Sx \cdot Sh \cdot I$$

if, Sc = Surviving fraction after combination of neutron irradiation and hyperthermia

Sx = Surviving fraction after neutron irradiation alone

Sh = surviving fraction after hyperthermia alone

I = Interacting power

According to Streffer & Müller we interpreted the power as follows; I>1 as sub-additive interaction, I=1 as independent additive action and I<1 as supra-additive interaction.

RESULTS

D_0 and n of MKN-45 for neutron irradiation were 0.8 Gy and 2.8, respectively (Fig. 1). The surviving fractions by 1.5 Gy of neutron, 41℃ for 30 min and 43℃ for 30 min were 0.36±0.34 (mean±standard deviation), 0.20± 0.18 and 0.17±0.16, respectively. Surviving fractions for the combination of 41℃, 30 min hyperthermia and neutron irradiation were 0.21±0.098, 0.19±0.018, 0.14± 0.035, 0.065±0.048, 0.089±0.004, 0.095±0.015, 0.11± 0.022, respectively, at the interval of -6, -4, -2, -0, 2, 4, 6 hours and 0.13±0.049, 0.14±0.050, 0.12±0.037, 0.058 ±0.029, 0.084±0.057, 0.075±0.049, 0.094±0.057, respectively, at each interval for 43℃, 30 min hyperthermia and neutron irradiation (Fig. 2). Interacting powers were 3.01, 2.74, 2.0, 0.93, 1.27, 1.36, 1.5, respectively, at the interval of -6, -4, -2, -0, 2, 4, 6 hours for 41℃, 30 min hyperthermia and neutron irradiation and 2.38, 2.45, 2.18, 1.04, 1.5, 1.34, 1.68, respectively, at each interval for

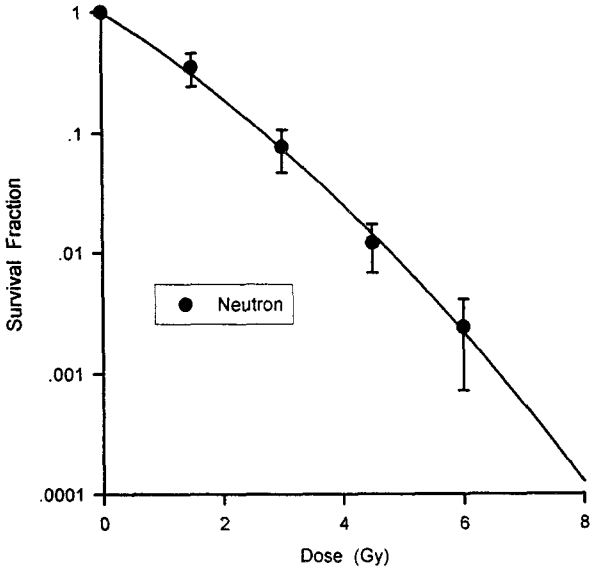

Fig 1. Survival curve of MKN-45 cells for single doses of neutron.

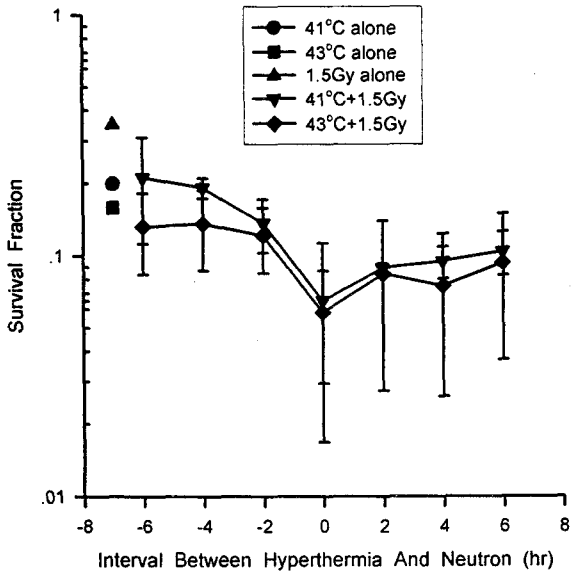

Fig. 2. Survival curves of MKN-45 cells as a function of sequence between neutron irradiation (1.5 Gy) and hyperthermia (41℃ or 43℃ for 30 min). Cells were heated at varying times before (negative values) and after (positive time values) irradiation.

43℃ (Fig. 3). The ratios of the highest interacting power to the lowest one were 3.24 and 2.36, respectively for 41℃ and 43℃. The combination effect was independent additive for hyperthermia just before neutron irradiation or hyperthermia after neutron irradiation up to 6 hour-intervals, and was sub-additive for hyperthermia followed

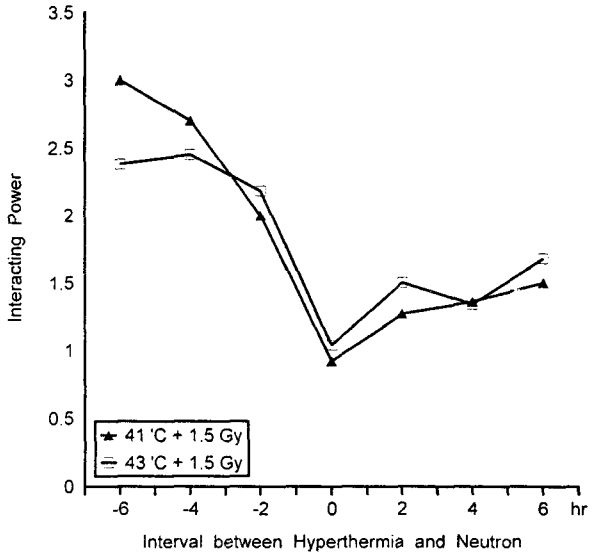

Fig. 3. Interacting power as a function of sequence and interval between neutron irradiation (1.5 Gy) and hyperthermia (41℃ or 43℃ for 30 min).

by neutron irradiation with four or more hour-interval.

DISCUSSION AND CONCLUSION

It is well known that high LET radiations have a characteristic of cell killing; repair of sublethal damage (SLD) or potentially lethal damage (PLD), variation of sensitivity through the cell cycle and oxygen enhancement ratio (OER) are lower compared with low LET radiations (X- or gamma-ray). From this characteristic it might be predicted that the combined effect of high LET radiations and hyperthermia is mainly subadditive or at best additive and the effect by sequence is lower than that of low LET radiations.

In an earlier in vivo study,[1] Hahn et al reported that hyperthermia (42.5℃ for 15 min) had no effect on the neutron (3.5MeV) response of an osteogenic sarcoma although X-ray damage was enhanced (TER 1.4~1.6), i.e. the TER was reduced to approximately 1 for neutrons. That study suggested that there would be no additional cell killing effect by combining high LET radiations with hyperthermia in tumors with hypoxic cells.

On the contrary to the results in tumors there are some reports that heat can enhance the effect of neutron in cultured cells and normal tissues in vivo. Gerner & Leith[3] showed that in chinese hamster ovary (CHO) cells

pre-irradiation hyperthermia of 43℃ (1 hr) changed both the radiation survival curve slope and the extrapolation number, and this was dependent upon the quality of the radiation. They suggested that heat may have a similar effect on the accumulation of sublethal damage following either 4MeV x-rays (low LET), accelerated helium ion (with a small component of high LET), or accelerated carbon ions (high LET), but that heat did not enhance the lethal damage caused by high LET radiation to the same extent as lethal x-ray damage. In the CHO cells which was given hyperthermia of 42℃ at 100 min after irradiation, Loshek et al[4] found that the interaction component and the radiation component has similar dependencies on radiation quality both for the deposition of damage and the repair or accumulation of that damage. They suggested that the high LET interaction damage is affected, but to a lesser degree than low LET interaction damage, by a mechanism of repair or accumulation of damage similar to the mechanism responsible for the shoulder of the unperturbed survival curve. The enhancing effect of hyperthermia on high LET radiations also observed in several in vivo studies of normal tissues. Hume et al[8] showed that when hyperthermia of 41℃ or 43℃ was given immediately before radiation (x-ray or neutron), thermal enhancement ratio (TER) were similar for the two tissues (baby rat cartilage and mouse intestine) and were not affected by the type of radiation used. Thus, the relative biological effectiveness (RBE) of fast neutrons compared with x rays was not markedly altered by combining radiation with hyperthermia. Law et al[9] reported that the heat treatments (41.5~43.0℃ for 1 hour) enhanced the response to both neutrons (mean energy approximately 7.5MeV) and x-rays (250kVp) in the skin of the mouse ear and foot, and the enhancement of neutron damage increased as the heating temperature was increased, as is well known for x-rays. When heat was given after irradiation TER for neutrons was similar to that for x-rays. When heat was given before irradiation the neutron TER was less than that for x-rays. Consequently, RBE of fast neutrons compared with x-rays was not altered by giving heat after irradiation but it was reduced by giving heat before irradiation. This discrepancy may be due to physiological effects rather than any differences in cellular responses. One effect of heat is to increase blood flow and hence improves tissue oxygenation.[10] Thus if heat is given before x-irradiation to a partially hypoxic tissue there would be an increased response due to improved oxygenation. As a result TER would be greater than that for heat given after x-rays. If a reduction in TER with high LET radiation results from a thermally induced increase in blood flow, it might be predicted that the TER would not depend on LET in oxic tissues (e.g. intestine) or avascualr tissues (e.g. cartilage) as was shown by Hume et al.[8]

For the sequence of low LET radiation and hyperthermia the greatest reduction in cell survival has been observed by irradiation during, or just before or after hyperthermia, and this results from maximal interaction of the two.[11, 12] Because the effect of interaction between high LET radiation and hyperthermia is lower than that of low LET radiation as was discussed above, the combined effect by the sequence of the two would be lower. In the present study, the range of interacting powers was narrower than that of low LET radiation which was reported previously.[5] As in the cases of low LET radiation, the variance of interacting powers according to the sequence and interval was higher at 41℃ than at 43℃. Interacting powers were decreased as the interval was closer. The maximal combined effect was observed at hyperthermia just before neutron irradiation, however it was independent additive (43℃) or slight supra-additive (41℃). In other intervals the interacting powers showed mostly independent additive to sub-additive. In the cases of hyperthermia 6 or 4 hour before neutron irradiation the interacting powers were high, which was sub-additive interactions. This might be due to heat-induced radiation resistance of the cells, which has been reported in cells to which low LET radiation was delivered at a certain period after hyperthermia.[5, 13, 14] As for the mechanism of low LET radiation resistance after hyperthermia, activation of recombinational DNA repair system or heat shock protein (HSP) has been proposed. However, further study is needed whether those or another factors are involved in the resistance to subsequent high LET radiations.

In summary, the combined effect of fast neutron irradiation (1.5 Gy) and hyperthermia (41℃ or 43℃ for 30 min) by sequence and time interval is largely independently additive. Mild hyperthermia (41℃ for 30 min) four or six hours prior to fast neutron irradiation may cause decreased sensitivity to subsequent neutron irradia-

tion.

REFERENCES

1. **Hahn EW, Canada TR, Alfieri AA, et al.** The interaction of hyperthermia with fast neutrons or x rays on local tumor response. Radiation Research 1976; 68:39-56
2. **Gerner EW, Leith JT, Boone MLM.** Mammalian cell survival response following irradiation with 4 MeV X rays or accelerated helium ions combined with hyperthermia. Radiology 1976; 119:715-720
3. **Gerner EW, Leith JT.** Interaction of hyperthermia with radiations of different linear energy transfer. Int J Radiat Biol 1977; 31:283-288
4. **Loshek DD. Orr JS, Solomonidis E.** Interaction of hyperthermia and radiation: radiation quality. British J Radiol 1981; 54:40-47
5. **Park WY, Kim WD, Min KS.** The change of cell survival according to the interval between hyperthermia and irradiation in MKN-45 cells. J Korean Soc Hyperthermia Oncol 1997; 2:95-103
6. **Yoo SY, Noh SW, Chung HW, et al.** Dosimetric characteristics of the KCCH neutron therapy facility. J Korean Soc Ther Radiol 1988; 6:85-91
7. **Streffer C, Muller WU.** Dose-effect relationship and general mechanisms of combined exposure. Int J Radiat Biol 1987; 51:961-969
8. **Hume SP. Myers R, Field SB.** A comparison of thermal enhancement ratios for fast neutron and x irradiation of two normal tissues in rodents. British J Radiol 1982; 55:151-155
9. **Law MP, Morris CC, Field SB.** The response of mouse skin to hyperthermia combined with fast neutrons or x-rays. Int J Radiat Biol 1984; 46:17-24
10. **Song CW.** Effect of local hyperthermia on blood flow and microenvironment: a review. Cancer Res (Suppl) 1984; 44: 4721S-4730S
11. **김일한.** 온열이 방사선조사후 발생한 잠재치사손상의 회복에 미치는 영향. 서울대학교 대학원 의학박사 학위논문, 1991
12. **Dewey WC, Hopwood LE, Sapareto SA, Gerweck LE.** Cellular responses to combinations of hyperthermia and radiation. Radiology 1977; 123:463-474
13. **Urano M.** Kinetics of thermotolerance in normal and tumor tissue: a review. Cancer Res 1986; 46:474-482
14. **Cai L, Jiang J.** Mild hyperthermia can induce adaptation to cytogenetic damage caused by subsequent X irradiation. Radiation Res 1995; 143:26-33

국문 초록

MKN-45 세포에서 속중성자와 온열치료의 순서 및 간격에 따른 병용효과

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목 적 : 온열치료는 엑스선 또는 감마선 등 LET 가 낮은 방사선에 대한 세포 및 조직에서의 반응을 증강시킬 수 있음이 이미 잘 알려져 있다. 그러나 다른 종류의 방사선과 온열치료의 상호작용에 대해서는 연구가 미미한 실정이다. 따라서 저자들은 속중성자와 온열치료의 순서 및 시간간격에 따른 병용효과를 파악하고자 이 연구를 시행하였다.

재료 및 방법 : 사람 위암세포주인 MKN-45 세포에서 1.5Gy 의 중성자조사 전후 각 6, 4, 2, 0(5분) 시간 간격으로 41℃ 또는 43℃ 에서 30분간의 온열치료 시행하여 세포생존율을 측정하였다.

결 과 : MKN-45 의 D_0 와 n 은 각각 0.8Gy 와 2.5 이었고, 1.5Gy 에서의 생존분획은 0.36(±0.34) 이었다. 시간간격에 따른 상호작용력은 대부분 1 과 2 사이였으나, 41℃ 의 온열치료후 4 또는 6시간에 시행한 중성자조사에서는 상호작용력이 각각 3.0 과 2.7 이었다.

결 론 : 속중성자와 온열치료의 병용효과는 주로 상가적(additive) 이나, 약온열치료(41℃, 30분) 가 4 또는 6시간 전에 시행된 경우 후속 중성자조사에 대한 내성이 유발될 수있다.

핵심용어 : MKN-45, 속중성자, 온열치료, 병용순서, 병용간격