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Effects of 2-Deoxy-D-Glucose on Metabolic Status, Proliferative Capacity and Growth Rate of FSall Tumor: Observations made by In Vivo ^{31}P-Nuclear Magnetic Resonance Spectroscopy and Flow Cytometry

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The effect of 2-deoxy-d-glucose (2-DDG) on C_3H mouse fibrosarcoma(FSall) was studied. Metabolic status, especially for energy metabolism, was studied using in vivo ^{31}P-MRS, proliferative capacity was observed on flow cytometry(FC) and growth rate was measured after transplantation of 10^6 viable tumor cells in the dorsum of foot of C_3Hf/Sed mice. One gram of 2-DDG per kg of body weight was injected intraperitoneally on 12th day of implantation. Average tumor size on 12th day of implantion was 250 mm^3. Growth rate of FSall tumor was measured by tumor doubling time and slope on semilog plot. After 2-DDG injection, growth rate slowed down. Tumor doubling time between tumor age 5-12 days was 0.84 days with slope 0.828 and tumor doubling time between tumor age 13-28 days was 3.2 days with slope 0.218 in control group. After 2-DDG injection, tumor doubling time was elongated to 5.1 days with slope 0.136.

The effect of 2-DDG studied in vivo ^{31}P-MRS suggested that the increase of phosphomonoester (PME) and inorganic phosphate (Pi) by increasing size of tumor, slowed down after 2-DDG injection.

Flow cytometry showed significantly increased S-phase and G_2+M phase fraction suggesting increased proliferative capacity of tumor cells in the presence of 2-DDG.

Authors observed an interesting effect of 2-DDG on FSall tumor and attempt to utilize as an adjunct for radiotherapy.

Key Words: 2-DDG, ^{31}P-MRS, FSall tumor

INTRODUCTION

2-Deoxy-d-glucose inhibits the glucose metabolism and blocks the anaerobic glycolytic pathway. Since malignant tumors have high rates of glycolysis[1], potential effect of 2-DDG on malignant tumor and potential role for cancer therapy have been questioned by several investigatiors[2~4]. In vivo studies on tumor bearing animals have shown that 2-DDG with radiotherapy enhanced animal survival[5] and investigator suggested that 2-DDG inhibits repair phenomenon in cancer cells and may enhance the repair of radiation damage in normal cells because of the effect on the energy metabolism. ^{31}P-MRS is an excellent tool to look at the energy metabolism in vivo. Recent technical advances on ^{31}P-MRS have promoted the study of tumor metabolism and the use of the tool in monitoring tumor response to therapy[6~9]. ^{31}P-MRS provides spectra with fairly well-separated reso-

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nances of important phosphorus metabolites, 3 levels of adenosine triphosphate (ATP), phosphocreatine (PCr), inorganic phosphate (Pi) and phosphomonoester (PME). ^{31}P-MRS studies in the tumor showed relatively high levels of PME and Pi, low levels of PCr and ATP[9]. Flow cytometric technique simplifies the biologic study to measure cell cycle fraction, the proliferative capacity and ploidy status[10].

METHODS AND MATERIALS

1. Animal Tumor Model

Syngeneic murine fibrosarcoma cells designated FSall were implanted on the dorsum of foot of the male or female C_3Hf/Sed mice, 8-12 weeks of age.

Animals were kept in the controlled environment and tumor cell lines were kept at −70°C freezer for preservation. For animal tumor model, single cell suspensions were prepared by trypsinization procedure and viable tumor cells were counted by hemocytometer using trypan blue exclusion method. The cells were resuspended in Hanks' balanced salt solution (HBSS) to obtain 10^6 viable cells per 0.05 ml and implanted into the mouse's dorsum of foot of right hind leg. Tumor volume was measured with Vernier caliper and calculated as follows; tumor volume=LWH/2, where L is length, H is height and W is width of the tumor. Approximately, one week after implant, tumor volume reached 50-80mm^3, measurable size.

The dorsum of the foot was chosen for tumor implant for ^{31}P-MRS, so that we can avoid spectral contamination from muscle for the spectra from the tumor. Total 60 animals were used for this experiment and divided evenly for control and 2-DDG treated groups.

2. ^{31}P-Nuclear Magnetic Resonance Spectroscopy (^{31}P-MRS)

^{31}P-MRS spectra were measured with 1.2cm diameter three turn surface coil using Bruker Biospec 4.7 Tesla/30 cm animal system. Surface coil was single tuned at 81.049 MHz. 1024 scannings were performed for averaging and recovery time was 1 second. So one experiment took 17 minutes of scanning time.

Series of ^{31}P-MRS spectra were obtained from tumor age 9 days old or 60 mm^3 of tumor volume and repeated periodically upto tumor age 28 days or tumor volume 1500 mm^3. 2-DDG was injected intraperitoneally on 12th day of implant for the group treated with 2-DDG.

Animals were anesthetized during MRS procedure with nembutal 50 mg/kg or thiopental 50 mg/kg of body weight, lintraperitoneally injected.

3. Flow Cytometry

For our experiment, DNA histogram for cell cycle fraction was measured on FAC Scan (Becton-Dickinson), excited with Argon Laser at 488 nm. Data was analysed by the software program, Cell Fit II.

RESULTS

Fig. 1 shows tumor growth curve plotted on semilog scale and authors measured the tumor growth rate by calculating the tumor doubling time D and slope λ from the equation, $V=V_0e^{\lambda D}$, $V=2Vo$, where V is doubled volume, Vo is initial volume, D is the doubling time in days and λ is the slope.

When tumor age was 12 dyas old, average tumor volume was 250 mm^3 and lgm of 2-DDG per Kg of body weight was injected intraperitoneally. 2-DDG treated group showed slower tumor growth rate. Tumor doubling time between 5-12 days of tumor age was 0.84 days with slope 0.828 and between 13 ~28 days of tumor age, tumor doubling time was 3.

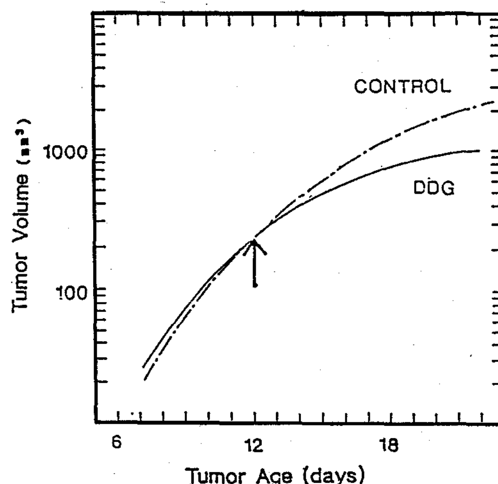
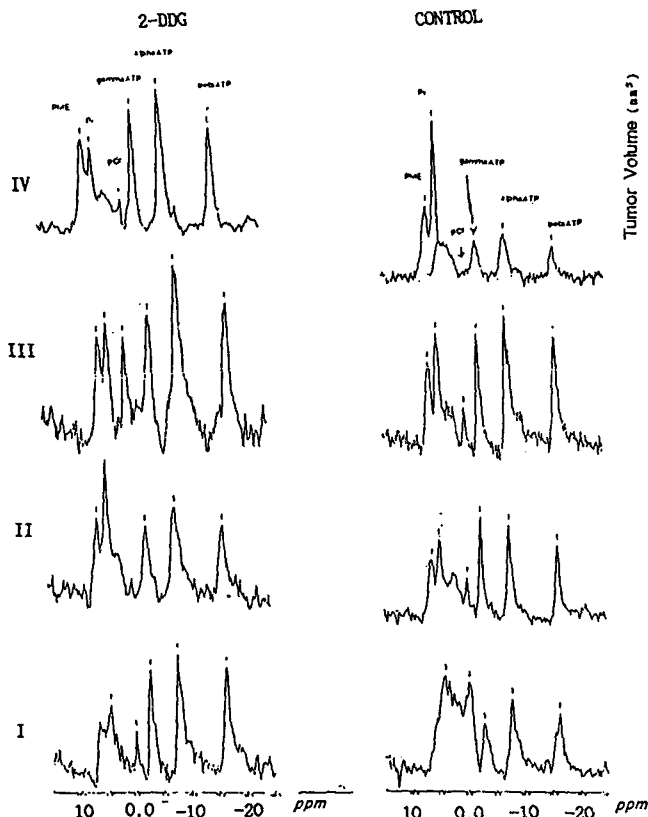


Fig. 1. Tumor Growth Curve, 2-DDG injected on 12th day of tumor implant (arrow indicates). Growth curve shows two portions of slope; initial slope, 0.828 with doubling time 0.84 days and slope at the 2nd portion of curve separated between DDG group and control group, 0.136 and 0.218 respectively.



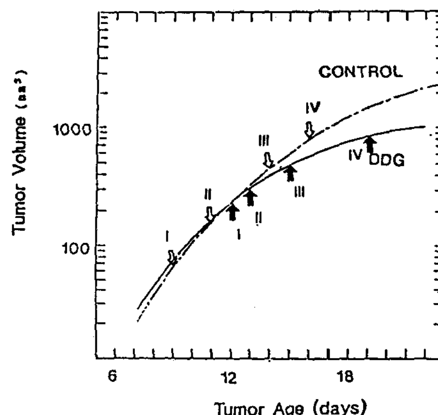


Fig. 2. Series of ^{31}P-MRS spectra in DDG group and control group. Tumor age and tumor volume ranged from 9 days, 60 mm³ to 19 days, 770 mm³ respectively by the order I---IV.

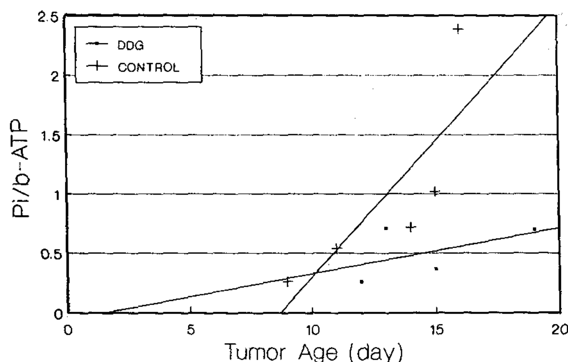


Fig. 3. Pi/βATP ratio increased with tumor growth and the rate of increase slowed down after 2-DDG injection.

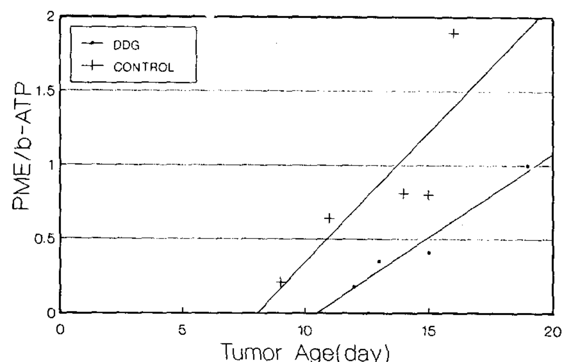


Fig. 4. PME/βATP ratio increased with tumor growth and the rate of increase slowed down after 2-DDG injection.

2 days with slope 0.218 in control group.

And in 2-DDG treated group, tumor doubling time was 5.1 days with slope 0.136. Intracellular pH was measured by chemical shift. Tumor pH ranged 6.9~7.2 and authors did not observe significant change by tumor size or 2-DDG.

Fig. 2 shows the ^{31}P-MRS spectral changes that obtained from tumors of different sizes and of different age, arranged by the order of tumor age. Along with tumor growth, PCr decreases and Pi and PME increase. Authors observed that 2-DDG slowed the rate of increase of Pi and PME along

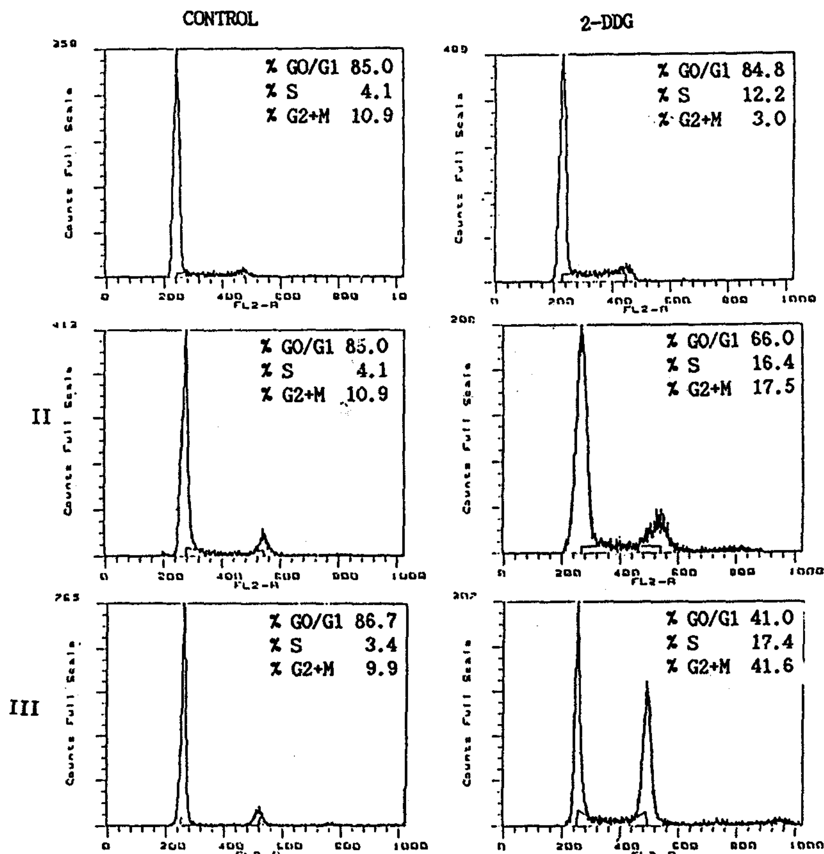


Fig. 5. DNA histogram plotted between cell cycles, obrtained from splenocyte for control(I), tumor volume 500 mm^3 (II) and tumor volume 800 mm^3 (III). DDG group showed increased S-fraction and G_2+M phase fraction as compared with control group. This increase of DNA component is more prominent in the tumor and more in larger tumor.

with tumor growth as compared with control group (Fig. 3, Fig. 4).

Fig. 5 shows the DNA histogram between cell cycles by the flow cytomytry and it shows significant increase of S fraction and G_2+M phase fraction after 2-DDG injectin as compared with the control group.

DISCUSSION

When the tumor volume grows larger, hypoxic cell fraction in the tumor increases and the fraction of hypoxic cell in FSaII was reported by Urano[11] that in tumor larger that 250 mm^3 of tumor volume, hypoxic fraction increased rapidly. 2-DDG may suppress the metabolism of tumor with high hypoxic fraction and high rate of glycolysis so that proliferative ability of tumor may be inhibited and this hypothesis can explain the efffect of 2-DDG on tumor growth rate. Jain and Kalis[12] observed severely reduced proliferation rate by the presence of high concentration of 2-DDG in HeLa cells through out the cell cycle and our observation for 2-DDG effect on tumor growth rate is in agreement with their observation. But our observation made by ^{31}P-MRS and flow cytometry appears rather contradiactory to the above hypothesis. Effect of 2-DDG in the tumor cells by $^{31)}$P-MRS and flow cytometry in our experiment can be interpreted that the cell degradation is somewhat suppressed and S-fraction and G_2+M phase fraction in the tumor tissue is greatly increased by the presence of 2-DDG. The following effect of 2-DDG on FSaII cell kinetics, 1) reduction of proliferation rate, 2) reduc-

tion of cell necrosis, 3) elevation of S-fraction and G_2+M phase fraction, may increase the radiotherapeutic effect. Authors believe that our study suggests the potential role of 2-DDG for radiosensitizer as well as adjunctive role for cancer therapy.

The weakness of our experiment is that the number of MRS and flow cytometry procedure performed for the group of animals were too small to make any statistically significant information. During 4 weeks of observation for 60 animals, we performed 20 MRS procedures and 5 flow cytometry procedures.

Our observations made in this experiment are encouraging and we plan to explore 2-DDG further for the potential role as a radiosensitizer.

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= 국문초록 =

2-DDG가 FSa II 종양의 성장속도와 증식 능력, 신진대사에 미치는 영향 ; ^{31}P-자기공명 분광기와 유세포 분석기를 이용한 연구

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2-DDG가 쥐의 섬유육종(FSaII)에 미치는 영향에 대한 연구로 에너지 신진대사는 체내에서의 ^{31}P-자기공명 분광기를 이용하여 관찰하였고 세포 증식 능력은 유세포 분석기를 사용하여 연구하였다. 성장속도는 10^6개의 세포를 C_3Hf/Sed 쥐의 발등에 이식한 후 3차원적으로 측정하여 관찰하였다. 2-DDG를 투여한 경우에는 이식후 12일에 복강내로 주사하였다. 이식후 12일의 종양의 평균 크기는 250 mm^3이었다. FSaII 종양의 성장속도는 semilog graph의 기울기와 종양의 doubling time으로 측정하였다. 2-DDG를 투여한 후 성장속도가 감속되었다. 5~12일 사이의 성장속도의 기울기가 0.828, 종양의 ldubling time이 0.84일이고 대조군에서는 13~28일 사이의 기울기가 0.218, doubling time이 3.2일인 반면 2-DDG 투여군에서는 성장속도의 기울기가 0.135이고 doubling time이 5.1일이었다.

^{31}P-자기공명 분광기를 이용하여 2-DDG의 영향을 분석해 본 결과 2-DDG 투여후 종양증식 속도의 감속과 더불어 phosphomonester (PME)와 inorganic phosphate (Pi)의증가속도가 감소하였다. 이것은 2-DDG 투여후 세포의 괴사가 감소하였다는 의미가 있다. 유세포 분석기를 이용하여 종양의 증식 능력을 분석한 결과는 2-DDG 투여후 S-phase와 G_2+M phase의 DNA 분포가 크게 증가하였다. 이것은 2-DDG 투여후 세포가 좀더 방사선에 민감한 cycle로 진행함을 의미하는 것으로 해석할 수 있다. 이에 저자들은 2-DDG가 FSaII 종양세포에 미치는 흥미있는 결과를 토대로 방사선 치료에 미치는 영향과 실제 이용 가능성에 대하여 더 연구하고자 한다.