

Here, we investigated the mechanism of STS-dependent protection. In mice, a 72-hour fast reduced IGF-I by 70% and increased the level of the IGF-I inhibitor IGFBP1 10-fold. Reduction of IGF-I/IGF-I signaling protected primary glia, but not glioma cells against cyclophosphamide and protected mouse embryonic fibroblasts (MEFs) against doxorubicin-dependent DNA damage. LID mice with a 70-80% reduction in circulating IGF-I levels displayed protection against 3 out of 4 chemotherapy drugs tested and melanoma-bearing LID mice treated with doxorubicin had a significantly improved long-term survival rate (60% vs. 0%, LID and control respectively) with less chemo toxicity. These results suggest that IGF-I is a potent inhibitor of protection in normal but not cancer cells.

EXAMPLE VI

Short-Term Starvation-Based Strategy for Differential Protection Against Multiple Chemotherapy Agents

Abstract

The side effects of chemotherapy are a major limiting factor in cancer treatment. Although progress has been made in the development of chemoprotectants, they are not widely used due to their drug- and tissue-specificity. Our previous research revealed the role of starvation and starvation-regulated genetic pathways in the protection of cells and organisms against a variety of toxins. Recently, we reported that short-term starvation (STS) selectively protected normal cells and mice against etoposide but provided no, or minor, protection to neuroblastoma cells in vitro and in vivo, respectively (differential stress resistance, DSR). Our DSR hypothesis is based on the fact that stress resistance is inhibited by oncogenic pathways and thus cannot be activated in cancer cells. We have investigated whether STS protects mice against other drugs and studied its effect on the resistance of different malignant cells to chemotherapy. The reported STS regimen consisted of a 48-60 hours fast prior to chemotherapy administration. Here we show that protection to cisplatin requires a 48-hour pre-chemo and 24-hour post-chemo fast. Using luciferase-expressing melanoma and neuroblastoma cells, we monitored the effect of chemotherapy in vivo. Our results confirmed that STS protects the host from chemotoxicity, and suggest that it does not protect neuroblastoma cells and may sensitize melanoma cells to multiple cycles of doxorubicin treatment. These results indicate that short-term starvation has the potential to be effective in the differential protection of normal and cancer cells against a wide range of chemo drugs and may enhance chemotherapy efficacy and health outcomes.

Materials and Methods

Cell Culture

Primary mixed glial cells were obtained from the cerebral cortices of 1 to 3 days old Sprague Dawley rat pups (Charles River). Cells cultured for 10-14 days in DMEM/F12 medium (Invitrogen) with 10% fetal bovine serum (FBS) were used. C6, A10-85, 9L and RG2 rat glioma cell lines and LN229 human glioma cell line, kindly provided by Dr. Chen (University of Southern California) and SH-SY5Y human neuroblastoma cell line were maintained in DMEM/F12 medium with 10% FBS at 37° C. under 5% CO₂.

STS Treatments of Mammalian Cells

Primary glia, glioma or neuroblastoma cells were seeded into 96-well microtiter plates at 20,000-30,000 cells/well and incubated for 2 days. Cells were washed with phosphate buffered saline (PBS) prior to treatments. All treatments were

performed at 37° C. under 5% CO₂. Glucose restriction was done by incubating cells in glucose free DMEM (Invitrogen) supplemented with either low glucose (0.5 g/L) or normal glucose (1.0 g/L) for 24 hours in 1% serum. Serum restriction was done by incubating cells in DMEM/F12 with either 10% or 1% FBS for 24 hours.

In Vitro Drug Treatments

Cyclophosphamide (CP, Sigma) was used for in vitro chemotherapy studies. Following STS treatments, cells were incubated with varying concentrations of cyclophosphamide (6-15 mg/ml) for 10 hours in DMEM/F12 with 1% FBS. Survival was determined by the MTT/LDH assay and presented as percent ratio of treated to control.

Stress Resistance in Mice

A/J, CD-1 and athymic Nude/nu mice, were used. Six week old female A/J mice (Harlan, Italy), weighing 15-18 g, and four week old female athymic (Nude-nu) mice (Harlan), weighing 20-22 g, were starved for 48 hours and then i.v. injected with 80 mg/kg and 100 mg/kg etoposide (Teva Pharma, Holland), respectively. Four week old female CD-1 mice, weighing 18-20 g, were starved for 60 hours and then i.v. injected with 110 mg/kg etoposide. In all experiments the mice were offered food after chemotherapy and were monitored daily for weight loss and general behaviour. Experiments were also performed with different chemotherapy agents cisplatin in CD-1 mice, and doxorubicin in A/J mice.

Differential Stress Resistance in Mice (DSR)

6-7 week old female A/J mice, weighing 15-18 g (Harlan, Italy) were housed in sterile enclosures under specific virus and antigen-free conditions. A/J mice were injected intravenously with murine neuroblastoma NXS2 cell line (200,000/mouse). After tumor cell injection, some groups of animals were starved for 48 hours and then i.v. injected with 80 mg/kg of etoposide, administered as a single dose. Control groups (NXS2 group) of mice without diet starvation were also investigated. To further investigate differential stress resistance, C57BL/B6 mice were injected with B16Fluc melanoma cells. Prior to injection, cells were washed and resuspended in sterile saline. Each mouse received 2×10^5 cells in 100 μ l followed by another 100 μ l of sterile saline to wash the remaining cells in the tail. Mice were randomly selected and followed throughout the experiment. Bioluminescence imaging were performed at USC Small animal imaging center. Signal intensity was quantified (Units of photon/S/cm²/steradian).

Results

See FIGS. 31-35.

CONCLUSIONS

A short-term starvation (STS) can induce stress resistance against chemo-toxicity in vitro and in vivo. STS induced stress resistance can be applied to various common chemotherapies. STS imparted differential stress resistance (DSR) against chemo-drugs in mammalian cells, and tumor-bearing mice. STS could sensitize cancer cells to chemotherapy.

All publications cited herein are incorporated by reference in their entirety.

What is claimed is:

1. A method of protecting an animal or human against chemotherapy or radiotherapy, comprising:

administering to an animal or human, prior to chemotherapy or radiotherapy, a diet capable of providing nutrition while providing no more than 11 kcal energy per kg body weight of the animal or human per day, and no more than 0.4 g protein per kg body weight of the