Potential of MRI Relaxometry in the study of Cisplatin Induced Cell Death in Squamous Cell Carcinoma of Skin

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OBJECTIVE
To correlate the changes observed in MR relaxation parameters (T1 & T2) during cisplatin induced cell death (apoptosis and necrosis) in an animal model of squamous cell carcinoma (SCC) of skin.

INTRODUCTION
Skin cancer is the most common form of cancer with male having 1:7 and female 1:5 lifetime chance of developing skin cancer. The squamous cell and basal cell carcinoma arise from keratinocytes, which correspond to about 20% of total skin cancers (1,2). Skin tumors respond differently to chemotherapy and chemo resistance often contributes to the failure of existing forms of drug therapy. MR imaging has been shown to have the potential to detect response in discretely localized regions of the skin tumor (3, 4). MR relaxometry can detect small changes in the tissue microenvironment because different tissues have different relaxation times, which can be used to generate contrast between different tissues in an MRI image. Therefore, monitoring the changes in relaxation parameters (T1 and T2) with the status of the cell death induced by chemotherapy would help in the selection of suitable treatment regimen and duration of the drug.

METHODS
Random bred of 4-6 weeks old Swiss-albino mice (20-25g) were procured from the experimental animal facility of our Institute. All MR imaging were performed on 4.7 T animal scanner (Bruker BIOSPEC, Germany) after anaesthetizing the mice with a combination of thiopentone and diazepam (40mg/kg +8mg/kg i.p). The various subgroups of mice studied were: (a) control-I (n=8), (b) control-II (n=8) and these are tumor bearing mice with 16 and 18 weeks old, respectively; (c) Test-I (n=8) mice with 2 wks of cisplatin therapy (2.5mg/kg body weight, i.p) and (d) Test II (n=8) which are with 4 weeks cisplatin therapy. A 35mm diameter volume resonator was used as transmitter/receiver coil. The field of view was 40 x 40mm in the acquisition matrix size of 256 x 256 corresponding to the spatial resolution of ~235 x 235µm with a 2 mm thickness. T1 experiments consisted of running two-dimensional spin-echo imaging sequence at variable recovery times (TR). A plot of ln[1-M(t)/M(0)] vs. TR was made for the calculation of T1. T2 experiments consisted of running a multi-spin multi-echo (MSME) sequence with different TE values. T2 was obtained from the plot of ln[1-M(t)/M(0)] vs. TR. The region of interest was selected and magnified to measure the tumor volume using the software provided by the manufacturer. After MRL for proliferation assay, mice were dissected 1 hour after the BrdU injection (125mg/kg in 0.9% NaCl, i.p). Proliferation index was calculated as the mean number of BrdU-positive cells/mm2 ±SD. Cisplatin induced cell death was determined by TUNEL assay by using Promega kit. All statistical analyses were done on SPSS 11.5 and study was approved by the Institute animal ethics committee (158/IAEC/02).

RESULTS
The tumor volume (Fig. 1A), T1 and T2 and proliferation index increased during tumor progression (from 0 to 14 wks) indicating correlation between MRI parameters and the histology data but there is no change in the apoptotic index. The results on tumor volume and T1, T2 of Control-I were compared with that obtained. after 2 weeks of cisplatin treatment (Test-I). Similarly, data of mice of Control-II were compared with 4 weeks treated mice with cisplatin (Test-II). Figure-1B reveals that tumor after 2wks cisplatin treatment showed no significant change in T1 but showed a decrease of 15% in T2 values. On the contrary, tumours after 4 weeks of treatment showed a drastic reduction in both T1 and T2 by ~34% and ~52%, respectively. Decrease of tumor volume (11% by two weeks and 60% by four weeks) after cisplatin treatment was corroborated by a drastic fall in the proliferation (Figure-2) index after cisplatin treatment due to cell death (Figure-2E, F). TUNEL assay showed initial apoptotic cell death (Figure-2,K) while necrosis after 4 weeks cisplatin treatment (Figure-2, L, R)

DISCUSSION
MRI relaxometry correlated with the histological findings in the mode of cell death induced by cisplatin with respect to time in the SCC of skin. Decrease in T2 (from 103 to 86 msec) and cell proliferation index (20 to 13%) along with high apoptotic index (87%) seen during the initial stages of cisplatin treatment may be due to tumor regression (235 to 110 mm³; compare Control I and Test I- see Figure 1A). Indeed, normotomic cell shrinkage associated with small changes noticed in T1 (929 to 918- msec, control I and Test I) during cell death have been considered as a major hallmark of apoptosis (5-7). However, after 4 weeks of cisplatin treatment, decrease in both T1 (1057 to 699 msec) and T2 (from 112 to 54-msec) is accompanied by shrinkage of skin tumor (340 to 40 mm ³), which is due to necrotic cell death (see Figure-2, J & L) (7). Tissue water mobility is closely correlated to local tissue morphology (cellularity, cell membrane integrity) and early changes of water mobility resulting from therapy-induced changes to tumor tissue morphology may serve as a sensitive biomarker for the prediction of therapy response (8,9). Our present study provides a direct correlation between changes in the relaxation parameters T1-and T2-and type of cell death like apoptotic/necrosis in SCC of skin. Thus, our animal model study indicate that early detection of chemotherapy response may be helpful in taking care of the side effects and may also guide appropriate therapeutic regimen, dose, or duration of drug.

REFERENCES: