# DOXORUBICIN CELLULAR RETENTION PATTERNS CORRELATES WITH THERAPEUTIC RESPONSE IN ASCITIC LYMPHOMA BEARING MICE

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Chemotherapy resistance (CR) greatly affects the risk of death in cancer patients. However, mechanisms of CR are not yet entirely elucidated. The mainstream of cancer research on this phenomenon call attention to genetically mediated mechanisms that lead to modified susceptibility to anticancer drugs. However non-genomic factors were also shown to trigger CR, for instance vascular permeability, stromal interstitial pressure, regions of hypoxia and some others. It has been also shown that the cellular uptake and cytotoxicity of anticancer agents might decrease with increasing cell density, an event observed *in vitro* and termed 'the inoculum effect'.

The aim of this study was to investigate Doxorubicin (Dox) plasma pharmacokinetics and cellular drug retention in C57BL/6NCr mice bearing ascitic EL4 lymphoma. Mice were inoculated intraperitoneally with $5*10^4$ EL4 lymphoma cells. Intravenous administration of Dox at a dose of 15mg/kg was given on day 3, day 5 or day 9 after tumor transplantation. These specific time points were chosen due to different tumor response characteristics attributable to tumor bearing mice (TBM) in our study. Cellular Dox content was determined by HPLC and FCM methods. Pharmacokinetic parameters were determined in plasma by HPLC and peripheral blood cell counts were done on automatic CBC analyzer.

Ascitic El4 lymphoma exhibited typical rapid proliferation in peritoneal cavity. Median survival time of untreated TBM was 14.5 days. Tumor cell numbers increased exponentially from $0.05x10^6$ (Day 0) to $202x10^6$ (Day 14). Therapeutic efficacy of Dox treatment was highly dependent on the relatively modest differences in tumor size. The median survival time of mice injected on Day 3 was 60 days with no signs of residual tumor. These mice were arbitrary designated as "cure" group. These mice also revealed the highest Dox cellular uptake in tumor cells during first 30 min after injection and tumor cells in peritoneal cavity were not detectable 72 h post-injection. TBM treated on day 5 exhibited signs of remission with median survival time of 26 days. Tumor cell counts were dramatically decreased in peritoneal cavity 72 hours after Dox administration. This group was designated as "relapse" group. Dox uptake 30 min after injection in "relapse" TBM was significantly lower as compared to "cure" group. During observable period of 12 days after drug administration cellular dox content was gradually decreasing in "relapse" TBM group. TBM treated with dox on day 9 exhibited a median survival duration of 14 days which was not different from group of untreated animals ("resistance" group). The cellular dox uptake in tumors was essentially undetectable in this group. Maximal plasma concentration of Dox was 1.33 µg/ml and it was detectable as early as 5 min post-injection. Plasma clearance half-time was 28.2 h, volume of distribution 128.4 l/kg. Plasma drug presence was below detection limit at 72 h post-injection. However, drug was retained in cellular samples of ascitic fluid, spleen and thymus up to 18 days following IV administration ("cure" group) or 10 days ("relapse" group). There were no significant differences in systemic hematopoietic toxicity observed between all three groups of TBM. Leukopenia (mostly due to decrease in lymphocyte and granulocyte counts) as well thrombocytopenia was observed on day 4 after Dox administration in all three TBM groups. Absence of differences in systemic effects between all three groups suggested that local tumor characteristics might be controlling susceptibility to dox treatment. In fact, the density of tumor cells in peritoneal cavity was rising by two orders of magnitude faster as compared to animal weight and ascites volume (ascitic fluid increase was 50-fold as compared to >5000-fold increase of cell number on day 14 after tumor transplantation). We hypothesized that the rapid increase of cell density at early avascular stage of tumor growth can govern the differences of cellular drug uptake, retention and eventually to drug susceptibility. This phenomenon is similar to "inoculum effect" reported to tumor cells exposed to cytotoxic drugs *in vitro*.

The intracellular tracking of dox in the cells of TBM might be a convenient means to further investigate this phenomenon. One additional clinical application out of these observations could be a possibility to develop a dox uptake test in blood. Importantly, dox lymphocyte uptake values exploring flow cytometric means could be performed on patient's blood. This method can be developed to show the individualized drug uptake and retention patterns in patient's blood.

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