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Drug resistance studies—implications of daunorubicin stability in in-vitro systems

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Extensive research into anthracycline-related multidrug resistance (MDR) has involved the use of many agents including daunorubicin (DNR). There are no published reports, however, on the drug's concentration after in-vitro alterations in physico-chemical variables which may affect the stability of DNR. Similarly, current reports on multidrug resistance comment solely on the cytotoxic effects of the parent anthracycline, neglecting the potential interference from degradation products. This study reports the effect of time, temperature, culture media, pH and protein binding on DNR concentration in an in-vitro system devoid of cellular material. It also highlights the effects of DNR cytotoxicity, as well as one of its derivatives, on a sensitive and MDR1-expressing cell line.

All analysis was performed, using a validated LC method, on protein-free DNR solutions (100 ng mL^{-1}) or deproteinated DNR filtrates (1μg mL^{-1}).

Over a period of 2 h, DNR loss (in RPMI) was 23, 35 and 70% when incubated at 37, 4 and 25°C, respectively. The drug loss after incubation at 37°C was less than expected because of a favourable pH change to more acidic conditions due to the 5% CO_2 environment in the incubator. The increased loss at 4°C and 25°C was attributed to an unfavourable pH change. Drug loss between the different diluent types was 17.5, 38.7, 43.5 and 50.5% after 0.5-h incubation for H_2O, PBS, RPMI and DMEM, respectively. In PBS, RPMI and DMEM there was an instantaneous drug loss with further loss occurring upon incubation. Loss during incubation occurred by both degradation and adsorption to the culture plate. Greatest stability was observed at a pH of 6 where drug loss was solely due to adsorption (38.5%), whereas at pH 7 and 7.5 there was significant loss due to degradation (82.6 and 93.4% total loss, respectively). Protein binding after incubating a 1-μg mL^{-1} DNR solution at 37°C in RPMI, resulted in a final 16.9% loss (Figure 1).

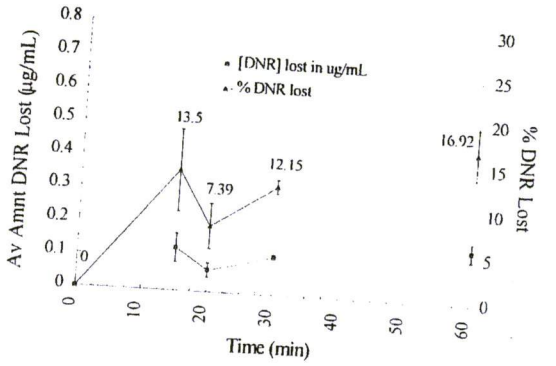

Figure 1. Protein binding for 1 μg mL^{-1} daunorubicin at 37°C (n = 3).

The investigations into the cytotoxic effects of DNR and its derivative are currently ongoing.

Under typical in-vitro experimental conditions significant loss of DNR occurred over short (0.5 h) incubation periods. Drug loss was attributable to simultaneous adsorption and pH-dependent degradation processes. This loss, including the presence of degradation products, could exert a profound effect on in-vitro MDR studies and must be considered in experimental design.