CANCER RESEARCH UK | MRC Medical Research Council

OXFORD INSTITUTE FOR RADIATION ONCOLOGY

UNIVERSITY OF OXFORD

DEPARTMENT OF ONCOLOGY

Bioanalysis Core Facility – HPLC and flow cytometry

Mike Stratford, Lisa Folkes & Mick Woodcock

Facilities

•High performance liquid chromatography-mass spectrometry (HPLC-MS) (Mike Stratford/Lisa Folkes)
  •Mass spectrometric (MS and MS-MS), photodiode array absorbance, fluorescence, conductivity and electrochemical detectors available.
•Expertise in spectroscopy (stopped-flow, atomic absorption, fluorescence and absorbance), steady state radiolysis and nitric oxide (NO) analysis.
•Flow cytometry (Mick Woodcock) - FacScan, FacSort, FacsCalibur + access to sorting facilities at the Kennedy Institute

Role

•Provision of bioanalytical and flow cytometric facilities to support research in the institute
•Drug confirmation and purification to support synthetic programs in the institute
•PK/PD support for Cancer Research UK and locally sponsored clinical studies - HPLC equipment run to GCLP standards

HPLC analyses

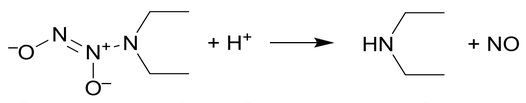
•Enzymatic and radiolytic release of bioreductive drugs
•Nucleotides and deoxynucleotides in cells
•Pharmacokinetics of chemotherapeutic agents
•Assessment of drug stability, purity and formulation
•DNA base damage after radiosensitization by NO
•Biogenic amines in brain microdialysates
•Clinical trials
  •red cell thioguanine nucleotides after 6-mercaptopurine
  •Gemcitabine metabolites after low dose Gemcitabine
  •AZD0424, CXD101, Doxorubicin pharmacokinetics

Non-HPLC analyses

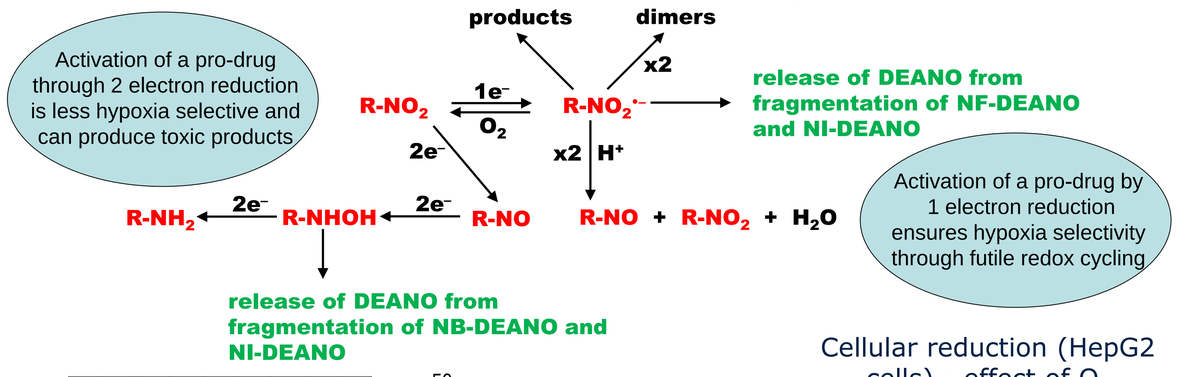
•Cisplatin in tumours by atomic absorption spectrometry
•Stopped-flow determination of rapid reaction rates
•Nitric oxide release

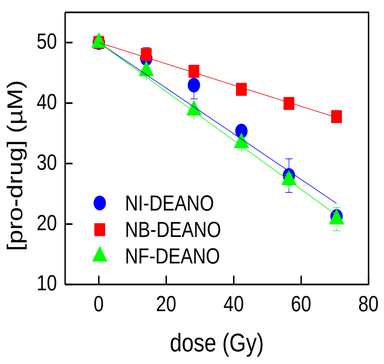
One-electron reduction by radiolysis

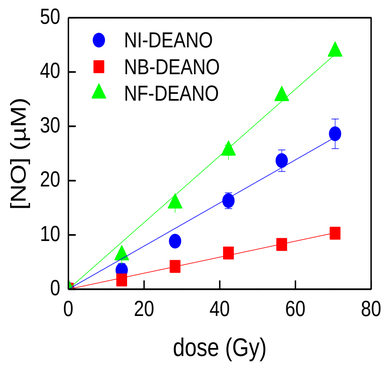
Radiation chemistry allows the effectiveness of bioreductive pro-drugs to be assessed by rapidly producing free radicals in a controlled and quantifiable manner. DEANO liberates the radiosensitizer NO after reductive release from nitroaromatic prodrugs.



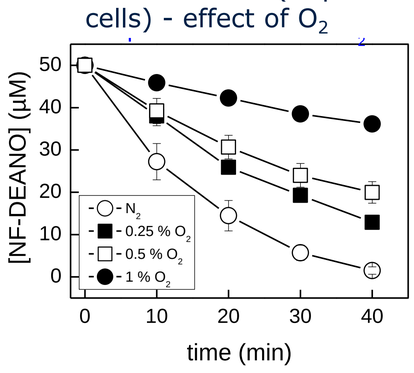
By measuring the loss of pro-drug/Gy/formation of NO/Gy, the fragmentation pathway of the pro-drug radical anion can be assigned, from the number of electron equivalents required to reduce the pro-drug.







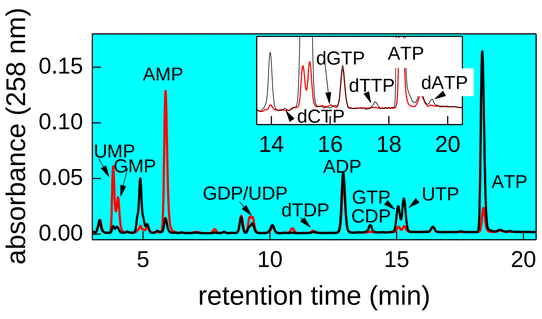
Cellular reduction (HepG2 cells) - effect of O_2



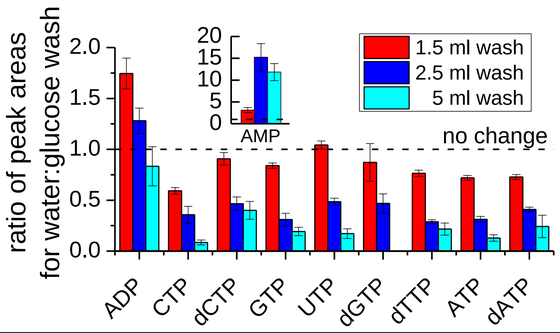
Cellular nucleotides and 2´-deoxynucleotides (dNTPs)

Analysis of dNTPs and nucleotides by HPLC commonly involves acid-lysis of washed cells using trichloroacetic acid (TCA). In *S. pombe* yeast we have found that when cells are washed with water, commonly used during routine cell maintenance, the change in osmolarity results in rapid loss of nucleotide triphosphates. We suggest that isotonic glucose solution is a milder method to wash these cells.

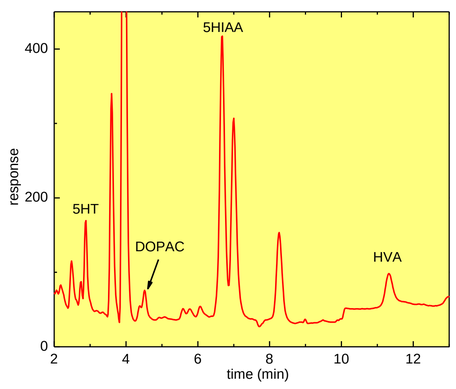
HPLC chromatograms of *S. pombe* extracts after washing with 5 ml 3% glucose or 5 ml water.

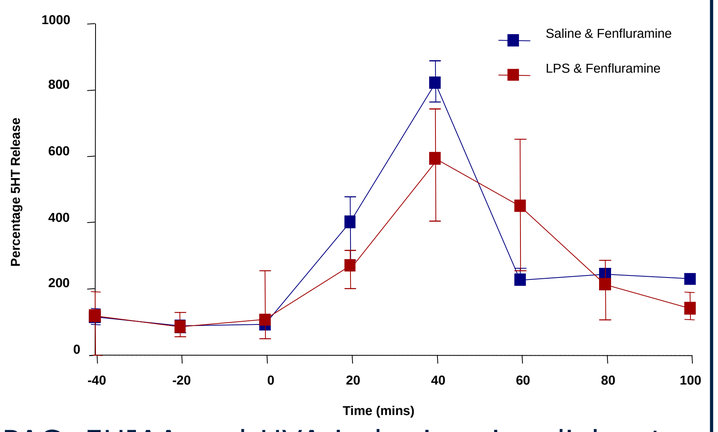


Effect of water or 3% glucose washing volumes on nucleotide content in *S. pombe*. Increasing water wash volume depletes the nucleotide triphosphate status of the cells



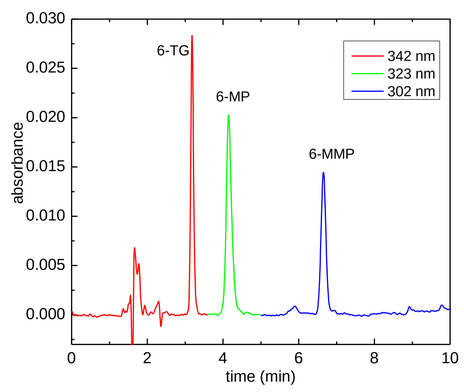
Biogenic amines using electrochemical detection

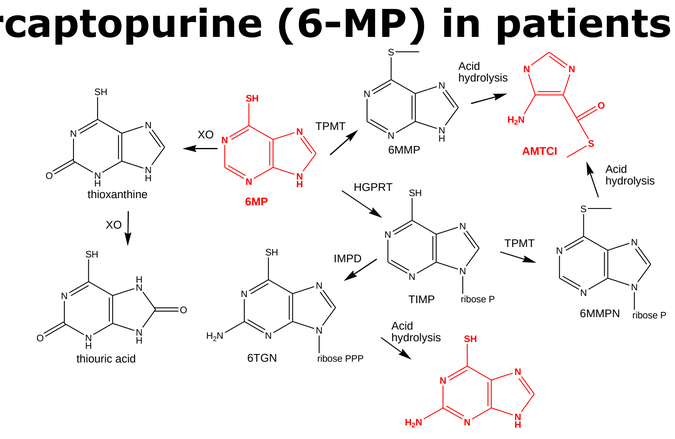




Hplc determination of 5HT, DOPAC, 5HIAA and HVA in brain microdialysates using electrochemical detection. Effect of LPS treatment (Couch *et al*, 2013).

Measurement of 6-Mercaptopurine (6-MP) in patients





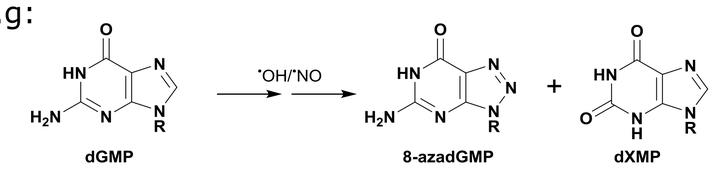
Clinical trial of 6-MP/low-dose methotrexate in BRCA-defective patients. TPMT activity affects the ratio of 6-TG (activation) to 6-MMP (inactivation) (measured as AMTCI), and thus the efficacy of 6-MP (Ricky Sharma).

Flow cytometry analyses

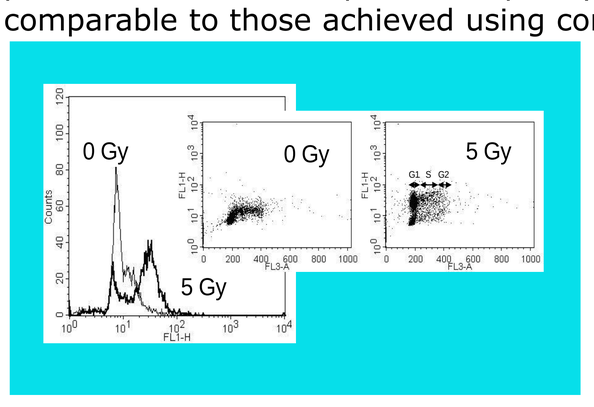
•Expression of Ki 67, Histone H3, Cohesin, Cenpf, Cyclins B and D, γH2AX
•Quantification of EGFP, RFP
•Cell cycle distributions (Pyronin Y, PI, 7-AAD, Brdu)
•Apoptosis measurements (Annexin V)
•Acridine orange RNA/DNA measurements

Radiosensitization by nitric oxide

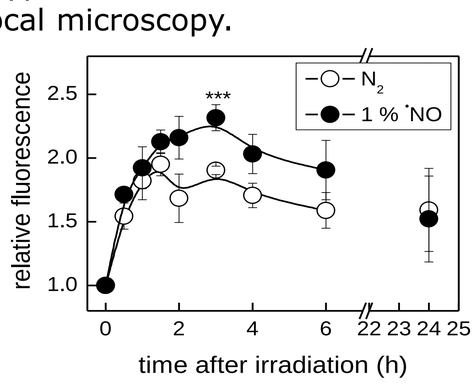
NO sensitizes hypoxic mammalian cells to low LET radiation. Using steady-state radiolysis and HPLC-MS we have shown that NO induces specific lesions to DNA bases in hypoxia which are not normally formed by ionizing radiation, e.g:



These modifications may be difficult to repair, particularly if formed in clustered damage sites specific to radiation. Flow cytometry using immunofluorescence staining for γH2AX was used to show that the extent of DNA double-strand break formation is higher in hypoxic cells in the presence of NO compared to purely hypoxic cells. These results were comparable to those achieved using confocal microscopy.



Flow cytometry histogram and dot plots showing increase in γH2AX fluorescence with ionizing radiation in V79-4 cells saturated with 1% NO/N_2, 2 h after 0 or 5 Gy (Folkes *et al* 2013).



Effect of time of incubation at 37 °C following γ-radiolysis (5 Gy) of V79-4 cells in N_2 or 1% NO-saturated cell suspensions on the formation of γH2AX staining in the total cell population, measured by flow cytometry.