The nuclear membrane in multidrug resistance: microinjection of epirubicin into bladder cancer cell lines

JONATHAN M. FEATHERSTONE*†, ALAN G. SPEERS†‡, BASHIR A. LWALEED*, MATTHEW C. HAYES*, ALAN J. COOPER*† and BRIAN R. BIRCH*

**Department of Urology, Southampton University Hospitals NHS Trust, Southampton, †Department of Biomedical Sciences, University of Portsmouth, and ‡Solent Department of Urology, St Mary's Hospital, Portsmouth UK*

Accepted for publication 29 November 2004

OBJECTIVE

To assess whether microinjecting epirubicin into cells showing multidrug resistance (MDR, common to many cancers, including bladder cancer, with resistance to, e.g. anthracyclines and mitomycin C) spares the nucleus, as when these drugs accumulate, distribution in MDR cells characteristically spares the nucleus, suggesting that the nuclear membrane is responsible for excluding cytotoxic drugs from MDR nuclei.

MATERIALS AND METHODS

Nuclear exclusion of drugs is an important feature of resistance in MDR cells, as many MDR-susceptible drugs have cytotoxic actions within the nucleus. Drug accumulation in 'classical' P-glycoprotein-mediated MDR cells is greatly reduced by efflux. Microinjection of epirubicin into the cytoplasm of MDR cells bypasses the P-glycoprotein efflux pump on the plasma membrane. Nuclear sparing would directly implicate the nuclear membrane in this phenomenon. Because of their fluorescence properties, which allow study by confocal microscopy and flow cytometry, anthracyclines have also been used extensively to investigate MDR. Thus sensitive (MGH-U1 and RT112) and MDR (MGH-U1R and MGH-U1-MMC) bladder cancer cell lines were used. Adherent cells from each cell line were individually microinjected with epirubicin (0.5 mg/mL) and a 77 kDa fluorescein isothiocyanate (FITC)-dextran (0.5 mg/mL). The pattern of nuclear epirubicin uptake in injected cells was then evaluated by confocal microscopy. The 77 kDa FITC-dextran allowed easier identification of injected cells and was also excluded from their nuclei.

RESULTS

Sensitive bladder cancer cell lines all showed a nuclear accumulation pattern of epirubicin, consistent with their normal uptake after exposure to epirubicin. The MDR cell lines showed the characteristic nuclear-sparing pattern of epirubicin uptake, similar to the normal uptake pattern after epirubicin exposure. The 77 kDa FITC-dextran showed clearly which cells had been microinjected, and was excluded from the nuclei of all injected cells. Cell viability was confirmed by acridine-orange staining after initial visualization of injected cells.

CONCLUSION

The nuclear membrane is responsible for the nuclear exclusion of epirubicin in MDR cells. Further work is necessary to determine the mechanisms involved.

KEYWORDS

bladder cancer, multidrug resistance, epirubicin, nuclear membrane, microinjection

INTRODUCTION

Multidrug resistance (MDR) in bladder cancer is common, with up to 75% of patients expressing markers of MDR at presentation [1,2]. Anthracyclines and mitomycin C belong to the MDR family of drugs and have been widely used as adjuvant intravesical chemotherapy for superficial bladder cancer [3–7]. After resection, adjuvant intravesical chemotherapy using these agents can reduce bladder cancer recurrence rates by 40–60% [4,7]. However, because of the development of MDR, many superficial bladder cancers will recur. MDR genes, including MDR1, MRP1, MRP2 and MRP3, have been shown to increase in cases of recurrent and residual cancer after chemotherapeutic treatment with doxorubicin [8,9].

Anthracyclines have been used extensively to investigate MDR because they autofluoresce, which allows their study by confocal microscopy and flow cytometry. Previous studies showed that anthracycline accumulation in 'classical' P-glycoprotein (P-gp)-mediated MDR cells is dramatically reduced by drug efflux [10–14]. Of the drug that accumulates, the distribution characteristically spares the nucleus [15–17], a phenomenon which has been shown in cell lines of different origins [16–18]. Primary cells cultured from bladder cancer explants also show heterogeneity in their nuclear uptake of epirubicin, with many having the MDR phenotype [19].

Anthracyclines have several nuclear cytotoxic actions, including intercalation-prompted, topoisomerase-II-dependent DNA cleavage, and DNA damage secondary to intracellular free-radical production. Mitomycin C is modified to its active metabolite intracellularly by a bioreductive alkylation reaction. The active moiety inhibits DNA synthesis, cross-links DNA, induces single-strand DNA breaks by alkylation of the O-6 guanine residue, and causes DNA damage secondary to superoxide and hydroxyl radical production. Thus, nuclear exclusion of these drugs will clearly reduce their effectiveness, contributing to resistance in MDR cells. The role of the nuclear membrane in this nuclear-sparing phenomenon in MDR cancer cells has not been clearly established. Microinjection of cytotoxic drugs into MDR cells provides a novel means of studying MDR status and mechanisms of MDR. Microinjection of

epirubicin into the cytoplasm of MDR cells bypasses the plasma membrane P-gp efflux pump; if MDR cells microinjected with epirubicin still show their characteristic nuclear sparing, this would provide direct evidence of the role of the nuclear membrane and establish another mechanism of MDR in bladder cancer.

MATERIALS AND METHODS

Drug-sensitive bladder TCC cell lines used were MGH-U1 (Human female TCC cell line, donated by Prof John Masters, UCL) and RT112 (Human female TCC cell line, purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen, Cat No. ACC 418, Germany). The MDR variants used were MGH-U1R (donated by Prof John Masters) and MGH-U1-MMC (MDR subline of MGH-U1, generated in-house). MGH-U1R was produced by prolonged low-dose exposure of MGH-U1 to doxorubicin [20] and MGH-U1-MMC by prolonged low-dose exposure of MGH-U1 to mitomycin C [21]. The inhibitory concentration required to reduce the viable biomass by half of that in controls for each of these cell lines, calculated from tetrazolium cytotoxicity assays for epirubicin and mitomycin C, respectively, were (μg/mL): RT112, 1 and (not assessed); MGH-U1, 2 and 1; MGH-U1R, ≈80 and ≈80; and MGH-U1-MMC, >80 and >80, respectively. The MDR cell lines had >40 times the resistance to epirubicin and >80 times the resistance to mitomycin C than their sensitive counterpart [21].

All cell lines were cultured in Dulbecco's Modified Eagle's Medium supplemented with 2 mmol/L L-glutamine, 10% fetal calf serum and 100 μg/mL of both penicillin/streptomycin solution (Sigma Chem Co., Poole, UK). Cells were passaged using trypsin-EDTA (Sigma) when 85% confluent. Cultures were maintained in a humidified incubator with 5% CO_2 at 37 °C.

For experiments, cells were harvested using trypsin-EDTA (Sigma) and the cell density adjusted to 1×10^4 cells/mL. Cells were subcultured in 40 mm Petri dishes, grown to 50% confluence (48–72 h in total) and then used for microinjection or incubation experiments. Before microinjection the culture medium was changed to one containing 10 mmol/L HEPES (1 : 100 HEPES buffering solution, Cat no. 83264, Sigma).

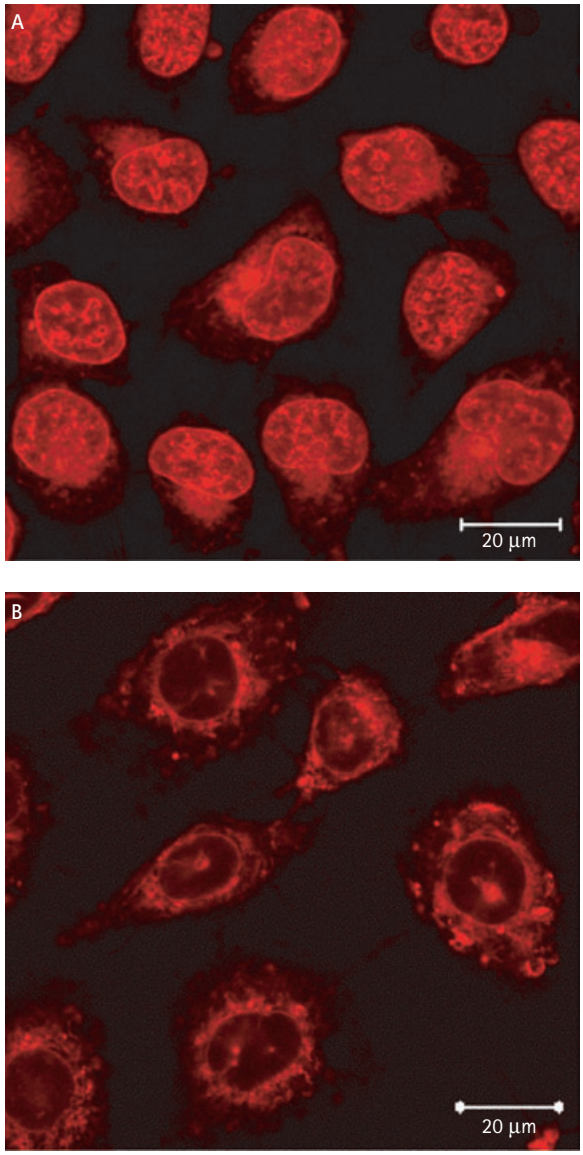
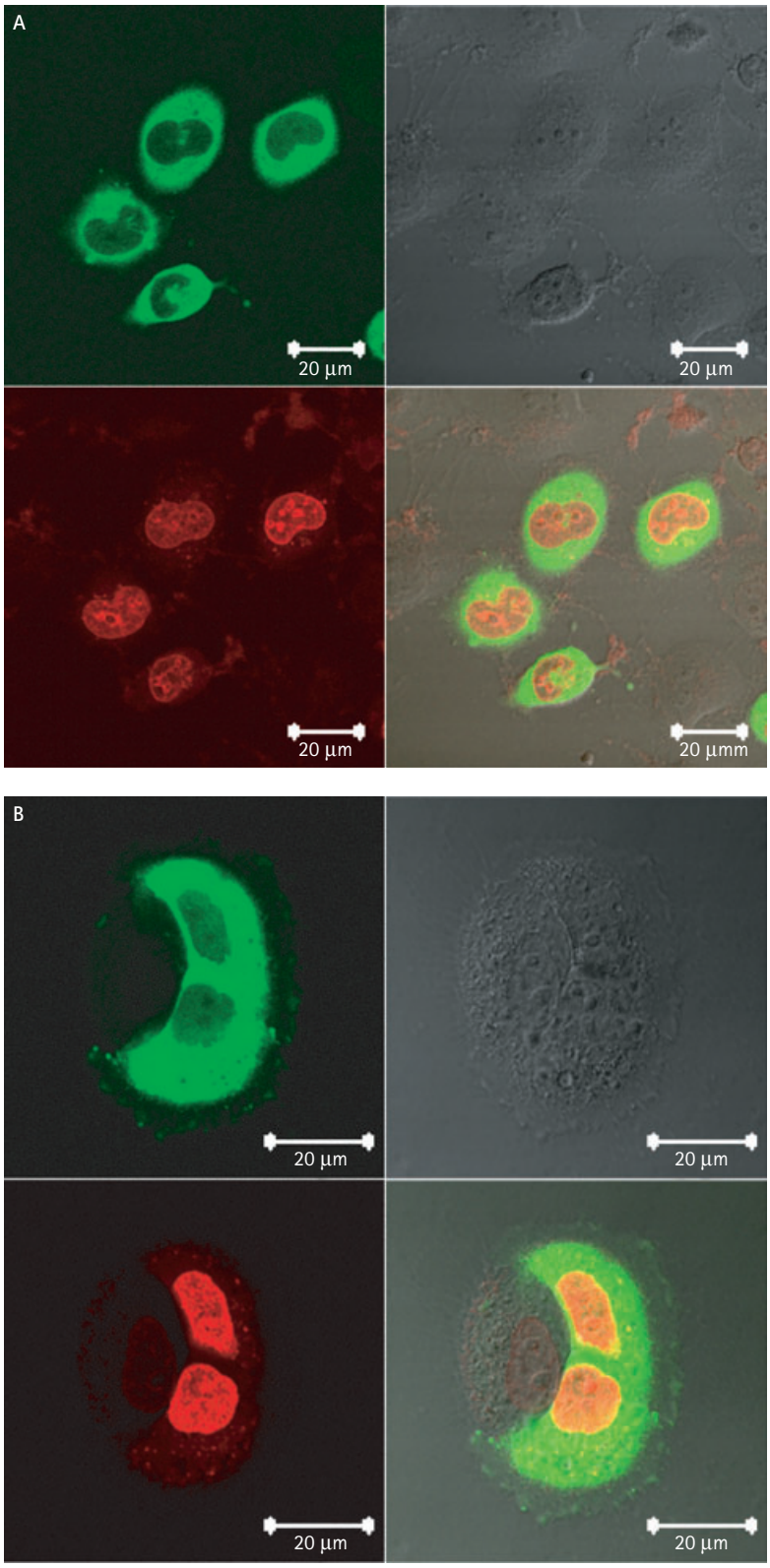


FIG. 1.
*MGH-U1 cells **(A)** and MGH-U1R **(B)** incubated with epirubicin (10 μg/mL) for 60 min; the fluorescence images show a 'sensitive' (A) nuclear uptake pattern of epirubicin and (B) characteristic nuclear sparing of epirubicin by MDR cells.*

Solutions were microinjected using an InjectMan® NI 2 micromanipulator combined with a Femtojet microinjector (Eppendorf, Germany). The micromanipulator was mounted on an Axiovert 200 microscope (Zeiss, Germany). Pre-drawn capillaries with a tip diameter of 0.5 μm were used for microinjection (Femtotip®/Femtotip®II, Eppendorf). The Femtojet system allows the pressure of injection (150 kPa), time of injection (0.5 s) and background pressure (50 kPa, to counteract capillarity) to be set and thus control the amount injected. There are variations in how well the capillaries breach the cell membrane, and hence the amount of drug injected cannot be accurately quantified and can vary significantly among cells. Epirubicin (0.5 mg/mL) was co-injected with a 77 kDa fluorescein isothiocyanate (FITC)-dextran (0.5 mg/mL) into the cytoplasm of adherent cells. The FITC-dextran allowed the injected cell population to be clearly identified. FITC-dextrans of 77 kDa are ordinarily excluded from cells and cell nuclei, as they are too large to passively diffuse across these membranes. The injection buffer used was 48 mmol/L K_2HPO_4, 4.5 mmol/L KH_2PO_4, 14 mmol/L NaH_2PO_4, pH 7.2 (Eppendorf, personal communication). All injection fluids were mixed in Eppendorf tubes and then centrifuged at 15 000 ***g*** for 3 min before transfer into capillaries, to ensure that any particles were separated from the injection liquid. Epirubicin precipitated

*FIG. 2. MGH-U1 cells **(A)** and RT112 cells **(B)** co-injected with epirubicin (0.5 mg/mL) and FITC-dextran (0.5 mg/mL). Bottom left: epirubicin fluorescence (red) showing a nuclear uptake pattern. Top left: FITC-dextran fluorescence (green) excluded from the nucleus. Top right: differential interference contrast image. Bottom right: Integrated image.*



slightly when mixed with injection buffer, but this precipitate was separated during centrifugation. Injection fluid was pipetted into capillaries using Eppendorf Microloaders, taking care not to stir up any precipitate. Once microinjected, cells were then incubated for 60 min in normal culture conditions to allow cell recovery and time for the epirubicin to distribute within the cells. To show the normal sensitive and MDR patterns of epirubicin uptake for comparison, MGH-U1 and MGH-U1R cells were incubated for 60 min with epirubicin 10 μg/mL in culture medium.

All cells were then viewed using a Zeiss LSM 510 confocal microscope, through a ×40 water-immersion lens. Injected cells were easily identified on direct fluorescence microscopy by their green FITC-dextran fluorescence. Confocal microscopy allowed direct visualization of the distribution of the various fluophores within the microinjected cells. Epirubicin fluorescence was excited by a 543-nm HeNe laser and emission of wavelengths of ≥560 nm (red) detected. FITC-dextran fluorescence was excited by a 488-nm argon laser and emission wavelengths of 505–530 nm (green) detected. The multitrack scanning mode on the confocal microscope was used to reduce cross interference in the fluorescence signals of these two fluophores.

Acridine orange (3 μg/mL) stains the nuclei of viable cells green and was added after initial image capture to confirm cell viability. Acridine orange has similar fluorescence properties to FITC and was detected with the same filter settings.

RESULTS

The epirubicin localization characteristics of MGH-U1 (sensitive) and MGH-U1R (MDR) cell lines after incubation with epirubicin 10 μg/mL are shown in Fig. 1A,B. MGH-U1 cells showed a clear nuclear uptake pattern of epirubicin (Fig. 1A), whereas MGH-U1R cells showed characteristic nuclear sparing of epirubicin (Fig. 2B).

After microinjection with epirubicin the sensitive cell lines MGH-U1 and RT112 showed clear nuclear uptake of epirubicin (Fig. 2A,B) consistent with their incubated uptake characteristics. However, the MDR cell lines, MGH-U1R and MGH-U1-MMC showed characteristic nuclear sparing of epirubicin

after microinjection (Fig. 3A,B), again consistent with their incubated uptake characteristics.

The 77 kDa FITC-dextran allowed injected cells to be clearly identified, especially the resistant ones (otherwise showing low fluorescence) but was excluded from the nuclei of all injected cells, allowing clear visualization of nuclear epirubicin uptake, if present. Galleries of confocal slices through the depth of some cells were obtained (Fig. 4A,B). Reconstructing these to produce a three-dimensional image of the cells visualized the epirubicin distribution three-dimensionally, allowing better localization of the exact cellular distribution of epirubicin and the FITC-dextran (Fig. 5A,B). Characteristically, the drug fluorescence associated with resistant cells was punctate, and on the surface or even outside the cells. The dextran fluorescence was evenly distributed through the cytoplasm. Acridine orange staining confirmed cell viability in all experiments.

DISCUSSION

Much has been reported about the nuclear-sparing phenomenon shown by MDR cells when incubated with various anthracyclines [15–18] but there are few reports assessing the role of the nuclear membrane in this effect [22]. The present study is the first to assess the role of the nuclear membrane in MDR in viable, adherent bladder cancer cells *in situ*.

Microinjection of drugs has clear advantages over standard exposure regimens for studying intracellular distribution in MDR cells. Previous investigators have questioned whether the nuclear-sparing phenomenon is merely a reflection of low levels of drug accumulation in MDR cells, because of drug efflux by P-gp, or whether the nuclear membrane is also active [22]. Microinjection of epirubicin bypasses the plasma membrane P-gp efflux pump, delivering a bolus of drug directly into the cytoplasm. There is still some efflux of the drug, as shown by the differences in overall drug fluorescence between sensitive and resistant cells, suggesting that the plasma membrane P-gp pump effluxes drug from the cell cytoplasm, and excludes drug while it crosses the plasma membrane, as suggested in previous studies [23,24].

FIG. 3. MGH-U1R cell ***(A)*** *and MGH-U1-MMC cell* ***(B)*** *co-injected with epirubicin (0.5 mg/mL) and FITC-dextran (0.5 mg/mL). Bottom left: epirubicin fluorescence (red) showing a nuclear-sparing pattern with cytoplasmic vesicular staining. Top left: FITC-dextran fluorescence (green) excluded from the nucleus. Top right: differential interference contrast image. Bottom right: integrated image.*

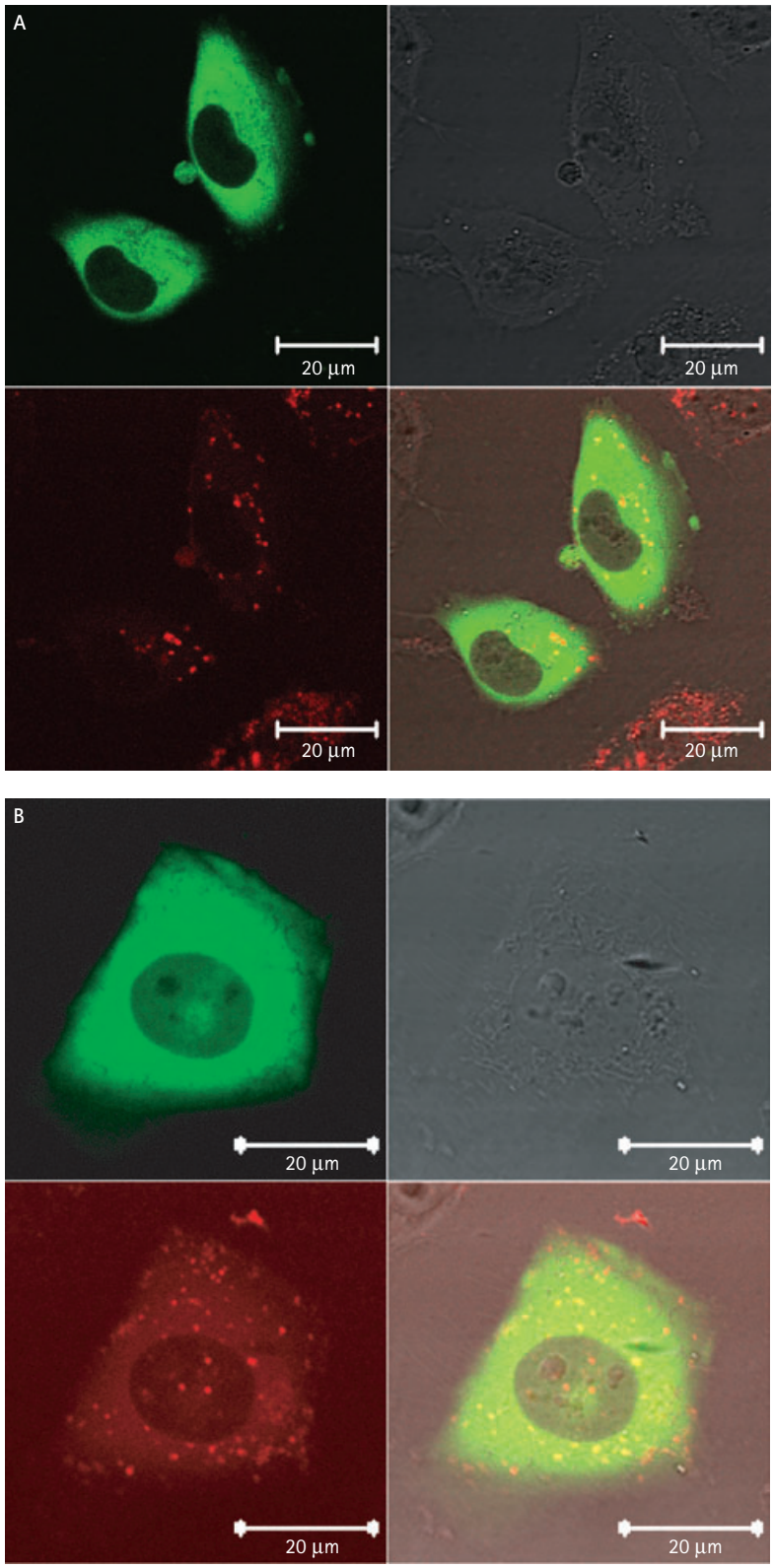
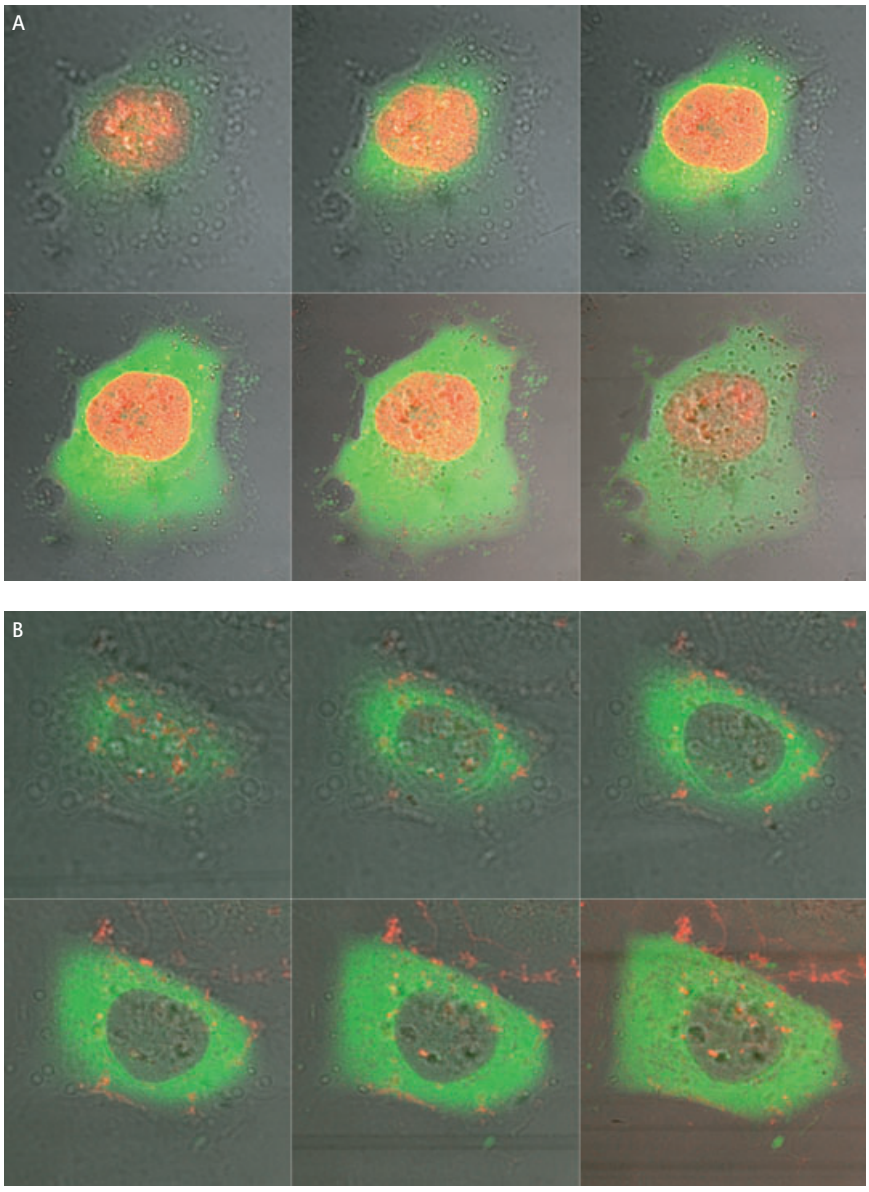


*FIG. 4. A gallery of confocal slices through a MGH-U1 cell **(A)** and a MGH-U1R cell **(B)** injected with epirubicin and FITC-dextran (integrated images).*



There was some delay in visualizing injected cells because of the location of the microinjection and confocal facilities at our institution, thus allowing time for some efflux to occur in the MDR cell samples. This delay also allowed time for the drug to be packaged in vesicles within the cytoplasm of MDR cells, as noted in previous confocal studies using standard anthracycline exposure methods [17].

The ability to reconstruct a three-dimensional image from serial stacks of confocal slices greatly enhances the visualization of epirubicin within injected cells, and allows clearer identification of nuclear uptake or sparing. The reconstructions strongly suggest that while almost all of the injected drug is sequestered into the nuclei of sensitive cells, resistant cell cytoplasm not only packages drug into vesicles, but takes it to the cell surface and expels it. There seemed to be very little drug in the cytosol.

The present results showed that cells microinjected with epirubicin had the same basic pattern of nuclear drug uptake or exclusion as cells incubated with epirubicin in the external milieu. They show that the nuclear membrane acts as a barrier to nuclear epirubicin uptake in MDR cells and must play a role in MDR. However, the mechanism(s) by which the nuclear membrane excludes epirubicin from the nuclei of MDR cells requires further investigation.

Possibilities include the expression of P-gp on the nuclear membrane causing drug efflux from the nucleus. One published study identified P-gp on the nuclear membrane of the MDR breast cancer cell line MCF-7 DX [25] but another reported that P-gp was absent from the nuclear membrane of a lymphoblastic tumour cell line that had the MDR phenotype [26]. Direct immunostaining of P-gp in the present MDR line using cold acetone/ethanol fixation and JSB-1 detected no clear localization to the nuclear envelope (C. Davies, personal communication). More intensive studies of the location of P-gp in the present MDR bladder cell lines are needed.

Another possible mechanism may involve the numerous nuclear pore complexes that allow 'molecular trafficking' between the nucleus and the cytoplasm. Nuclear pore complexes are large 120 MDa supramolecular assemblies that straddle the double-membrane of the nuclear envelope [27] and share the presence of the lung resistance protein with cytoplasmic vaults [28]. These nuclear pores allow passive diffusion of molecules up to 40 kDa and the transport of larger molecules, up to several megadaltons, by energy-dependent transport mechanisms, provided they carry a nuclear localization sequence that facilitates nuclear pore binding and transport [27].

Epirubicin is a small molecule of ≈0.5 kDa, so that by size criteria it should easily diffuse into nuclei via nuclear pores. However, the present results show that even when microinjected into MDR cells, epirubicin is excluded from the nucleus. This would seem to imply that either epirubicin is entering the nucleus and then being effluxed, or that it is denied entry into the nucleus in some way.

One possible explanation may be that epirubicin binds to proteins within the cytoplasm and is excluded from the nucleus if these proteins do not carry the nuclear localization sequence necessary for transport into the nucleus of MDR cells. This theory is

partly supported by previous work on the role of the nuclear membrane in adriamycin uptake in isolated sensitive and MDR nuclei, from a lymphoblastic tumour cell line [22]. This work showed that in isolated nuclei, nuclear transport of adriamycin (doxorubicin) is less in MDR cells than in sensitive cells. Furthermore, blockade of active nuclear transport by wheat germ agglutinin (*Triticum vulgaris* lectin) reduced sensitive nuclear uptake of adriamycin to about the level of MDR nuclear uptake, but had no effect on adriamycin accumulation in MDR nuclei. This suggests that sensitive cells have two mechanisms by which nuclear adriamycin is taken up, i.e. passive diffusion and active transport. Adriamycin is thus transported into the nucleus of sensitive cells bound to proteins, whereas MDR cells allow adriamycin into their nuclei by passive diffusion only, with no active transport. This absence of active transport in MDR nuclei has several possible explanations, the two most probable being either that adriamycin binds to different proteins that do not carry the necessary sequence for nuclear transport, or that the nuclear localization sequence receptor on the nuclear pore complex is different in MDR cells. However, the isolated nuclei of sensitive and MDR cells in this study accumulated 10 times more drug than their whole-cell counterparts, suggesting that this system is not a good model of intracellular nuclear adriamycin handling. It is also questionable whether or not isolated nuclei remain 'viable', as nuclear drug uptake in nonviable MDR cells has been shown to be similar to nuclear uptake in viable sensitive cells [17]. This observation also clearly indicates the need to assess cell viability in any study evaluating drug uptake and localization in MDR cells, to ensure that nuclear staining is not the result of cell death. All cells in the present study were clearly viable on staining with acridine orange.

Further evidence to support the theory that sensitive cells transport anthracyclines into their nuclei bound to proteins, comes from a study showing, in sensitive leukaemic cells, that adriamycin was transported into nuclei bound to proteasomes [29]. Proteasomes are intracellular proteinase complexes involved in nonlysosomal protein degradation; they can be localized in both the cytoplasm and the nucleus, and hence carry the nuclear localization sequence necessary for active transport from the cytoplasm into the nucleus. The protein binding of anthracyclines in cells must be investigated further to assess if there are differences between sensitive and resistant cancer cells that may account for their differences in nuclear uptake.

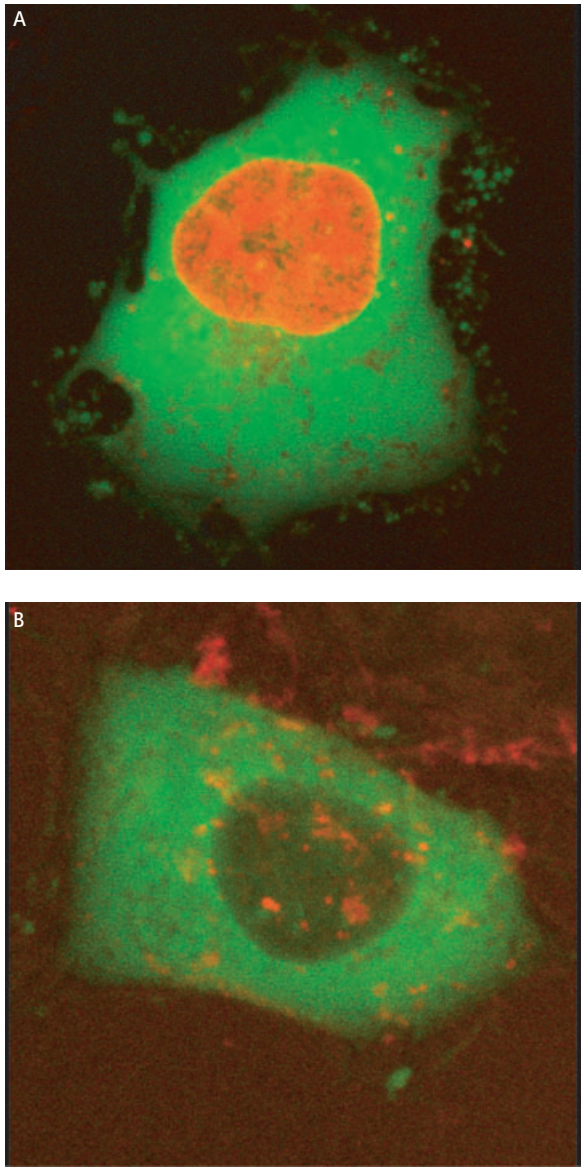


FIG. 5.
A three-dimensional reconstruction of confocal slices through a MGH-U1 cell ***(A)*** *and MGH-U1R cell* ***(B)*** *injected with epirubicin and FITC-dextran (fluorescence only).*

Confocal microscopy allows semiquantitative measurements of nuclear fluorescence but because the quantity of epirubicin microinjected cannot be uniform among cells we were unable to compare nuclear fluorescence measurements among the different cell types in this study.

Like MDR, nuclear sparing has been shown in MDR cell lines from numerous organs, appearing to transcend tissue type. It is therefore likely that this mechanism is an important reason for chemotherapeutic failure in all tumours showing MDR. Nuclear sparing has also been reported using other fluorescent chemotherapeutic agents, e.g. the anthraquinones, which include mitoxantrone [18,30]. Difficulties in visualizing the cellular distribution of nonfluorescent chemotherapeutic agents makes it impossible to confirm that in MDR cells, nuclear sparing influences the efficacy of all MDR-affected anticancer agents and not just those that are autofluorescent.

In conclusion, the nuclear membrane, perhaps in association with proteasome-like carrier molecules, is important in mediating resistance by denying drugs access to their

targets. If the mechanisms behind the role of the nuclear membrane in nuclear sparing and MDR can be elucidated further, they may provide further potential therapeutic targets for cancer treatment.

ACKNOWLEDGEMENTS

We are grateful to the British Urological Foundation and Sanofi-Synthelabo for their support in the form of a joint Surgical Research Scholarship.

CONFLICT OF INTEREST

J. Featherstone has received a BUF–Sanofi-Synthelabo Scholarship.

REFERENCES

1 **Park J, Shinohara N, Liebert M, Noto L, Flint A, Grossman HB.** P-glycoprotein expression in bladder cancer. *J Urol* 1994; **151**: 43–6
2 **Clifford SC, Neal D, Lunec J.** High level expression of the MDR-1 gene in the normal bladder urothelium: a potential involvement in protection against carcinogens. *Carcinogenesis* 1996; **17**: 601–4
3 **Abrams PH, Choa RG, Gaches CG, Ashken MH, Green NA.** A controlled trial of single dose intravesical adriamycin in superficial bladder tumours. *Br J Urol* 1981; **53**: 585–7
4 **Oosterlinck W, Kurth KH, Schroder F, Bultinck J, Hammond B, Sylvester R.** A prospective European Organization for Research and Treatment of Cancer Genitourinary Group randomized trial comparing transurethral resection followed by a single intravesical instillation of epirubicin or water in single stage Ta, T1 papillary carcinoma of the bladder. *J Urol* 1993; **149**: 749–52
5 **Koga H, Kuroiwa K, Yamaguchi A, Osada Y, Tsuneyoshi M, Naito S.** A randomized controlled trial of short-term versus long-term prophylactic intravesical instillation chemotherapy for recurrence after transurethral resection of Ta/T1 transitional cell carcinoma of the bladder. *J Urol* 2004; **171**: 153–7
6 **Richie JP.** Intravesical chemotherapy. Treatment selection, techniques and results. *Urol Clin North Am* 1992; **19**: 521–7
7 **Tolley DA, Parmar MKB, Grigor KM *et al.*** The effect of intravesical mitomycin C on recurrence of newly diagnosed superficial bladder cancer: a further report with seven years of follow-up. *J Urol* 1996; **155**: 1233–8
8 **Nakagawa M, Emoto A, Nasu N *et al.*** Clinical significance of multi-drug resistance associated protein and P-glycoprotein in patients with bladder cancer. *J Urol* 1997; **157**: 1260–5
9 **Tada Y, Wada M, Migita T *et al.*** Increased expression of multidrug resistance-associated proteins in bladder cancer during clinical course and drug resistance to doxorubicin. *Int J Cancer* 2002; **98**: 630–5
10 **Inaba M, Kobayashi H, Sakurai Y, Johnson RK.** Active efflux of daunorubicin and adriamycin in sensitive and resistant sublines of P388 leukaemia. *Cancer Res* 1979; **39**: 2200–3
11 **Krishan A, Sridhar KS, Davila E, Vogel C, Sternheim W.** Patterns of anthracycline retention modulation in human tumour cells. *Cytometry* 1987; **8**: 306–14
12 **Roepe PD.** Analysis of the steady-state and initial rate of Doxorubicin efflux from a series of multi-drug resistant cells expressing different levels of P-glycoprotein. *Biochemistry* 1992; **32**: 12555–64
13 **Coley HM, Twentyman PR, Workman P.** The efflux of anthracyclines in multidrug-resistant cell lines. *Biochem Pharmacol* 1993; **46**: 1317–26
14 **Duffy PM, Hayes MC, Cooper A, Smart CJ.** Determination and reversal of resistance to epirubicin intravesical chemotherapy. A flow cytometric model. *Br J Urol* 1996; **77**: 819–23
15 **Gervasoni JE, Fields SZ, Krishna S *et al.*** Subcellular distribution of daunorubicin in P-glycoprotein-positive and -negative drug-resistant cell lines using laser-assisted confocal microscopy. *Cancer Res* 1991; **51**: 4955–63
16 **Coley HM, Amos PR, Twentyman PR, Workman P.** Examination by confocal fluorescence imaging microscopy of the subcellular localisation of anthracyclines in parents and multidrug resistant cell lines. *Br J Cancer* 1993; **67**: 1316–23
17 **Duffy PM, Hayes MC, Gatrell SKE, Cooper A, Smart CJ.** Determination and reversal of resistance to epirubicin intravesical chemotherapy. A confocal imaging study. *Br J Urol* 1996; **77**: 824–9
18 **Davies CL, Loizidou M, Cooper AJ, Taylor I.** Effect of α-linolenic acid on cellular uptake of structurally related anthracyclines in human drug sensitive and multidrug resistant bladder and breast cancer cell lines. *Eur J Cancer* 1999; **35**: 1534–40
19 **Cooper AJ, Hayes MC, Duffy PM, Davies CL, Smart CJ.** Multidrug resistance evaluation by confocal microscopy in primary urothelial cancer explant colonies. *Cytotechnology* 1996; **19**: 181–6
20 **McGovern F, Kachel T, Vijan S, Schiff S, Lin C, Prout GR.** Establishment and characterization of a doxorubicin resistant human bladder cancer cell line (MGH-U1R). *J Urol* 1988; **140**: 410–4
21 **Hayes MC, Birch BR, Cooper AJ, Primrose JN.** Cellular resistance to mitomycin C is associated with overexpression of MDR-1 in a urothelial cancer cell line (MGH-U1). *BJU Int* 2001; **87**: 245–50
22 **Bobichon H, Cailleret L, Ploton D, Belhoussine R, Jardillier JC.** Nuclear transport as an ultimate step of multidrug resistance. *Life Sci* 1999; **65**: 2343–9
23 **Colin M, Madoulet C, Warren L, Cardillier JC.** Alterations of vinblastine influx in multi-drug resistant lymphoblastic leukaemic CEM cells. *Anticancer Res* 1996; **16**: 407–12
24 **Luker GD, Flagg TP, Sha Q *et al.*** MDR-1 P-glycoprotein reduces influx of substrates without affecting membrane potential. *J Biol Chem* 2001; **276**: 49053–60
25 **Calcabrini A, Meschini S, Stringaro A, Cianfriglia M, Arancia G, Molinari A.** Detection of P-glycoprotein in the nuclear envelope of multidrug resistant cells. *Histochem J* 2000; **32**: 599–606
26 **Bobichon H, Colin M, Depierreux C, Liautaud-Roger F, Jardillier JC.** Ultrastructural changes related to multidrug resistance in CEM cells. Role of cytoplasmic vesicles in drug exclusion. *J Exp Ther Oncol* 1996; **1**: 49–61
27 **Pante N, Aebi U.** Toward a molecular understanding of the structure and function of the nuclear pore complex. *Int Rev Cytol* 1995; **162**: 225–55
28 **Scheffer GL, Wijngaard PL, Flens MJ *et al.*** The drug resistance-related protein LRP is the major vault protein. *Nature Med* 1995; **1**: 578–82
29 **Kiyomiya K, Matsuo S, Kurebe M.** Mechanism of specific nuclear transport of adriamycin: the mode of nuclear

translocation of adriamycin-proteasome complex. *Cancer Res* 2001; **61**: 2467–71

30 **Consoli U, Van NT, Neamati N *et al.*** Cellular pharmacology of mitoxantrone in p-glycoprotein-positive and -negative human myeloid leukemic cell lines. *Leukaemia* 1997; **11**: 2066–74

Correspondence: Jonathan M. Featherstone, Department of Urology, Southampton University Hospitals NHS Trust, Tremona Road, Southampton, SO16 6YD, UK.
e-mail: JonFeath@aol.com

Abbreviations: **MDR**, multidrug resistance; **P-gp**, P-glycoprotein; **FITC**, fluorescein isothiocyanate.