Short Communication

Studies on the in vivo disposition of adriamycin in human tumours which exhibit different responses to the drug

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In vivo disposition of Adriamycin (ADR) is characterized by rapid uptake into most tissues and long tissue transit time due to high affinity but slowly reversible binding to nuclear DNA (Harris & Gross, 1975; Terasaki et al., 1984). The role of drug metabolism in this scheme has not been fully elucidated (Chan et al., 1978). There is reason to believe that uptake of ADR into solid tumours behaves independently of the factors that govern its distribution into tissues. For instance, ADR was detected in only the outermost 4 to 6 layers of cells of intra-abdominal ovarian tumours, indicating a penetration problem not normally associated with tissues (Ozols et al., 1979). Also, tumour cells develop resistance to ADR believed to involve active extrusion of the drug from cells (Dano, 1973). Studies on the uptake of ADR into solid human tumours in vivo have been limited because of the obvious problem of obtaining sufficient numbers of specimens for meaningful evaluation. Also, these studies have tended to compare drug concentrations in tumours with unrelated tissues. In this paper we have attempted to study several aspects of the disposition of ADR in solid human tumours in vivo: (a) dose/response, by sampling from tumours with different known responses to ADR; (b) tumour drug uptake, by comparing ADR concentrations in tumours with adjacent normal tissue and blood; (c) intracellular binding; and (d) tumour metabolism.

Tumour and normal tissue specimens were obtained from post-operative resections and open biopsies from a total of 36 patients admitted to Glasgow Royal Infirmary. Gastric carcinoma and adjacent normal mucosa were from partial and full gastrectomies; colorectal carcinoma and adjacent normal colon mucosa were from anterior resections and partial colectomies and breast carcinoma was from breast lumpectomies. Breast cancer metastases were from axilla node biopsies with evidence of tumour nodules. Liver biopsy specimens from gastric and colorectal cancer patients and 5 ml of venous blood from all patients were sampled simultaneously with tumour resection. A low dose of commercially available ADR (25 mg m⁻², Farmitalia, Milan, Italy) was administered i.v. operatively 30 min before tumour resection (27 min ± 16 min s.d.). Resections and biopsies were immediately taken to the pathology department from histological sectioning and 1–2 g tumour visibly clear of necrosis and normal tissue, 1–2 g mucosa and 0.5 g liver were immediately frozen to −60°C with solid CO₂ for determination of drug and metabolite content. ADR and metabolite concentrations of all samples were determined by HPLC as previously described (Cummings, 1985). Each tumour and tissue specimen was homogenised and extracted using two different methods, both of which employed Daunorubicin as internal standard (Cummings et al., 1984; 1986). The first involved mixing a 1 ml aliquot of homogenate directly with 5 ml of chloroform: propan-2-ol (2:1) for 30 min, followed by centrifugation (1000 g for 15 min) to separate three distinct phases. The upper aqueous layer was discarded, the lower organic layer was decanted over the middle tissue pellet, transferred to a clean test tube and evaporated to dryness. The residue was dissolved in a small volume of methanol and injected onto the HPLC column. Treatment of tissue homogenates directly with organic solvents does not release bound ADR (Ozols et al., 1979). Our direct extraction method was used to determine the concentration of the unbound ADR fraction in samples. The second method involved pretreatment of a 1 ml aliquot of homogenate with 0.2 ml of silver nitrate (33% w/v) for 10 min at 4°C before extraction with organic solvent as described above. Silver nitrate releases ADR bound to DNA by intercalation and precipitates proteins (Schwartz, 1973). We combined pretreatment with silver nitrate and organic solvent extraction to determine the concentration of the free and reversibly bound fraction of ADR and metabolites in samples. The bound fraction, which was an indicator of intracellular binding in samples, was then

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calculated as a percentage by comparing recoveries from both extraction techniques.

Results from all tumour and adjacent normal tissue specimens were summarised and are contained in Table I. There they are represented to highlight the four areas under study: dose response (published percentage response of patients to single agent ADR vs. mean tumour ADR concentration); uptake (mean intrapatient ratios of ADR concentration in tumours over ADR concentration in normal mucosa and serum); intracellular binding (mean percentage of total concentration in tumour and tissue bound to cellular components); and metabolism. Results obtained from all liver biopsies and blood samples are contained in Table II. The mean concentration of ADR determined in colorectal tumours (176 ± 89 s.d. ng g⁻¹ tumour) was significantly lower (P < 0.001, Student’s t test) than that determined in breast tumours (819 ± 482 s.d. ng g⁻¹ tumour) and gastric tumours (659 ± 231 s.d. ng g⁻¹ tumour). The levels of ADR in colorectal tumours (73.1 ± 38 s.d. percentage of normal mucosa concentration) and to a lesser extent in gastric tumours (47.7 ± 18 s.d. percentage of normal mucosa concentration) were close to equilibrium with the levels of ADR in adjacent normal mucosa. There was a strong correlation (r = 0.95) between the mean concentration of ADR in breast, gastric and colorectal tumours and the percentage of patients that responded to single agent ADR (not shown). When individual patient ADR concentrations in tumours were compared with individual ADR concentrations in serum, the levels in the three tumours were many times higher, indicating significant intracellular drug accumulation in all cases. The mean concentration of ADR determined in axillary node specimens was low (55 ± 48 s.d. ng g⁻¹ tissue) and less than sera, suggesting that at the time of sampling equilibrium with blood borne ADR had not yet been achieved. The determined fraction of the total ADR concentration bound to intracellular components in all tumour types was high (72–83%) and of the same order of magnitude as the reported binding of ADR to DNA in vitro and to tumour cells in culture (Schwartz, 1983). The determined fraction of the total ADR concentration bound to intracellular components in normal tissue and liver was significantly less than in tumours (P < 0.01, Tables I and II). There was no evidence of metabolites in any one tumour and normal mucosa specimen, although one metabolite has been reported to be present in human tumour biopsies sampled at different times from ours (Chan et al., 1978). In liver, where the levels of ADR were high (5570 ± 1500 s.d. ng g⁻¹), two metabolites were identified: adriamycinol 7-deoxyglycone (275 ± 516 s.d. ng g⁻¹) and adriamycin 7-

| Tumour type                      | Tumour | ADR concentration ng g⁻¹ | ADR to normal mucosa ratio | Intratumour concentration ng g⁻¹ | Published % patient responded to single agent ADR | Tumours | Number per tumour | Mean ADR concentration mg m⁻² | AD ratio | Culture | Metabolism | Mean ADR concentration mg m⁻² | AD ratio | Metabolism |
|----------------------------------|--------|--------------------------|-----------------------------|-----------------------------------|------------------------------------------|---------|------------------|-------------------------------|---------|---------|-----------|-------------------------------|---------|------------|
| Breast                           | 39%    | 819 (482)                | 6                           | 1340 (298)                        | 419 (242)                                | 36      | nil              | 5                             | nil     | nil     | nil       | nil                                          | nil     | nil        |
| Gastric                          | 22-24% | 659 (231)                | 6                           | 551 (232)                         | 67 (8)                                   | 7       | nil              | 10                            | nil     | nil     | nil       | nil                                          | nil     | nil        |
| Colorectal                       | 9%     | 176 (69)                 | 7                           | 319 (159)                         | 83 (6)                                   | 5       | nil              | 10                            | nil     | nil     | nil       | nil                                          | nil     | nil        |
| Axilla node with tumour nodules  | 5      | 55 (48)                  | 5                           | 56 (342)                          | 40 (21)                                  | 5       | nil              | 5                             | nil     | nil     | nil       | nil                                          | nil     | nil        |

*Significantly lower than breast and gastric carcinoma, P < 0.001; *Significantly higher than normal tissue, P < 0.01.
deoxyaglycone (65 ± 18 s.d. ng g⁻¹). There was a marked inter-patient variation in liver concentration of adriamycinol 7-deoxyaglycone, which is reflected in the large value of s.d. (Table II). Metabolite profiles in serum were different from liver. Adriamycinol was detected in all patients and the 7-deoxyaglycones in only 2–4 patients.

Studies of the in vivo disposition of ADR in human tumours have been performed after repeated sampling at a single time point (27 min ± 16 min s.d.). For a single determination of a drug concentration to yield information with which to make comparisons, ideally plateau concentrations should be measured. It is likely that plateau levels are reached in the different tumours and tissues at different times. Probably the best model available to study what the kinetics of ADR may be like in human tumours is the solid transplantable animal tumour. Results from animal studies show that peak levels are achieved in liver and intestine almost immediately after i.v. administration, whilst in tumours peak levels are achieved after 1–3 h (Yesair et al., 1972; Yesair et al., 1980). At 30 min, the time chosen in this study, approximately 60–100% of plateau levels are recorded in both normal tissues and tumours.

In one of the few related studies, human tissue and tumour specimens were obtained 1.5–4 h after i.v. administration of a dose of ADR similar to that used in the present study (10–60 mg m⁻², Chan et al., 1978). In a single colon adenocarcinoma sample, the concentration of ADR was 9.2 μg g⁻¹ 4 h after drug administration. In a single breast adenocarcinoma sample, the concentration of ADR was 2.49 μg g⁻¹ 2 h after drug administration. We can offer no explanation for the large disparity between these results and ours apart from the fact that different analytical methodology was used (TLC compared to HPLC) or at the time of sampling a genuine difference existed. The main finding of this study is that mean tumour drug concentration correlated with percentage figures published for response of each tumour type to single agent ADR. The low levels of ADR in colorectal tumours were not due to an inability to accumulate the drug from the circulation. Colorectal cancer, which is known to be refractory to ADR (Moertel, 1975) may benefit from new approaches in ADR cancer chemotherapy, such as regional drug administration or carrier-mediated drug targeting.

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### Table II

| Organ    | Number | ADR conc. (ng ml⁻¹) | 7-deoxyaglycone conc. (ng g⁻¹) | % bound | ADR mean (± s.d.) | 7-deoxyaglycone mean (± s.d.) |
|----------|--------|---------------------|------------------------------|---------|------------------|-----------------------------|
| Liver    | 14     | 5570 (1590)         | 174 (133)                    |         | 129 (26)         | 11 (2)                      |
| Serum    | 24     | ND                  | ND                           |         | ND               | ND                          |

*Not detected.*
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