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Perspectives and Commentaries

Pharmacology of Adriamycin: the Message to the Clinician

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(A COMMENT ON: Keyes SR, Hickman JA, Sartorelli AC. The effects of Adriamycin on intracellular calcium concentrations of L1210 murine leukaemia cells. *Eur J Cancer Clin Oncol* 1987, **23**, 295–302.)

THE ARTICLE of Keyes *et al.* [1] sets out to study *in vitro* the nature of Adriamycin (ADR)-induced alterations in calcium homeostasis in L1210 murine leukaemia cells. At drug concentrations of 5 and 10 μM intracellular calcium concentrations were elevated by 29% and 46%, respectively, but only after continuous exposure with drug for 4 h. Less than 4 h (a 2 h exposure) no effect was evident; greater than 4 h (a 6 h exposure) the effect appeared to be recovering back to baseline. Concentrations of 5 and 10 μM ADR were chosen because they were considered to be in the pharmacologically relevant cytotoxic range, albeit at the upper limits. Intracellular calcium may contribute to cytotoxicity when concentrations are changed beyond tolerable thresholds [1]. If intracellular calcium does have a role in the clinical pharmacology of ADR then clearly well defined levels of the drug must be maintained for a critical period of time close to or within actual deposits of tumour cells. Are these concentrations achieved or achievable clinically? In this commentary we will address this question, with its broader implication for many of the pharmacologic effects of ADR described *in vitro*, and discuss the relationship between known pharmacokinetic behaviour and known pharmacologic effect where a reasonable link has been established clinically.

METHODS OF ANALYSIS—PROBLEMS AND LIMITATIONS

ADR presents many problems for the analyst because of unique physico-chemical properties and precautions must be taken in order to prevent serious loss of the drug and its metabolites during analysis. ADR is chemically reactive, chemically unstable, and is sensitive to photo-degradation by light, acid catalysed hydrolysis and rapid base catalysed chemical decomposition. It can bind to inert materials such as glass vessels, tissue culture plastic, cellulose ester membranes and polytetrafluoroethylene (PTFE) filters [2] as well as forming chemical complexes with proteins, nucleic acids, phospholipids, amino acids, nucleotides, biogenic amines and itself. Repeated stability studies have consistently produced very different results, despite using similar experimental conditions (e.g. 35 min to 14 h for 5% decomposition at room lighting and temperature) [2]. One explanation for this large discrepancy is that different methods of analysis are used to measure the drug [most commonly fluorescence vs. u.v. absorption plus or minus high performance liquid chromatography (HPLC)]. Whilst ADR can be detected with superb selectivity and sensitivity by fluorescence, it degrades to many non-fluorescent products and intermediates, each with a unique u.v.-visible absorption spectrum. An extremely versatile analytical technique is required to determine ADR accurately.

We have examined drug purity in patient specimens by HPLC with computer-controlled diode array high speed scanning spectrophotometric detection (DAD), a technique which is capable of measuring simultaneously ADR, non-fluorescent degradation products, chemical intermediates and drug complexes [3]. Blood (plasma or serum) con-

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tained ADR in pure form. However in urine, tissue and tumour biopsy specimens, impurities were identified co-eluting with the drug and evidence of chemical complexation was apparent.

Simple solutions of ADR require no sample preparation but complex mixtures including blood, urine, tissue/tumour biopsies, tumour cells, tissue culture medium and enzymic incubations require extraction of the drug to reduce or eliminate interfering impurities. Weak interactions between ADR and cellular macromolecules (protein and DNA) may be broken by pretreatment of specimens with silver nitrate [4]. However a varying fraction remains covalently bound to tissue components and can never be extracted under any conditions.

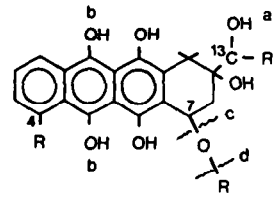
HUMAN PHARMACOKINETICS—THE RELATIONSHIP TO RESPONSE AND TOXICITY

After i.v. administration of a therapeutic dose of ADR to patients (30–60 mg/m^2) plasma drug levels fall (by rapid exponential decline, $t_{\frac{1}{2}} < 10$ min) from theoretically extrapolated zero time concentrations of 2–6 μM to level off at 20–50 nM by 1–4 h as equilibration with tissues occurs. The drug then enters its slow elimination phase with terminal half life ranging from 20–40 h. Thus plasma concentrations of 5–10 μM are rarely achieved and never maintained clinically.

Several factors are known to modulate the human pharmacokinetics of ADR, the most important of these being liver function status. With the hepatobiliary pathway generally being regarded as the major route of excretion of ADR and its metabolites in man, impaired liver function leads to deranged pharmacokinetics with the possibility of severe toxicity to the host due to an inability to clear the drug. Other modulating factors include age, dose, concomitant drug treatment and prior exposure to drug, the latter resulting in marked alterations in blood concentrations.

Bone marrow and GI-tract toxicity appear to be directly related to blood concentrations of ADR. Amelioration of these toxicities is achieved without loss of activity by either dose reduction to a 6–20 mg/m^2 weekly i.v. regime or rescheduling to a low dose infusion [5]. ADR-induced cardiotoxicity has been more closely linked to repeated drug treatment, limiting its administration to a recommended safe cumulative dose of 550 mg/m^2. Dose alterations similar to those mentioned above are also effective in reducing this unique toxicity, making it possible to exceed the above limit. In all these cases the belief is that toxicity is more related to peak plasma ADR concentrations rather than long terminal half life or area under the curve.

Clearly peak blood levels are important in determining ADR cardiotoxicity but other factors



a) C_{13} carbonyl reduction : alcohol

b) Quinone reduction : hydroquinone/semi-quinone

c) Reductive cleavage of C7 glycoside bond : 7- deoxyaglycone

d) Hydrolytic cleavage of C7 glycoside bond : 7- hydroxyaglycone

Fig. 1. Pathways of Adriamycin metabolism in man.

appear to be involved as well. Some patients show objective signs of cardiotoxicity after as low a cumulative dose as 200 mg/m^2 whilst others can exceed the safe limit without any adverse effects [6].

Attempts have also been made to relate ADR pharmacokinetics or blood concentrations at fixed time points to the outcome of response but these have been mostly unsuccessful [7, 8].

HUMAN DRUG METABOLISM—THE RELATIONSHIP TO RESPONSE AND TOXICITY

The major metabolite of ADR, which is consistently detected in patient plasma, is the C13 carbonyl reduced alcohol referred to as adriamycinol (AOL, Fig. 1). Formed by a ubiquitously distributed group of cytoplasmic aldo-keto reductase enzymes (activity also present in erythrocytes), AOL retains cytotoxicity but to a lesser degree than ADR. However, it remains an important metabolite because of its longer apparent half life which is also more sensitive to liver function status than ADR. After a therapeutic dose of ADR, peak AOL levels occur early on (5–15 min) and rarely exceed 200 nM but terminal half lives can extend out to 50 h [9].

Conjugate metabolites (a 4-O-sulphate and 4-O-glucuronide) have been positively isolated and identified in patient urine using TLC [10] but their presence in plasma awaits confirmation by newer, more sensitive HPLC methods.

Removal of the daunosamine sugar group can occur at two points, by two different mechanisms, to produce aglycone metabolites of ADR (Fig. 1). 7-Hydroxyaglycones are formed by hydrolytic cleavage which occurs *in vitro* by acid catalysed hydrolysis or, possibly, *in vivo* by microsomal hydrolyases. 7-Hydroxyaglycones of ADR can artefactually appear on TLC plates due to the acidic action of the silica gel on the glycoside bond [11]. On the other hand 7-deoxyaglycones cannot be formed *in vitro*. They are produced by reductive

Table 1. Tissue and tumour concentration equivalents of Adriamycin in man 30 min after 25 mg/m² i.v.

Tumour (μmoles/kg)		Normal tissue (μmoles/kg)	
Breast carcinoma*	1.4	Liver	9.6
Gastric carcinoma	1.1	Gastric mucosa	2.3
Colorectal carcinoma	0.3	Colorectal mucosa	0.6
Axilla node tumour nodules	0.1	Lung	1.1
		Spleen	0.3
		Serum	0.3†

*Data adapted from Ref. [13], S.D. not included for clarity (n = 36 patients in total).
†μMolar.

glycosidic cleavage from either the semi-quinone free radical or fully reduced hydroquinone intermediate after one or two electron reduction of the quinone nucleus (Fig. 1). Both these intermediates plus further short lived radicals and reactive intermediates (including a superoxide free radical) evolved during intra-molecular chemical rearrangements can participate in the anti-cancer action and cardiotoxicity of the drug, if produced locally.

Several pieces of circumstantial evidence suggest that the active cardiotoxic form of ADR is not the parent drug itself but these free radical products of quinone bioreduction. The presence of 7-deoxyaglycone metabolites in patient urine has been cited as proof for the formation of ADR free radicals in man [12]. From this point of view 7-deoxyaglycones may be important markers of clinical pharmacologic activity. Two have recently been identified in patient serum and their pharmacokinetics described [9]. These are the 7-deoxyaglycone of ADR (ADR-DONE) and AOL (AOL-DONE), and marked inter-patient variations are observed in their kinetic behaviour. In 4/25 patients peak serum concentrations of AOL-DONE (10–30 nM) occurred 4–8 h after drug administration. In 9/25 patients peak serum concentrations of AOL-DONE (10–200 nM) occurred during the first hour, and in 12/25 patients the metabolite was not detected. Only the two patients who had unexpectedly high levels of circulating 7-deoxyaglycones (AOL-DONE, 200 nM) experienced problems with early drug-induced heart failure (after less than a total cumulative dose of 300 mg/m²), suggesting that their appearance in blood may herald the development of cardiotoxicity.

DISPOSITION AND METABOLISM IN TARGET ORGANS—THE RELATIONSHIP OF TISSUE CONCENTRATIONS TO RESPONSE AND TOXICITY

Table 1 contains the range of ADR concentration equivalents achieved in human tissues and tumours 30 min after i.v. administration of 25 mg/m² [13]. These data require further clarification; the question being how close are these values to plateau levels? If a plateau is reached in different tissues and tumours at completely different times then the figures in Table 1 are not directly comparable, nor representative of what can actually be attained. Human pharmacokinetic studies and animal disposition studies are in agreement that well perfused tissues (like liver, heart and lung) reach equilibrium with blood-borne drug quickly, whilst it takes 1–3 h to reach equilibrium in solid tumours. Thirty minutes is the ideal time at which to monitor since both tissue and tumour drug concentrations represent between 60–100% of their maximum values.

Tumour biopsy drug levels at 30 min correlate strongly (r = 0.95) with known percentage response rates to single agent ADR chemotherapy [13]. No convincing evidence has been found for ADR metabolism by human tumours.

Returning to the central question of this commentary, are sustained levels of 5–10 μM ADR possible in human tumour cell deposits after administration of a therapeutic dose? The answer is probably no, based on the figures in Table 1 and accepting their validity as representing plateau concentrations.

At this point a cautionary note is due regarding Table 1. ADR is well known to penetrate only poorly solid tumour cell masses. From studies of drug uptake into avascular ascites tumour cell colonies and tumour cell spheroids, the majority of ADR appears to be confined to the outermost 4–6 layers of cells. Whilst the figures in Table 1 represent a gross value for approx. 1 g of biopsy material, higher concentrations may exist within the biopsy in localized regions close to blood vessels.

In human liver biopsies two metabolites are prominent: AOL-DONE and ADR-DONE, the two 7-deoxyaglycone metabolites of ADR which also appear in serum. Interestingly enough adriamycinol is never detected in liver. Consistent with the plasma/serum pharmacokinetics of 7-deoxyaglycones there is a marked inter-patient variation

in their liver concentrations (AOL-DONE, 0.5 μmoles/kg tissue ± 0.9). These data support our contention that a genuine difference exists in the way in which individual patients biotransform ADR to 7-deoxyaglycones and that this difference also manifests itself in plasma [9].

Murine models confirm that ADR cardiotoxicity is directly associated with the appearance of high levels of 7-deoxyaglycones in the heart itself. Here a close link between rate of formation and subsequent elimination from the heart and serum pharmacokinetics has been established [14, 15]. From the same studies it is also evident that serum/plasma pharmacokinetics of ADR *do not* reflect its rate of uptake into and elimination from solid tumour cell masses, which may explain the lack of success of clinical pharmacokinetic trials in trying to correlate response to plasma drug levels [7, 8]. The uptake of ADR into and elimination from solid tumours occurs more slowly than in normal tissues like liver and heart (90 min to achieve maximum concentration compared with 10 min for liver and heart, $t_{1/2}$ 30 h compared with 13 h for liver and 9 h for heart) [14].

SUMMARY

In attempting to describe the human pharmacology of ADR, one is aware of the gaps in our knowledge and shortcomings of the available data. Nevertheless, such information is essential if we are ever to be able to convert rationally *in vitro* observations into clinical pharmacologic effect or, as is more often the case, explain why the desired effect has not been produced.

Clinical pharmacokinetic studies to-date suggest that there is a clear relationship between ADR blood levels and toxicity. No such relationship between ADR blood levels and therapeutic response has been shown. The 7-deoxyaglycone tissue metabolites of ADR, which also appear in blood, may be more closely related to ADR cardiotoxicity and therefore may provide a better pharmacokinetic marker of its development. It appears that the only accurate pharmacokinetic indicator of response is the level of drug in the tumour itself.

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