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Stability of GR63178A, a Novel Pentacyclic Pyrroloquinone Anticancer Compound, in Aqueous Solutions and Biological Fluids

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Abstract—The effect of temperature, pH, light, drug concentration and aeration on the stability of aqueous solutions of GR63178A, a novel anticancer compound, has been investigated. The effect of light upon the stability of GR63178A in a clinical infusion system and in biological fluids, has also been investigated. A high performance liquid chromatographic assay was used to separate the parent compound from a number of degradation products. Data presented here demonstrate that GR63178A is stable in aqueous solutions at normal ambient temperatures in neutral or alkaline pH ranges. The compound is unstable when exposed to light ($t_{\frac{1}{2}}$ 3·2–233 min) and shows an inverse relationship between concentration and rate of degradation. The drug is also unstable under acidic conditions, but shows a more limited spectrum of degradation products than those arising from photolysis.

The pentacyclic pyrroloquinones are currently the subject of investigation regarding their potential anticancer properties. In common with many other established anticancer compounds, they possess a quinone moiety. Mitoquidone (GR30921; NSC 382057 D), the lead compound in this series, (Fig. 1) has been shown to be active against solid tumours, but not leukaemias, in mice (Fenton et al 1985) with limited toxic side-effects. GR63178A (NSC D611615; Fig. 1) is an analogue of mitoquidone with improved water solubility due to the possession of a dialkyl phosphate ester group as a sodium salt. Loss of this group from the molecule by hydrolysis would generate the 9-hydroxy derivative, GR54374X (Fig. 1). GR63178A has demonstrable antitumour activity in both murine tumours (sarcoma 180, MAC30T adenocarcinoma and colon 38) and human mammary, lung and colon tumour xenografts (Fenton et al 1989). No significant toxicity has been reported in rat, hairy mouse and nude mouse/xenograft systems at effective doses of

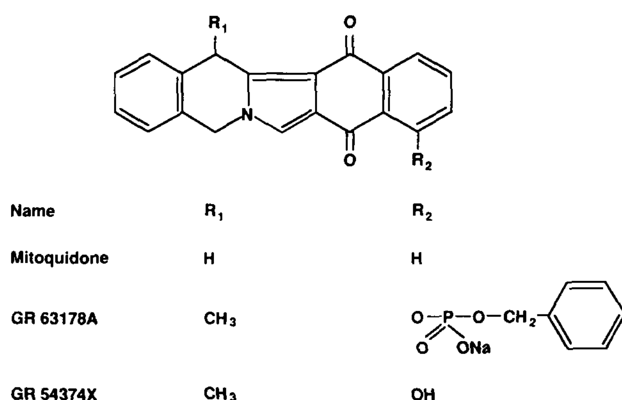

FIG. 1. Structure of mitoquidone and GR63178A.

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GR63178A. The compound has been the subject of phase I investigations (Eccles et al 1989; Verweij et al 1989) and has now entered extensive multicentre phase II trials at a dose of 120 mg m^{-2}, daily for five days every three weeks. The mechanism of action of GR63178A (or indeed mitoquidone) is not yet understood, but may involve either intercalation of the molecule's planar structure with DNA, or redox cycling of the quinone group and the subsequent generation of free radicals.

The object of this study was to investigate the stability of GR63178A in simple aqueous solutions, commonly used infusion media, and biological fluids. Such data will subsequently prove useful when designing phase II and other experimental studies.

Materials and Methods

Apparatus

Spectrophotometric analysis of solutions was performed using a Pye-Unicam SP1800 UV-visible spectrophotometer. Plastic universal containers used to incubate GR63178A with plasma samples were from Northern Media, Salisbury Way, Hessle, North Humberside, UK. HPLC analysis was performed on a Hewlett-Packard model 1090 liquid chromatograph equipped with a HP 9000/300 Chemstation computer with data acquisition and integrating software, and a multi-diode array UV-visible rapid scanning detector.

Chemicals

GR63178A (sodium salt, either pure or as formulated drug) and GR54374X (9-hydroxy analogue which is both a major degradation product and a metabolite; Fig. 1) were donated by the Infection and Oncology Department of the Medical Division of Glaxo Group Research Ltd, Greenford, Middlesex, UK. Methanol and acetonitrile were HPLC grade (Rathburn Chemicals, Walkerburn, UK). Ammonium acetate and acetic acid were also HPLC grade (Fisons, Loughborough, Leicestershire, UK). *N,N*-Dimethylacetamide (DMA) was from Aldrich Chemical Co., Gillingham, Dor-

set, UK. All other chemicals were of analytical reagent grade or better, and obtained from British Drug Houses plc, Poole, Dorset, UK or Sigma Chemical Co., Poole, Dorset, UK. Water was double-distilled and de-ionized in a quartz glass still. PVC Viaflex infusion bags (containing 5% Glucose for Infusion BP) and PVC infusion sets were from Baxter Healthcare Ltd, Caxton Way, Thetford, Norfolk, UK.

Sample preparation

All solution preparation and sample processing was undertaken in a darkroom equipped with a red safelight which had previously been shown not to cause degradation of the drug (results not shown).

Determination of molar extinction coefficient

Solutions of GR63178A from 1·0 mg mL^{-1} to 0·1 μg mL^{-1} (= 1·9 mM to 0·19 μM) were made up in water and their UV-visible absorbance spectra were scanned from 230–600 nm. The molar extinction coefficient was determined from the slope of the plot of absorbance at 370 nm (the visible λ_{max}) against concentration.

Solubility of GR63178A

Limits of solubility of GR63178A were determined in either simple aqueous solutions or phosphate-buffered saline (0·1 M) at pH 7·0. Excess GR63178A was placed in sealed brown glass sample tubes wrapped in silver foil and continually inverted overnight in a rotating mount. Resultant saturated solutions were centrifuged in an Eppendorf microcentrifuge for 10 min, and the supernatant was removed and diluted in duplicate either 1:10 or 1:100 with water or buffer. Absorbances of these solutions were measured in a Pye SP1800 UV-visible spectrophotometer at 370 nm. The actual concentrations of GR63178A were determined using the molar extinction coefficient.

Chromatographic conditions

Samples from incubations of simple aqueous solutions of drug were placed in brown glass injection vials without further dilution. Urine and plasma samples (0·5 mL) for HPLC analysis were mixed with equal volumes of DMA, and centrifuged for 2 min in an Eppendorf microcentrifuge, before being sealed in brown glass injection vials. Portions (150 μL) from all samples in brown glass injection vials were injected directly onto an Apex 1 C_{18} 5 μm column (25 cm × 4·6 mm i.d., Jones Chromatography, supplied by Crawford Scientific, Strathaven, UK). GR63178A and GR54374X are well resolved by this method (Fig. 2). Chromatographic conditions were as previously described (Cummings et al 1991). Other degradation products of extensively degraded samples also appear in the chromatogram. Peak purity was determined using absorbance spectra data generated by the diode array detector.

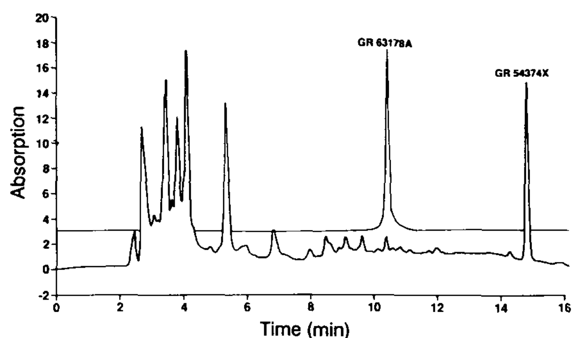

FIG. 2. HPLC of GR63178A (upper trace) and GR54374X in a photodegraded solution (lower trace).

Stability studies

All solutions were held in borosilicate glass volumetric flasks during incubation. For the investigation of factors affecting stability in the dark, solutions were held in dark brown borosilicate glass volumetric flasks in a darkroom at room temperature (21°C); alternatively, the temperature of the solutions were regulated where appropriate by placing solutions in a circulating water bath in the darkroom. The effects of pH on stability were assessed by dissolving GR63178A in water and adding HCl or NaOH (both 1·0 M) to give the required pH.

The effect of various light sources on stability was assessed on solutions of GR63178A held in clear borosilicate glass volumetric flasks. Uniform illumination was achieved in all conditions by rotating the flasks in-situ on a turntable at 40 rev min^{-1}. Light stability of GR63178A was investigated in the first instance by exposing a 100 μg mL^{-1} aqueous solution of the drug to a variety of illumination conditions. Ambient laboratory light indicates that samples were kept on an open bench under fluorescent lighting; samples were not in direct daylight, but laboratory light was supplemented by light from laboratory windows. Light levels from laboratory windows varied considerably, so control incubations of GR63178A in aqueous solutions were included in all incubations where stability in ambient laboratory light was determined. Direct daylight involved placing the solutions on a windowsill; as with ambient laboratory light, illumination conditions varied with the prevailing climate, so appropriate illuminated controls were utilized. A controlled light source indicates that samples were placed 20 cm from a single Philips 57202 E/99 HPR 125-Watt UV-visible bulb.

The effect of aeration on light stability was assessed by dissolving GR63178A in water rendered hypoxic by vacuum degassing (15 min at −80 kPa) followed by 30 min of helium spargeing.

Light stability in biological fluids was assessed by dissolving known amounts of GR63178A in plasma and urine samples taken from healthy volunteers. These solutions were held in opaque plastic collecting vessels and exposed to laboratory light on the laboratory bench. Aqueous controls were treated identically.

In experiments involving infusion apparatus, solutions containing formulated drug, as administered in the clinic, were used. Half the infusion bags (volume: 500 mL) were covered in a black plastic outer bag; samples were removed both directly from the bags and also from needles attached to the end of the infusion line, through which drug in solution was allowed to flow at a rate of 2 mL min^{-1}. In normal clinical practice, the recommended dose of GR63178A is 120 mg m^{-2}, given in 5% glucose at a final concentration of 0·5–

2·0 mg mL^{-1}. The required amount of drug is initially made up in 5 mL of Water for Injection BP, and then added to a Viaflex infusion bag containing 5% Glucose for Infusion BP. The pH of the infusion bag glucose solutions had a mean value of 4·02 (range between batches: 3·84–4·15).

Results

Molar extinction coefficient

The absorbance spectrum of GR63178A in water demonstrated a visible λ_{max} at 370 nm (Fig. 3). From the slope of the plot of GR63178A concentration against absorbance at this wavelength, a molar extinction coefficient, for GR63178A in water, of $6{\cdot}126 \times 10^3$ was determined. This value was used to quantitate some of the data presented below.

Solubility of GR63178A

Maximum solubility of GR63178A in water alone was 1·55 mg mL^{-1}. Solubility in phosphate-buffered saline at pH 7·0 was lower at 45·5 μg mL^{-1}. No insoluble precipitate was apparent in 5% Glucose for Infusion medium at 0·8 mg mL^{-1} or in plasma or urine at 100 μg mL^{-1}.

Effect of light and concentration

A solution of GR63178A (100 μg mL^{-1}) in water was exposed to ambient laboratory lighting, to direct daylight or to a controlled light source. On analysis by HPLC, loss of GR63178A was seen under all illuminated conditions; no loss of drug was apparent in samples stored in the dark (Fig. 4). The half-life of the drug, in ambient laboratory light appears to be relatively long ($t\frac{1}{2}$, 233 min, Fig. 4; other experiments: median $t\frac{1}{2}$, 235 min, range 220–250 min) whereas shorter half-lives were seen with a controlled light source ($t\frac{1}{2}$, 12·8 min) and in direct daylight ($t\frac{1}{2}$, 3·2 min).

The half-life of GR63178A was shown to be directly proportional to the concentration of the drug (Fig. 5). However, in laboratory light this effect was only significant at concentrations < 100 μg mL^{-1}. At concentrations of 0·5 and 1 mg mL^{-1}, half-lives for the parent compound were not significantly different. At 1 mg mL^{-1}, an apparent increase in % GR63178A remaining was seen after 120 min; the HPLC profile of GR63178A at this concentration demonstrated some peak splitting. Examination of both daughter peaks' UV-visible absorbance spectra, using the diode array detector, indicated > 99% peak purity (by spectral overlay) with

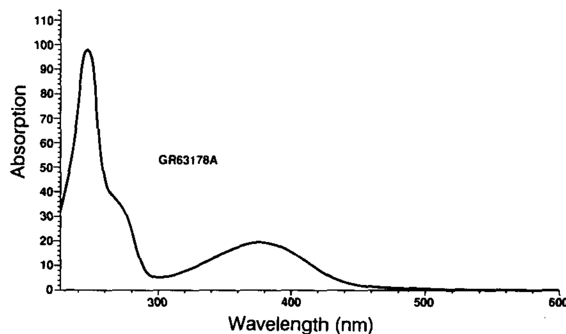

FIG. 3. UV-visible absorbance spectrum of GR63178A.

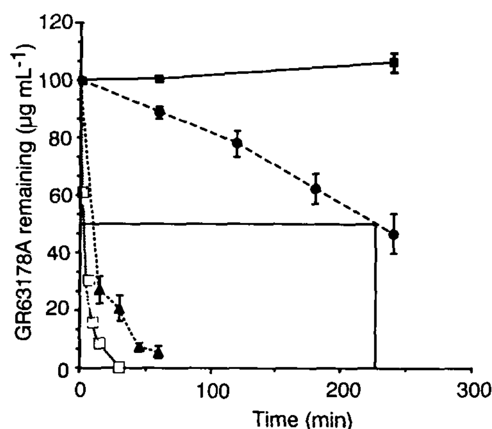

FIG. 4. Degradation of GR63178A by light. GR63178A in aqueous solution, 100 μg mL^{-1}. ■ Dark, ● ambient, ▲ controlled, □ direct. (All points are means ± s.e.m. of three determinations.)

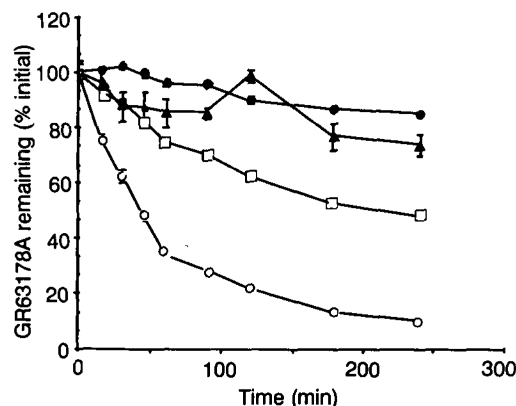

FIG. 5. Effect of GR63178A concentration on rate of degradation by ambient laboratory light. ▲ 1 mg mL^{-1}, ● 0·5 mg mL^{-1}, □ 100 μg mL^{-1}, ○ 10 μg mL^{-1}. (All points are means ± s.e.m. of three determinations.)

identical absorbance spectra. A single peak was observed if the sample was diluted with water; subsequent analysis of samples with high drug concentrations were performed after dilution. At 100 μg mL^{-1}, the initial rate of degradation (as measured by loss of parent drug from the incubation) was 0·420 μg mL^{-1} min^{-1} (0·805 μmol mL^{-1} min^{-1}, $t\frac{1}{2}$ = 139 min) compared with 0·178 μg mL^{-1} min^{-1} (0·341 nmol mL^{-1} min^{-1}, $t\frac{1}{2}$ = 28 min) at 10 μg mL^{-1}. Thus, although the amount of parent drug lost from the incubation was lower in percentage terms at higher concentrations, the actual amount of drug lost (μg mL^{-1}) was still greater at higher concentrations of compound compared with lower concentrations.

Several photodegradation products were detected by this HPLC assay (Fig. 2). Major peaks included a strongly retained product eluting after 14·6 min, which co-eluted with GR54374X standard and possessed an identical UV-visible spectrum. Additionally, several early-eluting peaks were seen, in two groups, at t_r 7–13 and 2–6 min. Peaks eluting at 7–13 min possessed a broad UV-visible absorbance maximum at 380–400 nm, while peaks eluting in the earlier grouping had sharper absorbance maxima of shorter wave-

Table 1. Effect of temperature on the stability of GR63178A in aqueous solution in the dark (100 μg mL^{-1}).

Temperature (°C)	4	21	37	50
GR63178A remaining after 24 h (% of initial)	99·90	98·61	98·97	99·79
s.e.m. (n = 3)	0·32	0·09	0·07	0·09

length than GR63178A. Several minor peaks were detected in completely degraded samples during the course of a single analysis. All these peaks increased in size as a function of time of exposure to light.

Effect of temperature, pH and aeration

No significant loss of parent drug over 24 h was seen in solutions of 100 μg mL^{-1} GR63178A kept in the dark at 4, 21, 37 and 50°C (Table 1).

Aqueous solutions of 100 μg mL^{-1} GR63178A were held in the dark at room temperature (21°C) at pH 2·0, 7·0 or 12·0 and sampled at 0, 1, 6 and 24 h. Neither neutral nor basic solutions showed significant loss of the drug over 24 h; apparent increases above 100% were not statistically significant. However, after 6 h, acidic solutions exhibited a green feathery precipitate, and appeared to lose over half the parent drug peak area (Fig. 6). Some chemical degradation was apparent; chromatography of acid-degraded samples showed a more limited degradation product profile (compared with photodegradation) which included a major peak co-eluting with GR54374X, and a number of peaks with t_r values between 2 and 4 min (Fig. 7).

Solutions of 100 μg mL^{-1} GR63178A exposed to laboratory light or a UV-visible light source, having first been dissolved in degassed and helium-sparged water, showed slight reduction in the rate of degradation compared with controls (aerated solution). However, this was not statistically significant and this small reduction was not apparent in solutions exposed to a controlled light source (Fig. 8).

Stability in biological fluids

Urine. Solutions (100 μg mL^{-1}) of GR63178A were made up in urine samples from healthy volunteers and held in urine containers under ambient laboratory lighting at room temperature for up to 6 h. GR63178A proved more stable in urine than in similarly illuminated aqueous controls (Fig. 9). The variation in pH of the urines (range 6·29–7·51 after addition of GR63178A) did not significantly affect the degradation rates.

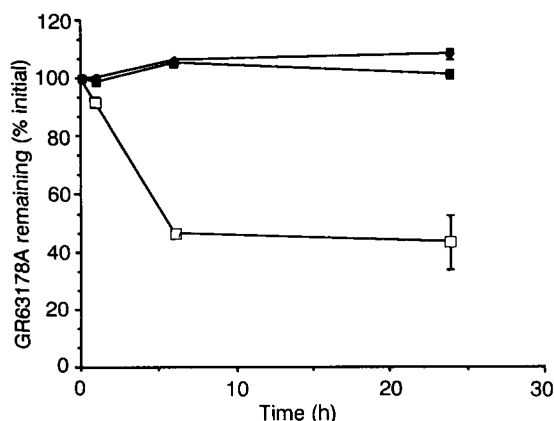

FIG. 6. Degradation of GR63178A with varying pH. GR63178A, aqueous solution of 100 μg mL^{-1} kept in the dark. □ pH 2·0, ◆ pH 7·0, ■ pH 12·0. (All points are means ± s.e.m. of three determinations.)

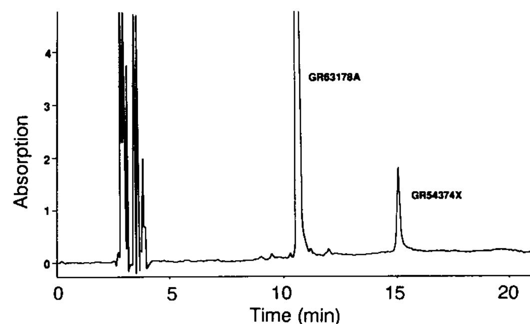

FIG. 7. HPLC of acid-degraded solution of GR63178A (100 μg mL^{-1}).

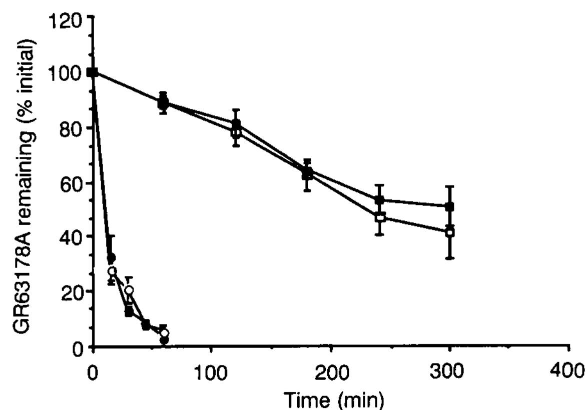

FIG. 8. Degradation of GR63178A by light in aerated and degassed solutions. GR63178A, aqueous solution of 100 μg mL^{-1}. □ Aerated solution, ambient laboratory light; ■ degassed solution, ambient laboratory light; ○ aerated solution, controlled light source; ● degassed solution, controlled light source. (All points are means ± s.e.m. of three determinations.)

Plasma. Human plasma samples containing 100 μg mL^{-1} GR63178A were held in stoppered plastic universal containers and exposed to laboratory lighting. Samples were removed at 0, 1 and 4 h and analysed. No significant loss of GR63178A was seen during this exposure (compared with aqueous controls; Fig. 8). The transient rise in plasma GR63178A concentrations at 60 min was not significant. Under these conditions, aqueous controls exhibited extended half-lives compared with control solutions held in clear borosilicate glass containers in other experiments.

Stability in infusion apparatus

GR63178A was dissolved in 5% Glucose for Infusion (500 mL) to give a final concentration of 0·8 μg mL^{-1}. No loss of GR63178A was seen during 120 min incubation of the drug in the infusion bag, either when exposed to ambient labora-

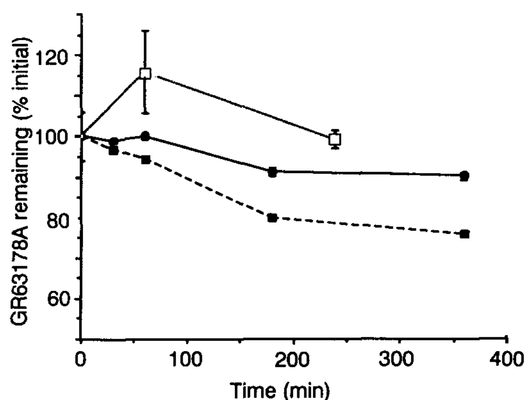

FIG. 9. Degradation by ambient laboratory light of GR63178A in urine and plasma. GR63178A, 100 μg mL^{-1} in solution. ■ Water (illuminated control), ● urine, □ plasma. (All points are means ±s.e.m. of at least three determinations.)

tory lighting or when covered with a black plastic bag. Indeed, an increase in the drug detected was apparent after 30 min. Furthermore, no net loss of drug was seen in solution exiting from the needles at the end of the infusion lines, all of which had been exposed to ambient laboratory lighting (Table 2).

Discussion

Many anticancer compounds related to GR63178A exhibit variable stability; both adriamycin and daunorubicin are known to decompose at pH values of <4·0 or >7·0, to aglycone derivatives (Beijnen et al 1986). Both of these compounds are also unstable in solution when exposed to light, as are mitomycin C, actinomycin D and neocarzinostatin (Tavoloni et al 1980; Daugherty & Hixon 1981; Bosanquet 1986). Thus, it is important that the chemical stability of GR63178A is characterized in both simple aqueous solutions and biological fluids, as this information is critical for non-clinical experimental design and protocol preparation for clinical investigations.

GR63178A, as pure drug alone, proved to be much less soluble in phosphate-buffered saline than in distilled water. This lower solubility is probably due to the sodium ions in this medium, favouring the non-dissociation of the drug, which would therefore remain uncharged and insoluble.

Photodegradation occurs with many other anticancer compounds: the half-life of adriamycin in aqueous solution decreases from 20 to 1·6 h as the concentration decreases from 100 to 10 μg mL^{-1} (Tavoloni et al 1980). Light stability studies have demonstrated that, under ambient laboratory lighting conditions, the rate of GR63178A degradation is inversely proportional to its concentration and becomes significant at concentrations lower than those recommended for use in the clinic. At 1 mg mL^{-1}, the fine splitting of parent drug HPLC peaks may indicate a slow solution process at high concentrations of pure drug, possibly with undissolved drug existing as a fine suspension or colloid which is subsequently solubilized on the HPLC column by mobile phase. This is supported by the observation that a dilution of samples leads to the resolution of the drug as a single peak, and by the data from the diode array detector indicating peak purity. This may explain the similarity in degradation rates of the two highest concentrations tested here, and possibly also account for the apparent increase in the levels of undegraded drug seen in the 1 mg mL^{-1} incubation after 120 min (Fig. 5), as previously undissolved drug becomes solubilized.

GR63178A appears to be stable over a range of temperatures and pH values; only in low pH conditions was compound lost from the solution; this loss was apparently due, in part, to a decrease in the solubility of the parent drug in acid solution, and hence did not continue with further incubation; the HPLC analyses of these samples did indicate some chemical degradation as well. The products of acid degradation included a compound with the same retention time as, and an identical absorption spectrum to the 9-OH metabolite GR54374X, and also a range of hydrophilic compounds of undetermined structure. Levels of aeration do not appear to affect the drug's stability nor do they assist the photolytic degradation of GR63178A in aqueous solutions. Under normal storage conditions in the dark, therefore, drug in aqueous solution can be considered stable.

GR63178A (100 μg mL^{-1}) demonstrated a longer half-life in biological fluids than in simple aqueous solution. Indeed, plasma samples showed no net loss of parent compound over a 4 h period. Previous work has demonstrated that >99% of GR63178A becomes bound to plasma proteins (Glaxo Group Research, unpublished results 1988); this may have a role in stabilizing the drug. Treatment of plasma samples with DMA (which causes protein precipitation) did not appear to reduce levels of detected drug, suggesting that the protein binding is reversible. All solutions were kept in opaque plastic containers of the type used for urine collection in the Clinical Oncology ward. However, as most urine samples from the clinic will contain relatively low concentrations of the drug, and therefore degradation rates may be rapid, these data lead to the recommendation that samples taken from the ward and held in these containers should be shielded from ambient light.

Table 2. Stability of GR63178A in infusion bags and infusion lines under ambient laboratory lighting (0·8 mg mL^{-1}).

Time (min)	GR63178A remaining (% initial ± s.e.m.) Infusion bag (illuminated)	Infusion line (illuminated)	Infusion bag (covered)	Infusion line (from covered bag)
0	100	100	100	100
30	106·91 ± 0·81	109·41 ± 0·31	109·22 ± 3·14	107·64 ± 2·25
60	99·51 ± 0·52	97·85 ± 0·43	99·57 ± 1·04	97·52 ± 0·81
90	99·25 ± 0·95	98·42 ± 0·57	103·31 ± 3·69	102·82 ± 3·11
120	103·96 ± 1·27	102·44 ± 0·75	104·49 ± 0·83	103·92 ± 0·81

The formulated drug in 5% glucose infusion medium showed no solubility problems, despite the low pH of the solution; this is clearly important in terms of administration of the drug to patients, but the high concentrations thus achieved also assist greatly in enhancing the stability of the compound to light. The small transient increase in the concentration of drug in all samples in infusion media at 30 min was not accompanied by chromatogram peak splitting, and may instead indicate an initial complexing of drug (possibly with a formulation constituent) which renders a proportion of the drug undetectable initially, but which is apparent at 30 min. This does not, however, explain the return of the apparent drug concentrations, after 1 h, to the original values; the cause of this is not obvious, and is difficult to speculate upon as the nature of the excipients in the drug formulation was not known, but loss was probably not due to degradation of GR63178A, as no degradation products were evident in the chromatography. Care should therefore be taken when interpreting measurements of formulated drug concentrations. Data presented here suggest that, under such illumination conditions, the provision of black plastic bags is not necessary to prevent drug degradation, and solutions remain stable for at least 2 h.

In conclusion, the work described here has demonstrated that solutions of GR63178A are prone to degradation by light and this effect is concentration-dependent, being more rapid at concentrations of 100 $\mu g\ mL^{-1}$ or less. We have also shown that GR63178A can be subjected to wide ranges of temperature with no apparent breakdown, provided that it is kept in the dark; refrigeration is not required for stability to be maintained over 24 h. Acidic media should be avoided, although buffering solutions may affect the solubility of the drug. Infusion apparatus for administration of the drug to patients should be assembled away from direct daylight. Biological samples, which may contain very low concentrations of GR63178A, should be protected completely from light as far as is practically possible, and processed under a red darkroom safelight.

The precautions outlined above should be considered when pre-clinical and clinical experimental work with GR63178A is undertaken.

References

Beijnen, J. H., van der Houwen, O. A. G. J., Underberg, W. J. M. (1986) Aspects of the degradation kinetics of doxorubicin in aqueous solution. Int. J. Pharm. 32: 123–131

Bosanquet, A. G. (1986) Stability of solutions of antineoplastic agents during preparation and storage for in vitro assays. II: Assay methods, adriamycin and other antitumour antibiotics. Cancer Chemother. Pharmacol. 17: 1–10

Cummings, J., French, R. C., MacLellan, A., Smyth, J. F. (1991) Chromatographic characterisation of six human metabolities of the new anticancer drug GR63178A. Cancer Chemother. Pharmacol. 28: 27–32

Daugherty, J. P., Hixon, S. C. (1981) Photolytic destruction of adriamycin. J. Pharm. Pharmacol. 33: 556

Eccles, D. M., Cummings, J., Stewart, M. E., Nicolson, M., Evans, S., Cornbleet, M. A., Leonard, R. C. F., Smyth, J. F. (1989) Phase I and pharmacology studies of GR63178A, a water soluble analogue of mitoquidone. 6th NCI-EORTC Symposium on New Drugs in Cancer Therapy (090)

Fenton, R. J., Kumar, K. A., Spilling, C. R., Elves, M. (1985) Studies with GR30921 (NSC382057D) a new anticancer pyrroloquinolone, in animal tumour models. Anticancer Res. 5: 592

Fenton, R. J., Kumar, K. A., O'Sullivan, S. M., Neate, M. S., Spilling, C. R., Knox, P. (1989) In vivo anti-tumour activity of the Mitoquidone analogue, GR63178A. 6th NCI-EORTC Symposium on New Drugs in Cancer Therapy (089)

Tavoloni, N., Guarino, A. M., Berk, P. D. (1980) Photolytic degradation of adriamycin. J. Pharm. Pharmacol. 32: 860–862

Verweij, J., Wootton, C. M., van der Berg, M. E. L., Stoter, G. (1989) Phase I study of GR63178A (NSC D611615) administered three times per week. 6th NCI-EORTC Symposium on New Drugs in Cancer Therapy (092)