Cancer Chemother Pharmacol (1991) 28: 27–32
0344570491000511

Cancer Chemotherapy and Pharmacology



# Chromatographic characterisation of six human metabolites of the new anticancer drug GR63178A

**Jeffrey Cummings, Raymond C. French, Alex MacLellan, and John F. Smyth**

Imperial Cancer Research Fund, Medical Oncology Unit, Western General Hospital, Edinburgh, EH4 2XU, UK

**Summary.** GR63178A is the second pentacyclic pyrroloquinone to enter clinical trials as an anticancer drug. We developed a reversed-phase, gradient-elution high-performance liquid chromatography (HPLC) method along with a Bond Elut $C_2$ mini-column sample-preparation technique for the analysis of GR63178A, its 9-hydroxy-metabolite GR54374X and internal standard GR70440A in human plasma and urine. The limit of detection is 2 ng/ml for both GR63178A and GR54374X. Analysis of GR63178A is complicated by its light instability, whereby a number of chromatographically distinct, stable degradation products can form. These can be practically eliminated if clinical specimens are frozen immediately and all subsequent sample preparation is performed in a darkroom. Using this methodology, a total of six metabolites (including GR54374X) were detected in human plasma and urine specimens. The five new metabolites were characterised according to polarity (HPLC retention time), UV-visible absorption maxima and the effect of incubation with β-glucuronidase and aryl-sulphatase. Application of this methodology to the analysis of GR63178A will aid in the development of this novel synthetic anticancer drug.

## Introduction

GR63178A (NSC D611615, Fig. 1) is a more water-soluble analogue of the pentacyclic pyrroloquinone mitoquidone (NSC 382057D, Fig. 1), the first of a new class of synthetic compounds to be clinically evaluated for anticancer activity. In preclinical screens GR63178A demonstrated activity against a broad panel of murine solid tumours as well as human xenografts growing in nude mice, but the expression of its full activity required administration on days 1–21 after tumour implantation [5]; it proved to be inactive against L1210 and P388 murine leukaemias [5]. Phase I clinical trials of mitoquidone were suspended due to formulation problems before either the maximum tolerated dose could be achieved or a tumour response, recorded [6]. Three different phase I protocols have been performed using GR63178A and these demonstrated that the major side effects associated with its administration are pain, often at the site of primary disease or metastases, and nausea and vomiting rather than bone marrow suppression, which was absent [1, 4, 7].

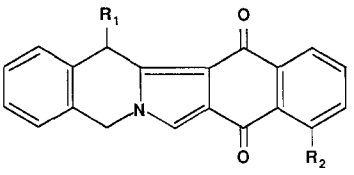
At present the mechanism of action of GR63178A (or mitoquidone) remains unknown, but it may be related either to the intercalation of its planar structure with DNA or to redox cycling of its quinone group, resulting in the generation of toxic free radicals. We have recently reported that GR63178A is inactive (non-cytotoxic) in vitro against a panel of human and murine cancer cell lines [4]. This raises the possibility that the drug must be activated in vivo, either through biotransformation or by modulation of the immune system or through a physiological mechanism such as the alteration of tumour blood flow in a manner analogous to flavone 8-acetic acid [3]. In the present report we describe an HPLC method for the detection of GR63178A and six putative metabolites in human plasma and urine.

## Materials and methods

*Apparatus.* The liquid chromatograph used throughout was a Hewlett Packard Model 1090 equipped with a PV5 ternary low-pressure mixing solvent delivery system; a variable-volume (10–250 µl) automatic injector (set at 100 µl) and autosampler, a heated column compartment and a multi-diode array rapid-scanning UV-visible spectrophotometric detector (Hewlett-Packard Analytical, Manchester, England).

*Chemicals, reagents and drug standards.* All of the methanol and acetonitrile used were of HPLC grade (Rathburn, Chemicals, Walkerburn, Scotland); all of the ammonium acetate and acetic acid applied were also of HPLC reagent grade (Fisons, Loughborough, England) and *N,N*-dimethylacetamide (DMA) was of AnalaR grade (BDH, Poole, England). Aryl-sulphatase (type V, low in β-glucuronidase activity) and β-glucuronidase (type VII) were obtained from Sigma Chemical Company (Poole, England). Water was deionised and bidistilled in a quartz glass

*Offprint requests to:* J. Cummings



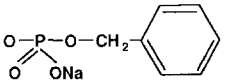
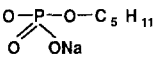
| Name | $R_1$ | $R_2$ |
|---|---|---|
| Mitoquidone | H | H |
| GR 63178A | $CH_3$ |  |
| GR 54374X | $CH_3$ | OH |
| GR 70440A | $CH_3$ |  |

**Fig. 1.** Structural formulae of GR6317A, its metabolite GR54 374X and internal standard GR70440A

still, and all other reagents, chemicals and solvents were of the highest grade commercially available.

Pure GR63 178A (sodium salt), GR54374X and GR70440A (sodium salt, internal standard; Fig. 1) were kindly supplied by the Infection and Oncology Department of the Medical Division of Glaxo Group Research Ltd (Greenford, England). Due to light instability, standard solutions (0.01–100 µg/ml) were prepared in a darkroom under red light, transferred immediately to dark glass vials with aluminium caps and PTFE liners and than analysed on the same day. As diluents water was used for GR63 178A and GR70440A and DMA was used for GR54374X. Calibration curves were constructed by injecting 100 µl standard to yield between 10 µg and 1 ng on the column. The limit of detection lay at a signal-to-noise ratio of 3 : 1.

*Chromatographic conditions.* The stationary phase was Apex I octadecyl 5 µm silica obtained prepacked in stainless steel columns measuring 25 cm × 4.6 mm in internal diameter (Jones Chromatography; supplied by Crawford Scientific, Strathaven, Scotland). Gradient elution was carried out at a flow rate of 1 ml/min according to the following programme, using a starting mobile-phase composition of 50% 0.1 M ammonium acetate/acetic acid (pH 4.6, A), 20% acetonitrile (B) and 30% methanol (C) and a complete run time of 25 min:

| Time (min) | %A | %B | %C |
|---|---|---|---|
| 0 | 50 | 20 | 30 |
| 3 | 50 | 20 | 30 |
| 10 | 20 | 70 | 10 |
| 15 | 20 | 70 | 10 |
| 20 | 50 | 20 | 30 |

Mobile-phase components were kept permanently degassed by continuous sparging with helium during chromatography and were also filtered prior to use. The column was maintained at a constant temperature of 40° C, and chromatographic peaks were monitored at 380 nm. The diode array detector was programmed to record a UV-visible absorption spectrum (190–600 nm) of each chromatographic peak with a height of >0.1 mAU.

*Sample preparation.* Blood (immediately separated into plasma) and 6-h urine specimens were collected from patients who had received 40–160 mg/m² GR63 178A in a 20-min i.v. infusion. All samples collected were frozen to –20° C. GR63 178A and its metabolites were extracted onto 500 mg Bond Elut $C_2$ 40 µm silica gel in 2.4-ml reservoirs placed in a Vac Elut SPS-24 manifold operating under negative pressure (analytichem International; supplied by Crawford Scientific). To 1 or 2 ml plasma or urine, 1 µg GR70440A was added as an internal standard; 3 ml methanol was added to the Bond Elut columns, which were then washed with 3 ml water. The plasma or urine was immediately loaded slowly onto the column, which was further washed with 5 ml water and then allowed to dry. GR63 178A and metabolites were eluted in 2 ml methanol, which was evaporated to dryness at 50° C in a Univap centrifugal evaporator (Uniscience, London). The dried extracts were finally reconstituted in 200 µl mobile phase (starting composition) and 100 µl was injected onto the column. All steps of the extraction procedure were performed in a darkroom under a red light. Urine specimens could also be directly injected onto the column after a simple sample-preparation step; 1 ml urine was mixed with 1 ml methanol and then spun at 1,000 *g* for 10 min prior to the injection of 100 µl onto the column. Direct injection of urine specimens enabled the determination of the extraction efficiency of the unidentified metabolite (see Table 3).

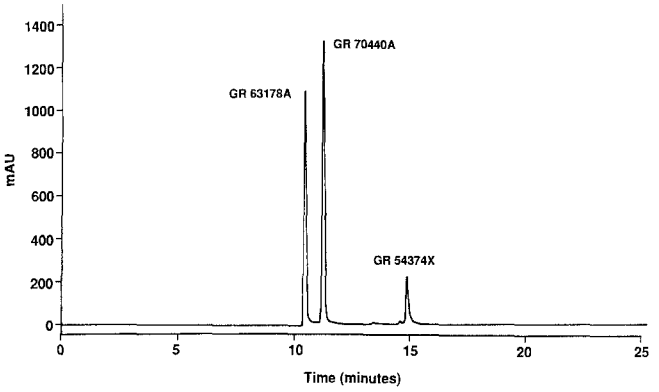



**Fig. 2.** HPLC separation of a mixture of standards of GR63 178A, GR54 374X and GR70 440A

*Enzyme incubation of urine.* Urine specimens (1 ml) were incubated with either 9 ml water (control) or 9 ml 4 mM sodium phosphate (pH 6.8) containing 1,000 IU β-glucuronidase or with 9 ml 10 mM MOPS (pH 6.8) containing 1,000 IU aryl-sulphatase at 37° C in a dark environment. At time 0 and at 1, 2, 24 and 48 h, 1 ml incubation mixture was removed, extracted on Bond Elut $C_2$ and analysed by HPLC as described above.

## Results

### *High-performance liquid chromatography*

Figure 2 shows the separation of GR63 178A, its 9-hydroxy-metabolite GR54374X and the internal standard GR70440A by HPLC. The chromatographic characteristics of this separation are shown in Table 1. The retention times (t*R*) of all three components varied by <1% throughout the day and by 1%–2% from day to day. The limit of detection for each component was 1 ng for direct injection onto the column and 2 ng/ml following extraction. All calibration curves were linear over the concentration range of 0.01–100 µg/ml (from 1 ng to 10 µg on the column), with $r^2$ being close to 1 (Table 1).

### *Extraction procedure*

The efficiency of the Bond Elut $C_2$ extraction method in control experiments, in which different concentrations of GR63 178A, GR54374X and GR70440A were added to volunteer plasma, is shown in Table 2. Although recovery

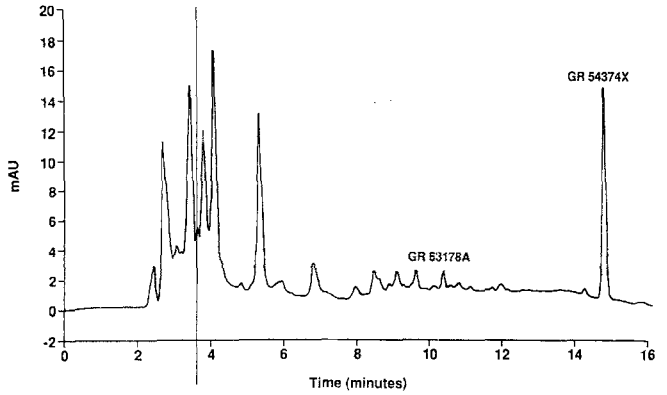



**Fig. 3.** Chromatogram of 100 μg/ml GR63178A exposed for 30 min to natural daylight on a sunny summer day in Edinburgh. Note that 99.9% of the GR63178A has broken down and a number of chromatographically distinct degradation products have formed, of which the peaks to the *right of the line* are extracted by our Bond Elut $C_2$ sample-preparation technique

was high in each case, the extraction efficiency of GR54374X was significantly lower than that of the other two components (Table 2), and this should be borne in mind during analysis of clinical specimens. The $C_2$ technique did not extract any substance that could potentially interfere with the identification of GR63178A and its metabolites in plasma (see Fig. 4) or urine. Over the concentration range of 0.1–100 μg/ml added to plasma, the mean within-day co-efficient of variation for quantitation (CV) was 5.4% for GR63178A and 6.8% for GR54374X and the between-day value was 7.3% and 11.3%, respectively.

*Stability and degradation of GR63178A*

GR63178A is unstable in the presence of light, especially within the concentration range seen in clinical specimens (≤100 μg/ml) after administration of phase I doses. This instability, illustrated chromatographically in Fig. 3, became evident after a 100 μg/ml aqueous solution of GR63178A was exposed to natural daylight for 30 min on a sunny summer day in Edinburgh. A non-degraded standard of 100 μg/ml GR63178A would normally give a peak height of around 1,000 mAU; the peak height of GR63178A in the sample exposed to light was 1 mAU, and a number of chromatographically distinct products were resolved by the gradient-elution HPLC method, including a significant peak corresponding to GR54374X. Furthermore, these products were stable once they had been removed from light, and the majority (peaks to the right of the line in Fig. 3) were extracted by the sample-preparation technique with an efficiency of >60%. Thus, if present in clinical specimens, light-degradation products could easily be mistaken for metabolites, making the identification of genuine metabolites more complicated. Conversely, it must be proven that genuine metabolites are not degradation products.

Using the gradient-elution HPLC method together with a diode array detector, we studied the stability of GR63178A and characterised its degradation products.

**Table 1.** HPLC characteristics of GR63178A, GR54374X and GR70440A

| | GR63178A | GR54374X | GR70440A |
|---|---|---|---|
| Retention time ($t_R$, min) | 10.3 | 14.6 | 11.1 |
| Standard deviation, within day | 0.01 | 0.01 | 0.02 |
| Standard deviation, between-day | 0.01 | 0.04 | 0.02 |
| Detection limit on column (ng) | 1 | 1 | 1 |
| After extraction (ng/ml) | 2 | 2 | – |
| Linearity calibration curve over the range 0.01–100 μg/ml ($r^2$) | 0.999 | 0.998 | 0.998 |

**Table 2.** Extraction efficiency of the Bond Elut $C_2$ sample-preparation technique

| Amount added to volunteer plasma (μg/ml) | Efficiency (± SD[a]) | | |
|---|---|---|---|
| | GR63178A | GR54374X | GR70440A |
| 0.1 | 80.5 ± 12.5 | | |
| 1 | 92.5 ± 2.5 | 61.9 ± 5.4 | 95.1 ± 4.8 |
| 10 | 87.3 ± 2.8 | 68.6 ± 8.2 | 80.1 ± 2.7 |
| 100 | 103 ± 3.7 | | |

[a] $n$ = 10 for each concentration

The complete stability profile will be described in a separate report. In brief, once GR63178A (100 μg/ml) had been removed from light, it was stable at pH 13, and 50° C or under darkroom conditions for several hours. The light-catalysed instability of this drug was inversely proportional to its concentration and directly proportional to the light intensity. Thus, at ≥0.5 mg/ml (concentrations used in infusion bags for administration of the drug to patients) and under laboratory lighting, little degradation occurred and no degradation products were detected. Where degradation was apparent, each variation in pH and lighting conditions produced a unique degradation-product profile.

To control for degradation products appearing artefactually in clinical samples, we incubated 100 μg/ml GR63178A in volunteer plasma and urine in normal specimen containers for several hours under laboratory lighting conditions. Samples were analysed for degradation products at several time points for up to 6 h. No degradation products were detected in plasma. Urine also protected GR63178A from instability, albeit to a lesser extent; after 6 h incubation, approximately 10% of the drug had broken down and trace amounts of six degradation products were detected, as well as the 9-hydroxy metabolite. Each peak represented <0.1% of the peak height of the parent drug. These studies reveal that clinical samples should be free of metabolite artefacts, provided that samples are frozen as soon as possible and that all subsequent sample preparation is performed in a darkroom under red light.

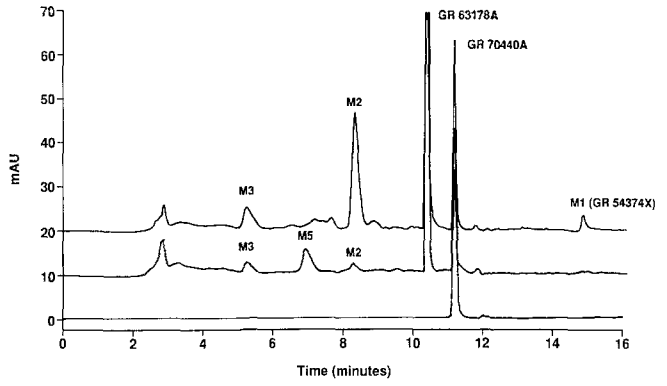



**Fig. 4.** Chromatograms of extracted plasma samples taken from patients who had received GR63 178A during a phase I trial. *Lower chromatogram*: pre-dose plasma containing 1 μg/ml GR70440A as an internal standard. *Middle chromatogram*: plasma taken 2 h after the administration of an 80 mg/m$^2$ i.v. infusion containing 2.3 μg/ml GR63 178A, 1 μg/ml GR70440A and significant levels of M2, M3 and M5 (see Table 3 and Discussion). *Upper chromatogram*: plasma taken 2 h after the administration of a 160 mg/m$^2$ i.v. infusion containing 3.8 μg/ml GR63 178A, 1 μg/ml GR70440A and significant levels of M1 (GR54 374X), M2 and M3

### *Analysis of clinical specimens and chromatographic characterisation of six human metabolites of GR63 178A*

In Fig. 4, three chromatograms of plasma taken from three separate patients who had been treated with different doses of GR63 178A illustrate the different metabolite profiles that can be observed. The lower chromatogram was obtained from a pre-dose plasma sample, and it shows that no peaks were concomitantly extracted with the internal standard that could have interfered with the identification of GR63 178A and its metabolites. The middle chromatogram shows the elution profile of a plasma sample taken from a patient at 2 h after the administration of a dose of 80 mg/m$^2$; this specimen contained 2.3 μg/ml GR63 178A as well as significant levels of three metabolites (M2, M3 and M5; see Table 3). The upper chromatogram represents another plasma sample taken at 2 h after the administration of a dose of 160 mg/m$^2$; this sample contained 3.8 μg/ml GR63 178A as well as significant levels of three metabolites (M1, M2 and M3; see Table 3).

Both the HPLC method and the extraction technique were sufficiently sensitive to detect GR63 178A (and its metabolites) in plasma at 24 h following the administration of doses of $\geqslant$80 mg/m$^2$ but not after the starting dose of 40 mg/m$^2$. Direct injection of human urine revealed that GR63 178A is excreted intact along with a series of more water-soluble metabolites and traces of "plasma metabolites" such as GR54 374X and M2 (Fig. 5). Treatment of urine specimens with β-glucuronidase aided in the identification of metabolites. Additionally, aryl-sulphatase was found to hydrolyse the phosphate ester linkage in GR63 178A and distinguish between a parent-drug-derived metabolite and a GR54 374X-derived metabolite. Thus, by judicious use of enzymes and of the diode array detector (for spectral characterisation to determine whether a metabolite shared spectral characteristics with either the parent drug or GR54 374X), a partial identity could be ascribed to most of the metabolite peaks detected.

A combination of six major peaks (M1–M6) was detected in human plasma and urine samples, as were several minor peaks that remain uncharacterised. The chromatographic properties of these six species are summarised in Table 3. M1 corresponded to the 9-hydroxy metabolite GR54 374X (see Fig. 1). It showed strong visible absorption and a λ-maximum that was shifted 20 nm to the longer wavelength of GR63 178A; in contrast, the photo-degradation products of GR63 178A displayed weak visible absorption and broad λ-maxima that were centred around 380–400 nm. Although M1 was a major plasma metabolite, it was rarely detected in urine. Upon treatment of urine specimens with β-glucuronidase (and, to a lesser extent, aryl-sulphatase), three of the six peaks disappeared (M3, M4 and M6), liberating similar peak areas of GR63 178A and GR54 373X.

Both M3 and M4 exhibited strong visible-absorption bands that were identical in shape to that of GR54 374X but displayed λ-maxima that were shifted 10 nm longer and are presumed to be conjugates of GR54 374X (see Fig. 6). M6 exhibited both a visible-absorption band that was identical in shape to that of GR63 178A and a similarly shifted

**Table 3.** Chromatographic characterisation of the major human metabolites of GR63 178A

| Metabolite | Retention time (min) | Presence in urine | Presence in plasma | UV absorption maxima (nm) | Visible absorption maxima (nm) | Extraction efficiency (%) | Urine incubations[a] | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Effect of aryl sulphatase (1,000 IU) | Effect of β-glucuronidase (1,000 IU) |
| M3 | 5.3 | + | + | 252 | 402 | 71 | ↓ | ↓↓ |
| M4 | 5.5 | + | – | 252 | 402 | 68 | ↓ | ↓↓ |
| M6 | 6.3 | + | – | 244 | 382 | 65 | ↓ | ↓↓ |
| M5 | 7.1 | + | + | 242 | 490 | 55 | ↓↓ | ↑↑ |
| M2 | 8.2 | – | + | 252 | 402 | ND | ND | ND |
| GR54374X (M1) | 14.8 | – | + | 248 | 392 | 65 | ↑↑ | ↑↑ |
| GR63178A | 10.3 | + | + | 244 | 372 | 90 | ↓↓ | ↑↑ |

[a] Human urine specimens were incubated as detailed in Materials and methods and then analysed by HPLC to observe the effect of enzyme treatment on each chromatographic peak. ↓, peak decreased in size; ↑, peak increased in size

+, Present in the majority of samples; –, not present in the majority of samples; ND, not determined

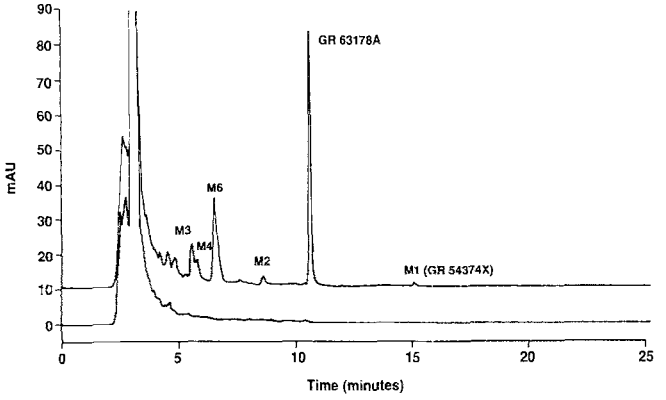



**Fig. 5.** Chromatograms of non-extracted (direct injection) urine specimens taken from a patient who had received 80 mg/m$^2$ GR63178A. *Lower chromatogram*: pre-dose urine. *Upper chromatogram*: 0- to 6-h collection in which a total of 1.6 mg GR63178A was excreted along with significant levels of M1, M2, M3, M4 and M6

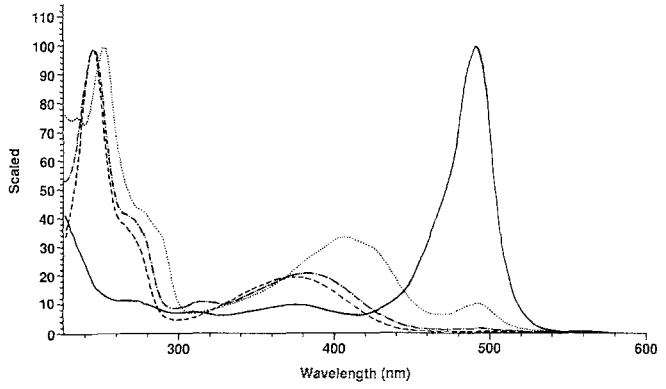



**Fig. 6.** UV-visible absorption spectra of GR63178A and its putative human metabolites. The spectra were generated during HPLC by a diode array detector from chromatographic peaks that appeared in human plasma and urine specimens. The spectra have been normalised and overlain for direct comparison. – – –, GR63178A; · · · · · ·, M2, 3 and 4; ———, M5; – · – · –, M6 (see Table 3 for maxima). In the M3, 4 spectrum (· · · ·), we believe that the maximum at 490 nm is artefactual due to a slight overlap in elution between M3 and 4 and to the possible occurrence of a glucuronide form of M5 in the urine specimen from which the spectra were obtained

λ-maximum and is presumed to be a conjugate of the parent drug. M3 was the only conjugated species detected in plasma. M2, found predominantly in the plasma, also showed a band sharing the characteristic shape of the strong visible-absorption band of GR54374X, but its λ-maximum was shifted 10 nm to the longer wavelength, as in M3 and M4 (Fig. 6). On the basis of retention time, M2 is clearly much more water-soluble than either GR54374X (8.2 vs 14.8 min) or GR63178A itself. Of the six species identified, M5 was by far the most unusual. Its visible-absorption maximal wavelength was shifted 118 nm to the longer wavelength of GR63178A (Fig. 6); levels of this metabolite decreased on treatment with arylsulphatase but increased on incubation with β-glucuronidase.

## Discussion

The two aims of the study were: (1) to develop an HPLC method for GR63178A and (2) to identify its metabolites such that the role of drug metabolism in the clinical pharmacology and mechanism of action of this drug could be determined. We elected to use a gradient-elution system with a run time of 25 min rather than a shorter isocratic system for two reasons: because of the very large number of possible degradation products that could interfere with the identification of metabolites and because of the likelihood that a considerable number of metabolites may be formed, based on previous clinical experience with mitoquidone [6] and on the preclinical pharmacology of GR63178A itself [2].

Six putative metabolites were detected. Stringent criteria must be applied to these chromatographic peaks before they can be considered to represent genuine drug metabolites. The six peaks satisfied the following criteria: (a) peaks were detected in specimens that had been frozen soon after their collection and whose preparation had been performed in a dark environment; (b) with the exception of GR54374X, no putative metabolite exhibited both a $t_R$ value and a UV-visible spectrum that were similar to those of any degradation product; (c) after i.v. administration of GR63178A, the pharmacokinetics of all six peaks showed behaviour typical for a metabolite, indicating first an increase and then a decrease in concentration rather than mirroring the continuous decay of the parent drug; and (d) the same metabolite peaks (or their conjugates) were detected in both urine and plasma specimens from each patient.

Our metabolism data suggest that conjugation with glucuronic acid probably represents a significant route for elimination of GR63178A in man. Three putative conjugate metabolites were detected in human urine specimens; based on spectral data, two of these appear to be glucuronides of GR54374X, whereas the other seems to be a glucuronide of the parent drug. Considering the structure of GR54374X (see Fig. 1), it is difficult to identify an additional site for direct conjugation with glucuronic acid apart from the obvious 9-hydroxy group, unless phase I oxidation has occurred. Nevertheless, treatment of urine with β-glucuronidase liberated only the parent drug and GR54374X, as well resulting in a small increase in M5. No new species appeared such as M2. Moreover, it is surprising that the retention time of these conjugates of GR54374X, a species that is much more hydrophobic than the parent drug, should be shorter on a reversed-phase HPLC column than that shown by a conjugate of the parent drug. Confirmation of the identity of these species must await their purification and structural elucidation, which is presently being carried out by our laboratory in conjunction with the Drug Metabolism Department of Glaxo Group Research.

The unique absorption spectrum of M5, with its visible maximum of 490 nm and its orange/red colour (as opposed to the green/orange colour of GR63178A and GR54374X), raises doubts about its authenticity as a genuine metabolite. However, this species is not a degradation product and it did satisfy our four criteria for a metab-

olite. Our recent studies indicate that M5 may be related to quinone bioreduction and redox cycling of both GR63178A and GR54374X; however, at this stage it is impossible to rule out the possibility that it is endogenously produced in response to GR63178A administration rather than being drug-derived.

At least five uncharacteriseed metabolites of mitoquidone were detected in human plasma during a phase I trial of this drug; moreover, large amounts of mostly polar metabolites were found in the urine, with only traces of the parent drug being identified [6]. In that study, some patients reported that they had passed dark-coloured urine, which was believed to be related to either metabolites or degradation products of mitoquidone. Our present data on the metabolism of GR63178A, a benzyl phosphate ester analogue of mitoquidone, are broadly supportive of the above-mentioned observations and show that biotransformation is clearly an important factor in the clinical pharmacology of these new pentacyclic pyrroloquinones. We are presently investigating the role of drug metabolism in the antitumour activity of GR63178A by following its disposition in murine tumours that are sensitive or resistant to the drug. Our new HPLC methodology will hopefully aid in the further clinical evaluation of this compound and in the elucidation of its mechanism of action.

## References


1. Cassidy J, Lewis C, Setanoians A, Adams L, Kerr DJ, Calvert AH, Pateman AJ, Rankin EM, Kaye SB (1989) Phase I trial of GR63178A. Proceedings, 6th NCI-EORTC Symposium on New Drugs in Cancer Therapy, Amsterdam, March 7–10
2. Clinical investigators manual, 2nd Edn (1988) Anticancer compound GR63178A (NSC D611615). Infection and Oncology Department, Medical Division, Glaxo Group Research Ltd, Greenford, UK
3. Cummings J, Smyth JF (1989) Flavone 8-acetic acid: our current understanding of its mechanism of action in solid tumours. Cancer Chemother Pharmacol 24: 269
4. Eccles DM, Cummings J, Stewart ME, Nicolson M, Evans S, Cornbleet MA, Leonard RCF, Smyth JF (1989) Phase I and pharmacology studies of GR63178A, a water-soluble analogue of mitoquidone. Proceedings, 6th NCI-EORTC Symposium on New Drugs in Cancer Therapy, Amsterdam, March 7–10
5. Fenton RJ, Kumar KA, O'Sullivan SM, Neate MS, Spilling CR, Knox P (1989) In vivo anti-tumour activity of the mitoquidone analogue, GR63178A. Proceedings, 6th NCI-EORTC Symposium on New Drugs in Cancer Therapy, Amsterdam, March 7–10
6. Speth PAJ, Gore ME, Pateman AJ, Newell DR, Bishop JAM, Ellis WJ, Green JA, Crumbrell LP, Linssen PCM, Miller A, Smith IE, McVie JG, Mulder PHM de, Paulo BE de, Griggs JV, Brown GW (1988) Phase I and pharmacokinetic studies with the pentacyclic pyrroloquinone mitoquidone. Cancer Chemother Pharmacol 21: 343
7. Verweij J, Wootton CM, Berg MEL van der, Stoter G (1989) Phase I study of GR63178A (NSC D611615) administered three times per week. Proceedings, 6th NCI-EORTC Symposium on New Drugs in Cancer Therapy, Amsterdam, March 7–10