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# Pharmacokinetics of 7-con-*O*-methylnogarol in patients with solid tumors

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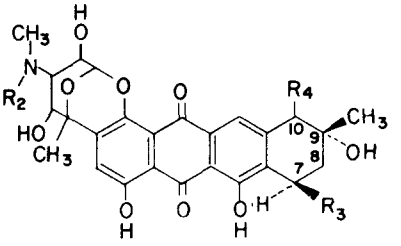
**Summary.** The pharmacokinetics of 7-con-*O*-methylnogarol were investigated by HPLC assay with fluorometric detection in nine cancer patients with normal hepatic and renal function, after a 2-h infusion of 160 or 200 mg/m$^2$. The drug disappeared from plasma biexponentially with a mean elimination half-life of 38 ± 3 h; the mean apparent volume of distribution and the plasma clearance were 805 ± 91 l/m$^2$ and 14 ± 2 l/h per m$^2$. Within 48 h of administration, urinary excretion of the drug and its metabolite 7-con-*O*-methyl-*N*-demethylnogarol accounted for 2%–15% and 0.1%–6% of the dose, respectively. Neither 7-con-*O*-methylnogarol nor its *N*-demethyl derivative was conjugated with glucuronic acid or sulfate in detectable amounts.

## Introduction

7-con-*O*-methylnogarol (7-OMEN) is a semisynthetic analogue of the anthracycline nogalamycin, which was found to be effective against many experimental murine tumors but was dropped because of its renal and pulmonary toxicity [14].

In view of its different structure (Fig. 1) and mode of action, 7-OMEN can be considered distinct from doxorubicin. In contrast to doxorubicin, 7-OMEN is reported to be present mostly in the cytoplasm of treated cells; in addition, it does not inhibit nucleic acid biosynthesis and it binds to DNA only weakly [2, 10]. Reports of different spectra of activity in animal tumors (e. g., it is active against colon 38 carcinoma of the mouse, whereas doxorubicin is not) and lower cardiotoxicicitiy than doxorubicn [13] prompted further interest in 7-OMEN and led to its clinical investigation.

While detailed pharmacokinetic data recorded in animals are available [4, 5, 12–15], only scant information exists on the clinical pharmacokinetics of this drug. We report on the plasma pharmacokinetics of 7-OMEN and its major metabolite, 7-con-*O*-methyl-*N*-demethylnogarol (nor-7-OMEN; Fig. 1) and on their urinary excretion in nine cancer patients given 7-OMEN 160–200 mg/m$^2$ as an i. v. infusion.



| COMPOUND | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|
| Menogaril | $CH_3$ | $OCH_3$ | H |
| N-Demethyl Menogaril | H | $OCH_3$ | H |

**Fig. 1.** Structures of 7-con-*O*-methylnogarol (menogaril) and its metabolite

## Patients and methods

*Patients.* Nine adult patients with an age range of 47–72 years, who had solid tumors and were being treated at the Centro di Riferimento Oncologico, Aviano (PN), Italy, with 7-con-*O*-methylnogarol as one of the ongoing studies of the Early Clinical Trials Group (ECTG-EORTC), entered this study. Patients 1, 3, 4, and 8 were women; patients 2, 5, 6, 7 and 9 were men. Patient 1 was suffering from melanoma; patients 2, 3, 5, 6 and 7 from squamous cell lung cancer; patients 8 and 9 from adenocarcinoma of the lung; and patient 4 from breast cancer. Performance status (PS) was 0 for patients 1, 4, and 5; 1 for patients 2, 6 and 7; and 2 for patients 3, 8 and 9. All patients had normal renal and hepatic function (serum creatinine ≤ 1.5 mg/ml; creatinine clearance ≥ 60 ml/min; bilirubin ≤ 1.5 mg/ml).

*Drug treatment.* 7-Con-*O*-methylnogarol was supplied through the ECTG by Upjohn International Inc. in vials containing 50 mg drug, 100 mg mannitol, and 16.6 mg lactic acid. The vials were reconstituted in 10 ml sterile water and further diluted in 5% dextrose to give a concentration less than 1 mg/ml. The drug was administered by i. v. infusion over a period of 2 h; this treatment was repeated every 4 weeks. Depending on each patient's PS the dose given was 200 mg/m$^2$ (PS = 0 or 1) or 160 mg/m$^2$ (PS = 2).

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*Sample collection.* Samples (8 ml) of heparinized venous blood were obtained at the following times: 0 (baseline), 1 h after the beginning of the infusion; 0, 10, 20, 30, 60 min and 2, 4, 6, 8, 12, 24, 48, 72 h after the end of the infusion. The blood samples were immediately centrifuged and the plasma was stored at −20 °C until analysis.

Urine fractions were collected 24 and 48 h after the end of the infusion and were stored at −20 °C until analysis.

*Drug assay.* In this study a modification of the method described by Brown et al. [3] was used to measure the concentrations of 7-OMEN and the metabolite nor-7-OMEN in plasma and urine. 7-OMEN and nor-7-OMEN pure standards, generously donated by Upjohn International Inc., were used to construct calibration curves. Plasma or urine (250 µl, diluted 1:5) was added to 250 µl acetonitrile $-H_3PO_4$ 0.1 *M* (4:1 vol/vol) containing 15 µg/ml daunomycin as internal standard. Samples were centrifuged at 3000 RPM for 10 min at 4 °C, and then 10–20 µl of the supernatant fraction was injected into a Perkin-Elmer 3B series HPLC apparatus equipped with a 650-10 LC Perkin-Elmer fluorescence detector set at 475 nm excitation and 560 nm emmission. Separation was achieved using an isocratic solvent system of water-acetonitrile-$H_3PO_4$ 0.1 *M* (33:30:37) at a flow rate of 1 ml/min in a 25-cm-long µBondapak $C_{18}$ column purchased from Waters Ass. The sensitivity of this assay was 3 ng/ml for 7-OMEN and 6 ng/ml for nor-7-OMEN. The coefficient of variation was less than 10%.

To evaluate the presence of conjugates with glucuronic acid or sulfate we incubated 0.5 ml urine with 0.5 ml acetate buffer (pH = 4.5) and 50 µl β-glucuronidase/arylsulfatase (from *E. coli* K12, Boehringer Biochemia Robin, Milan, Italy). Comparison was made with a sample without β-glucuronidase sulfatase, and the same HPLC assay was used.

*Pharmacokinetic analysis.* The plasma concentrations after i. v. infusion were processed using a two-compartment open model described by the equation $C = A \exp(-\alpha t) + B \exp(-\beta t)$, where C is the plasma concentration at time t, A and B are the intercepts on the ordinate at time zero, and α and β represent the slope of the respective exponential segments. All pharmacokinetic parameters were processed using a non-linear fitting procedure using the weighted least-squares criterion and a microcomputer program [16]. The half-life associated with β-phase, total clearance, and volume of distribution were computed from the following relationship:

$$T_{(½)}\beta = 0.693/\beta;\ Cl = Dose/AUC_{o\text{-}oo};\ V_d = Cl/\beta.$$

$AUC_{o\text{-}oo}$ was calculated using the trapezoidal rule, extrapolating from the last sampling time to infinty with the formula: $C^*/\beta$, where $C^*$ is the last plasma concentration measured.

## Results

Figure 2 shows the curves for disappearance of 7-OMEN from the plasma of nine cancer patients who received 160 or 200 mg/m² as a 2-h i. v. infusion. After an initial rapid decline the drug levels decreased slowly, with a terminal half-life between 29 and 64 h. The volume of distribution ($V_d$) ranged between 441 and 1391 l/m² and the plasma clearance, between 10 and 32 l/h per m² (Table 1).

Plasma levels of nor-7-OMEN were very low but detectable in patients 2, 3, 6, 7, 8 and 9, with peak levels be-

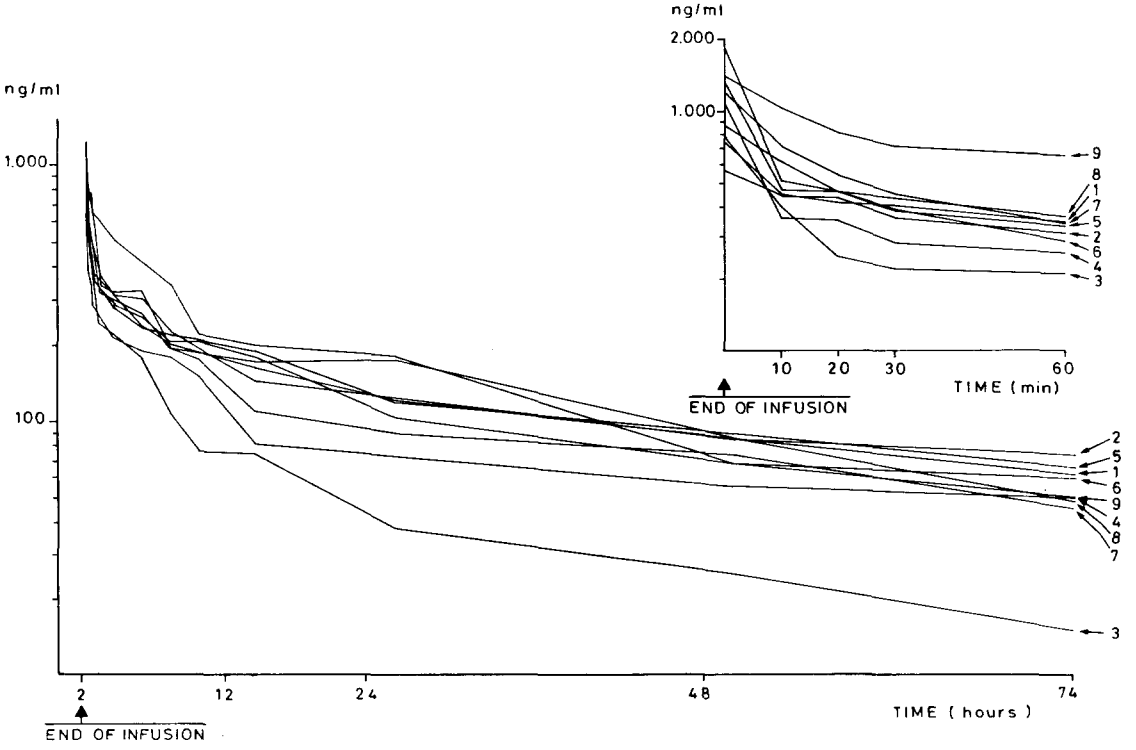


**Fig. 2.** 7-con-*O*-methylnogarol plasma level after a 2-h infusion of 160 (patients 3, 8, 9) or 200 (patients 1, 2, 4, 5, 6, 7) mg/m². On the *small panel*, plasma levels of 7-con-*O*-methylnogarol are represented on a different scale up to 1 h after the end of infusion

**Table 1.** Plasma pharmacokinetic parameters of 7-con-*O*-methylnogarol

| Patient no. | Dose ($mg/m^2$) | $t_{1/2\alpha}$ (h) | $t_{1/2\beta}$ (h) | $V_d$ ($l/m^2$) | Cl ($l/h$ per $m^2$) |
|---|---|---|---|---|---|
| 1 | 200 | 1.3 | 39 | 735 | 12.5 |
| 2 | 200 | 2.0 | 64 | 937 | 10.0 |
| 3 | 160 | 1.7 | 29 | 1391 | 32.0 |
| 4 | 200 | 0.1 | 37 | 838 | 15.0 |
| 5 | 200 | 0.4 | 35 | 781 | 12.5 |
| 6 | 200 | 0.9 | 32 | 680 | 14.3 |
| 7 | 200 | 2.0 | 45 | 1002 | 15.0 |
| 8 | 160 | 0.5 | 33 | 441 | 11.0 |
| 9 | 160 | 2.0 | 29 | 445 | 10.0 |
| $\bar{x}\pm SE$ | | $1.2\pm0.2$ | $38\pm3$ | $805\pm91$ | $14.5\pm2.1$ |

**Table 2.** Urinary excretion of 7-con-*O*-methylnogarol and 7-con-*O*-methyl-demethylnogarol

| Patient no. | Urinary 7-OMEN (% of dose) | | | Urinary nor-7-OMEN (% of dose) | | |
|---|---|---|---|---|---|---|
| | 0–24 h | 24–48 h | 0–48 h | 0–24 h | 24–48 h | 0–48 h |
| 1 | 1.6 | 3.3 | 4.9 | 0.5 | 0.3 | 0.8 |
| 2 | 1.3 | 0.8 | 2.1 | 0.05 | 0.04 | 0.09 |
| 3 | 3.5 | 0.2 | 3.7 | 2.0 | 0.14 | 2.14 |
| 4 | 1.8 | 1.2 | 3.0 | 0.28 | 0.15 | 0.43 |
| 5 | 6.3 | 5.0 | 11.3 | 0.4 | 0.38 | 0.78 |
| 6 | * | 4.9 | | * | 0.63 | |
| 7 | 8.2 | 2.9 | 11.1 | 1.97 | 1.0 | 2.97 |
| 8 | 1.6 | 0.7 | 4.5 | 5.2 | 1.2 | 6.4 |
| 9 | 12.3 | 2.1 | 14.4 | 0.12 | 0.1 | 0.24 |
| $\bar{x}\pm SE$ | $4\pm1$ | $2.3\pm0.6$ | $7\pm2$ | $1.3\pm0.6$ | $0.5\pm0.1$ | $1.7\pm0.7$ |

* Urinary fraction collected between 0 and 24 h was lost

tween 40 and 105 ng/ml and AUC values between 1.0 and 3.8 μg/ml × h. In patients 1, 4 and 5 nor-7-OMEN was undetectable (< 6 ng/ml). As can be seen from Table 2, the urinary excretion of unchanged 7-OMEN and of its *N*-demethyl derivative amounted to 2%–15% and 0.1%–6% of the dose, respectively.

After incubation of urines with β-glucuronidase arylsulfatase no increase was found in 7-OMEN or nor-7-OMEN concentrations, suggesting that no conjugation with glucuronic acid or sulfate occurs.

## Discussion

In cancer patients receiving 7-OMEN at doses of 160 or 200 $mg/m^2$ as a 2-h infusion a biexponential decay of drug plasma levels was observed. This biphasic drug disappearance was characterized by a very rapid distribution phase followed by slow elimination, with a terminal half-life between 29 and 64 h.

Only one previous report on the clinical pharmacokinetics of 7-OMEN has been published [7]. Other data have been published in the form of abstracts, which give too little detail to allow comparison with our data.

In spite of this, it appears that the pharmacokinetic parameters found by us differ from those previously reported [6–9, 11]. The $t_{1/2\beta}$ and $V_d$ values we found appeared to be greater and the clearance smaller. For example, the mean $t_{1/2\beta}$ values found by Egorin et al. [6, 7], Kuhn et al. [9] and Long et al. [11] were 13.2, 14.3 and 11.9 h, respectively, whereas Grochow et al. [8] reported a very variable $t_{1/2\beta}$ ranging between 6.5 and 60 h. This difference may be attributable to our use of higher doses and different infusion times than in most previous studies. In fact, Egorin et al. [7] gave 3.5–31.5 $mg/m^2$ in 1 h and 42, 50 and 56 $mg/m^2$ in 2 h; Grochow et al [8] gave 8–140 $mg/m^2$ and did not specify the duration of infusion; Long et al [11] infused 42–126 $mg/m^2$ in 72 h

The fact that we found a lower clearance after higher doses suggests the possibility of dose-dependency of the kinetics of 7-OMEN. Other possible reasons could be a different sensitivity of the analytical assays and/or different sampling times, but except for Egorin, who sampled 24 h after drug injection, other authors unfortunately included no information on these two points in their reports, so that adequate comparison is impossible.

As previously reported [6–9, 11], the excretion of unchanged 7-OMEN accounts for a small proportion of the dose, which was less than 6% in the nine cases investigated. In contrast to some other anthracyclines [1], 7-OMEN does not undergo conjugation with glucuronic acid or sulfate.

Nor-7-OMEN was present in variable amounts in the urines of the patients investigated in this study, but its excretion accounted for a very small fraction of the 7-OMEN dose, a finding consistent with previous reports [6–9, 11]. In some cases nor-7-OMEN was detectable in plasma, but at much lower levels than 7-OMEN. Since the cytotoxic potency of this metabolite is reportedly similar to that of the parent drug [15] it appears unlikely that this metabolite, which is present at such low levels in plasma, plays a major role in the antitumor activity of 7-OMEN.

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