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The bioavailability in man of ICRF-159 a new oral antineoplastic agent

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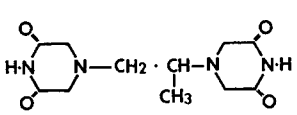
The bioavailability of the antineoplastic agent, ICRF–159, has been examined in 12 patients receiving the drug in single and subdivided dose schedules in an attempt to account for the differences in toxicity found with the different schedules clinically. Recovery of radioactivity in the urine after single large doses (13·3–19·4 g) was 8·5 ± 3·0% of the administered dose. After doses of 3·8–5·55 g recovery was 22·7 ± 10·5% and after the same dose subdivided into 3 equal aliquots it was 52 ± 8·7%. Unrecovered radioactivity was largely accounted for in the faeces. Plasma radioactivity levels in 2 patients after high and low dose were equivalent. Toxicity of the drug paralleled urinary recovery of radioactivity. It is concluded that schedule dependence of toxicity of ICRF–159 is at least partly due to bioavailability factors.

ICRF–159 (ICI 59118, NSC 129943) is (±)-1,2-bis(3,5-dioxopiperazin-1-yl) propane (I). The antineoplastic activity of this compound was discovered by Creighton, Hellman & Whitecross (1969) and it was introduced into clinical practice in England by Hellman, Newton & others (1969). In England the drug has generally been given daily, but in an initial clinical trial in the United States a once only single dose administration and two schedules of weekly administration were evaluated (Creaven, Cohen & others, 1974) one in which the weekly dose was given as a single administration and one in which it was given in three equal aliquots 6 h apart. The first of the two weekly schedules proved to be much less toxic than the second and the single dose administration was barely toxic in spite of very large single doses (250 mg kg^{-1}) being given. These results strongly suggested that bioavailability might be limited at high doses. We have, therefore, administered ICRF–159, to which [^{14}C] labelled ICRF–159 had been added, to twelve patients and studied urinary and faecal concentrations of the drug as an estimate of bioavailability.



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MATERIALS AND METHODS

ICRF–159 and ICRF–159 randomly labelled with [^{14}C] in the carbonyl groups (specific activity 8·73 mCi $mmol^{-1}$) were obtained through Drug Research and Development, National Cancer Institute, Bethesda, Maryland, U.S.A.

Drug administration. Doses were calculated on the basis of body surface area (BSA): 10·5 g m^{-2} BSA given as a once only single dose (Schedule A), 3·0 g m^{-2} BSA

given as a single weekly dose (Schedule B) and 3·0 g m^{-2} BSA given as 1·0 g m^{-2} at 9·00 a.m., 3·00 p.m. and 9·00 p.m. on one day each week for six weeks (Schedule C). The single dose and the drug in Schedule B was given on an empty stomach; with Schedule C the first dose only was given on an empty stomach. Tablets of ICRF–159 were crushed, made up into a slurry with water and 50 μCi of [^{14}C]-labelled ICRF–159 was added. The slurry was drunk and the container was washed out with water and the washings were drunk. This was repeated till the total dose had been consumed. The container was washed with 0·1 N HCl and the solution counted. Any radioactivity found was subtracted from the calculated dose.

Patients. Patients who received the 10·5 g m^{-2} BSA single dose and who subsequently, after clearing of all toxicity, received the 3·0 g m^{-2} $week^{-1}$ schedule had absorption studies carried out on the first dose of this schedule. One patient (no. 4) had all three treatments and had absorption studies carried out on all three.

Patients were not given the single dose once we had determined that this was giving neither adequate drug absorption nor reproducible drug toxicity. Consequently only two patients who received Schedule C had previously had either of the other treatments.

The patients had histologically confirmed cancer and adequate renal function as determined by a blood urea nitrogen (BUN) concentration of less than 25 mg 100 ml^{-1} and a serum creatinine concentration of less than 1·5 mg 100 ml^{-1}. Other requirements included a serum bilirubin of not more than 1·5 mg 100 ml^{-1} and SGOT of <100 I.U., absence of gross oedema, pretreatment white blood cell count (wbc) in excess of 5000 mm^{-3}, and platelet counts in excess of 100 000 mm^{-3}, and a sufficient interval after previous chemotherapy to avoid possible additive toxicity (2–8 weeks depending on prior treatment). All patients (results Table 1) gave informed consent and the study was approved by the Human Investigations Subcommittee of the Research and Education Committee of this Hospital.

Sample collection. Following administration of the drug, 5 ml of blood were collected through an indwelling heparin lock placed in an arm vein into a heparinized tube at 0, 0·5, 1, 1·5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48 and 72 h. Plasma was separated by centrifugation and refrigerated to await assay of radioactivity.

Urine was collected for 96 h after the drug administration, refrigerated, and assayed for radioactivity and metabolites. Faeces were collected for 3 days, frozen, and assayed.

Radioactive counting. For the assay of total radioactivity, plasma or urine (0·1 ml) was counted in a Beckman model LS–250 liquid scintillation counter in 10 ml of toluene containing 100 g of BBS-3 solubilizer (Beckman Instruments), 8 g of 2-(4′-t-butylphenyl)-5-(4″-biphenyl)-1,3,4-oxadiazole, and 0·5 g of 2-(4′-biphenyl)-6-phenylbenzoxazole $litre^{-1}$. Faeces were homogenized in water (1 litre) and assayed as for plasma. Samples of the homogenates were also combusted using an Oxymat JA101 oxidizer (Intertechnique Ltd., Westwood, N.J.).

Chromatography. The following chromatographic systems were used: ethyl acetate–n-propanol (15:1) and n-butanol–acetic acid–water (3:1:1) on Whatman No. 3 paper, ethyl acetate–methanol (10:1) on cellulose thin layer and n-amyl alcohol–pyridine–water (2:1:1) on silica gel G thin layer. Paper and cellulose were cut up in 1 cm strips and counted as above. Silica was scraped off in 1 cm sections and counted.

RESULTS

The recovery of radioactivity in the urine as a percentage of the administered dose on three schedules of administration of ICRF–159 is shown in Table 1. Recovery of radioactivity in the faeces was more erratic but averaged 73·5% in four patients on Schedule A, 62% in three patients after Schedule B, and 50% in three patients after Schedule C. Excretion of radioactivity in the urine after the drug was administered on the three different schedules of administration to one patient is shown in Fig. 1. Plasma radioactivity in patients 1 and 2 (Schedules A and B) are shown in Fig. 2. The haemotological toxicity after ICRF–159 on the three different schedules is shown in Table 1. Chromatography of the urine revealed that the radioactivity was excreted as two materials R_F 0·05 and 0·65 when chromatographed in n-butanol–acetic acid–water (3:1:1). However, it was not always possible by co-chromatography to resolve the faster running material and added standard ICRF–159 (R_F 0·55). The bulk of the faecal radioactivity chromatographed with the same R_F as ICRF–159.

Table 1. *Patients studied with* [^{14}C] *ICRF–159 at doses* (g m^{-2}) *of A* 10·5 g *single, B 3* g *weekly, C* 3 × 1 g *1 day a week for 6 weeks.*

No.	Age	Wt (kg)	Diagnosis (previous treatment)	Recovery of radioactivity (% of dose) 0–96 h Schedule A	B	C	Pre-treatment	Toxicity W.B.C. mm^{-3} × 10^3 Schedule A*	B†	C†
1	57	56	Fibrosarcoma retroperitoneum (S, Ifosfamide, Adriamycin, Cytoxan, Methotrexate, Dactinomycin)	4·1	12·6	—	8·5	5·5	5·9	—
2	63	47	Adenocarcinoma, salivary gland (S,R)	12·9	33·7	—	5·0	3·9	3·6	—
3	49	63	Clear cell carcinoma, kidney	8·6	15·0	—	7·7	5·9	5·0	—
4	42	77	Adenocarcinoma, lung	6·6	29·5	42·1	8·1	8·1	8·4	6·9
5	56	56	Anaplastic large cell carcinoma lung (R)	8·5	—	65·1	Not evaluable for toxicity			
6	55	60	Clear cell carcinoma, kidney (R, CCNU, Streptozotocin, Bleomycin)	10·1	—	—	Not evaluable for toxicity			
7	58	60	Epidermoid carcinoma, oesophagus (R)	—	—	46·4	5·2	—	—	3·1
8	48	65	Fibrosarcoma, back (Adriamycin)	—	—	59·2	12·0	—	—	3·9
9	55	62	Adenocarcinoma, rectum (R, 5FU, Cytoxan)	—	—	46·5	9·5	—	—	2·1
10	77	68	Adenocarcinoma, colon (5FU)	—	—	42·3	5·9	—	—	2·1
11	59	59	Epidermoid carcinoma, lung (R, HN2, Methotrexate, CCNU)	—	—	58·7	6·5	—	—	2·1
12	49	71	Anaplastic carcinoma, primary site unknown (VP16–213)	—	—	55·7	8·5	—	—	2·3
			Mean	8·5 ± 3·0	22·7 ± 10·5	52 ± 8·7				

* Nadir after a single dose. † Nadir after 3 weekly doses (total 9·0 g m^{-2}). S = Surgery. R = Radiotherapy. CCNU = 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea. 5FU = 5 Fluorouracil. HN2 = Mechlorethamine. VP16–213 = 4′-demethylepipodophyllotoxin 9-(4,6-0-ethylidene-β-D-glucopyranoside).

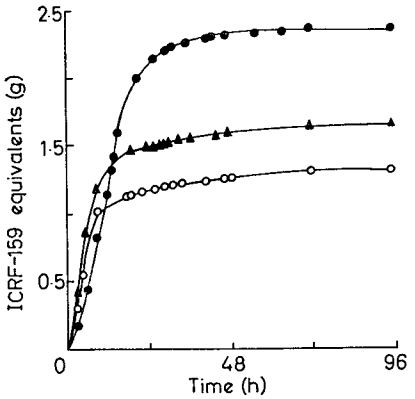

FIG. 1. This patient received a single dose 10·5 g m^{-2} and both weekly schedules of ICRF–159 (3 g m^{-2} week^{-1}). The total recovery of radioactivity in the urine after 10·5 g m^{-2} Schedule A ○——○, 3 g m^{-2}, Schedule B ▲——▲, and 3 g m^{-2} Schedule C ●——● are shown.

DISCUSSION

The present study attempts to elucidate the basis for the marked schedule dependency of the haematologic toxicity of ICRF–159. The drug on a daily or divided daily schedule produced marked leukopaenia (Hellman & others, 1969) whereas single dose administration produced only mild and erratic leukopaenia in spite of the large doses given, and the attempt to produce consistent dose-limiting haemotological toxicity with single dose administration had to be abandoned (Creaven & others, 1974). These authors also found that the drug in a subdivided schedule (Schedule C) produced consistent moderate haematological toxicity even though much less total drug was given. Although marked schedule dependence based on cell cycle specificity is a characteristic feature of some antineoplastic agents, there still remained the possibility that the effect might be due to limitation of drug absorption at high doses especially in view of the fact that no absorption studies had been carried out in man. The results strongly suggest that limitation of absorption at high dose concentrations is occurring. Of the four patients studied at 10·5 g m^{-2} and later at 3·0 g m^{-2}, three had essentially the same total recovery of drug after both doses. Moreover, the faecal recovery is consistent with the idea that much

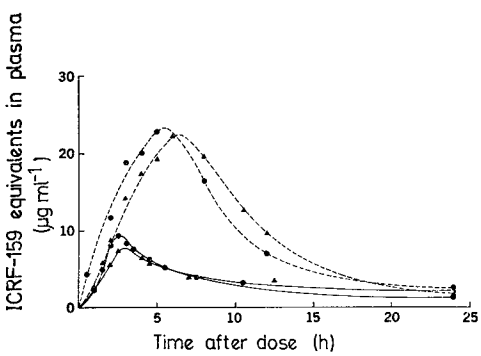

FIG. 2. Plasma radioactivity in patient 1 (solid line) and patient 2 (broken line) after 10·5 g m^{-2} (circles) and after 3 g m^{-2} (triangles).

smaller proportions of the 10·5 g m^{-2} (Schedule A) and the 3·0 g m^{-2} dose given on Schedule B are being absorbed than of the 3·0 g m^{-2} dose given on Schedule C. The results could possibly be explained on the basis of a much greater biliary excretion after the Schedule A dose than after the smaller doses. However, this appears unlikely in view of the fact that the bulk of the faecal radioactivity is chromatographically identical with ICRF–159 whereas all of the urinary radioactivity appears to be metabolite.

The amount of radioactivity was insufficient to allow a quantitation of the unchanged drug and the metabolites in the plasma. We were, therefore, unable to verify changes in absorption by measurements of area under the plasma concentration curve of unchanged drug. However, for the plasma curves of total radioactivity (Fig. 2) the ratio of areas under the curve for patient 1 is 1 : 1 and the ratio of recovered radioactivity in the urine after the two doses is 1 : 0·9; for patient 2 the ratio of the AUC's is 1 : 1 and the ratio of recovered radioactivity is 1 : 0·8. Moreover, the height of the peak of plasma radioactivities and the time after dosage at which the peak occurs appear to be essentially the same for the two doses in these patients which lends support to the idea that the same quantity is being absorbed in each case.

We feel that the evidence presented here indicates very strongly that ICRF–159 has a limited absorption at high doses and that in future clinical studies of the drug, Schedule C or some similar schedule of divided drug dosage should be used rather than large single doses. The study does not, however, exclude the possibility that some of the schedule dependence of toxicity may be due to cell cycle specificity of the drug.

No studies were carried out to elucidate the mechanism of the limitation of absorption of ICRF–159. Since the kinetics of absorption seem from Figs 1 and 2 to be essentially the same for the Schedule A and Schedule B and since the total amount of drug absorbed is, at these dose concentrations, independent of the administered dose it would seem to be due to an intrinsic limitation rather than to a slower absorption at the higher dose. However, no definite conclusion can be reached without further detailed studies of the mechanism involved.

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