IN VIVO PHARMACOKINETICS OF [^{14}C]-ETANIDAZOLE

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SUMMARY

Etanidazole, like other nitroaromatic radio sensitizers, displays its biological activity mostly through two molecular groups: the nitro group and the side-chain attached to the nitrogen in the imidazole ring in position 1.

To monitor the behaviour of the two moieties in vivo, labelled Etanidazole, containing a ^{14}C atom in position 2 of the side-chain was prepared and administered to four patients at a 2 gm^{-2} dose: the concentration of Etanidazole in different fluids and tissues was followed either by titrating the NO_2 group by polarography or determining the total radioactivity, carried by the side-chain. Two different profiles of Etanidazole concentration were found, in blood and urine, as determined by the two methods. The concentration was found to be higher when measured as total radioactivity than when measured by polarography; this difference was greater in blood (49%) than in urine (27%). Since the two methods involve determination of different portions of the Etanidazole molecule, we hypothesized a degradation of the drug to explain these differences.

Key words: [^{14}C]-Etanidazole; polarography; radiometric measurements; metabolites; pharmacokinetics.

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INTRODUCTION

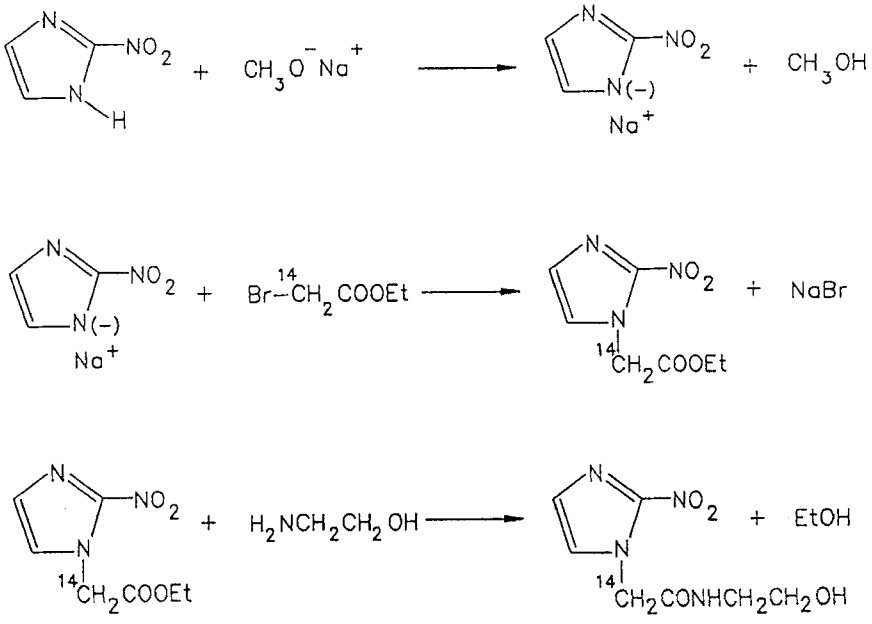
Nitro-heterocycles, such as nitroimidazoles, are widely used in the chemotherapy of cancers as radiosensitizer agent; because of their electron affinity they trigger a redox cycle by shunting electrons from redox intermediate to oxygen. Nitroimidazoles, particularly, metronidazole (a 5 nitro derivative), misonidazole and etanidazole (2 nitro derivatives) proved to be effective oxidant agents and their reduction has been exhaustively studied in vitro and is a well known process. The in vivo activity appears to be related, not only to the redox properties of the nitro group, but also to the side chain bound to the N_1 atom of the imidazole ring, that can affect the hydrophilic-hydrophobic balance of the whole molecule, resulting in the modification of the drug absorption. But beside these properties, common to all the molecules of this class, the pharmacokinetics of Etanidazole is not yet fully understood. The first report on this topic was made by Coleman and co-workers in 1981 and 1984 (1, 2) and by Newman (3) and Watts (4) in 1987. Our group started a research program on Etanidazole in 1986 (5) and together with many parallel results to those previously reported, we found also many differences (7, 8), in particular it was found that etanidazole enters hypoxic cells (7); inside the cells the nitro group is present in a reduced form. Etanidazole loses the NO_2 group under irradiation. These results were obtained using polarography (9), which was very useful in measuring Etanidazole concentration in samples obtained after extraction from blood of patients. In order to investigate the excretion of the drug and the metabolite formation we carried out experiments using labelled Etanidazole with a ^{14}C atom in the side chain and following its pharmacokinetics either by radiochemical (side chain) or polarographic (NO_2 group reduction) methods (10).

EXPERIMENTAL

Materials

2-Nitroimidazole (I) was purchased from Sigma. Etanidazole was supplied by Pharma AG, Austria. Both compounds were used as received, after having tested their purity by TLC. Ethyl 2-bromo [2-^{14}C]-acetate was purchased from Amity PG; total activity: 1 mCi.

[^{14}C]-Etanidazole was synthesized according to the Scheme 1.



Scheme 1. Synthesis of [^{14}C]-Etanidazole

1 g of 2-Nitroimidazole (I) dissolved in 5 ml of dimethylformamide (Fluka, Buch, Switzerland) was added to 0.011 moles of sodium methylate (Carlo Erba, Milan, Italy): the solution turned yellow-orange. The [^{14}C]-labelled ethylbromoacetate was then added first, under stirring, followed by the equivalent amount of the cold reagent.

After 24 h at 70°C a slight excess of ethanolamine was added and the mixture refluxed for 24 h. The solvent and the excess reagents were vacuum distilled and the oily residue was purified by column chromatography (silica gel, eluent dichloromethane). After evaporation of the eluent, the residue crystallized from ethanol.

Total yield 65%, specific activity 0.5 μCi/mg; the labelled compound did not differ from an authentic pure sample of etanidazole: r_f = 0.65 (silica gel, dichloromethane).

Methods

In vivo experiments

Four patients (males, mean age 64±5 y) under radiotherapic treatment for cancer were chosen, after their permission had been given. [^{14}C]-Etanidazole was administered by intravenous injection (2g/m^2) over 30 min; each sample injected contained 3 g of cold drug plus 25 mg of labelled drug, corresponding to 7.10^6 Bq, dissolved in 5 ml of isotonic saline solution. The solutions were prepared in the Hospital Pharmacy and tested for sterility, abnormal toxicity and pyrogenicity (11): all of them were found free of contaminants and according to USP.

The protocol was the same as used in previous trials (12).

Preparation of physiological samples

Faeces were collected at 12, 24, 48 hours, mixed with 0.1M sodium hypochlorite (1% p/v) and homogenized. 2 g of the homogenate were supplemented with 14 ml of ethanol and centrifuged; 10 ml of supernatant were added to 10 ml of a mixture of 0.1M KCl and 10 ml of 0.1M phosphate buffer solutions and submitted to polarographic analysis; 1 ml of the supernatant was directly measured for radioactivity (Table 1).

Urine samples were collected after 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 hours; 1 ml of each untreated sample was taken for polarographic measurements and radioactivity analysis by liquid scintillation counting (Table 2).

Table 1. Amount of Etanidazole (μg/g±1) recovered in the faeces as a function of time, as determined by polarography (a) and liquid scintillation counting (b) in four different patients.

Time after infusion (hour)	patient 1		patient 2		patient 3		patient 4	
	a	b	a	b	a	b	a	b
0-24					15	17	1	2
24-48	6	3	0	8	9	4	0	5
48-72			0	30	5	1		
72-96			0	16	5	2		
96-120					9	4		

Table 2. Amount of Etanidazole (μg/ml±1) recovered in the urines of four patients as a function of time, as determined by polarography (a) or liquid scintillation counting (b).

Time after infusion (hour)	patient 1		patient 2		patient 3		patient 4	
	a	b	a	b	a	b	a	b
0-2	-	-	2	2	7	7	1	1
2-4	-	-	2	3	4	4	1	2
0-6	4	4						
4-6	-	-	2	2	2	2	3	3
6-8	-	-	1	2	1	1	2	2
6-12	2	2						
8-12	-	-	1	1	1	1	1	1
12-18	1	2						
12-24	-	-	1	1	1	1	0	0
18-24	2	3						
24-48*	1	1						
48-120	<1	<1						

Table 3. Amount of Etanidazole (μg/ml±1) recovered in the plasma of four patients as a function of time, as determined by polarography (a) or liquid scintillation counting (b).

Time after infusion (hour)	patient 1		patient 2		patient 3		patient 4	
	a	b	a	b	a	b	a	b
0.25	-	-	57	99	44	98	53	96
0.5	66	74	49	73	33	83	36	78
0.75	-	-	36	59	29	79	41	66
1	49	55	31	67	25	53	36	57
1.5	-	-	27	63	24	56	28	42
2.0	37	42	25	54	14	45	26	45
3.0	-	-	21	40	12	43	24	48
4.0	28	29	19	23	10	32	23	44
6.0	23	26	14	14	6	23	17	35
12	8	7	-	-	3	13	10	25
18	-	-	-	-	1	6	1	2
24	2	2	0	1	1	4	1	2
48	0	0	0	0	0	2	0	1
72	-	-	0	1	0	1	-	-
96	-	-	0	1	0	1	0	1
120	-	-	0	1	0	0	0	1

Blood samples were withdrawn at the following times, after administration of the drug: 0, 0.3, 0.5, 1, 2, 4, 6, 24, 48, 72, 96, 120 hours. The blood samples were allowed to clot and the serum removed after centrifugation. 2 ml of serum was added to 14 ml of 95% v/v ethanol under stirring to precipitate proteins and extract the drug.

After centrifugation for 10 min at 3000 g, 10 ml of supernatant was removed and diluted with 10 ml of 0.1M KCl and 10 ml 0.1M phosphate buffer (pH 7) solutions for the polarographic and radiometric determinations (Table 3).

Radiochemical measurements

The measurement of the radioactivity was carried out by liquid scintillation. 1 ml of the final ethanol solutions (obtained after the appropriate treatments) was added to 9 ml of scintillation counting solution (Ready-Solv CP Beckmann) and counted in a liquid scintillation counter (LSC1 Nuclear Enterprise).

Samples of the same final ethanol solution were also eluted by TLC and radioactive/quantitative measurements of Etanidazole recovered in blood and urine at different times were made by TLC Linear Analyzer (mod. LB 2821, Berthold). 10μl of the final ethanol solution were eluted on Silica gel 60F plates (Merck), using the solvent mixture dichloromethane/methanol 4:1 (v/v). The presence of the nitro group was revealed by the absorbance at 320 nm in the TLC Scanner Densitometer (Camag). Experimental data were transformed into weight of total Etanidazole (and metabolite) using a linear relationship previously assessed for all the analytical methods. Results in terms of μg/ml or μl/g Etanidazole recovered in each biological fluid are reported in Table 1.

Polarographic measurements

The ethanolic final solutions were analyzed by differential pulse polarography (scan rate 2 mV/s, pulse height 50 mV, sweep amplitude from -180 to 1180 mV vs SCE), using AMEL polarograph mod. 472. Under these experimental conditions the reduction peak of the nitro group of Etanidazole was found at -600 mV. No interfering peaks were observed at 600 mV in the blood sample at t = 0. The calibration curve was made by addition of prefixed amount of Etanidazole to the blank samples. The response was linear up to 1000 μg.

RESULTS AND DISCUSSION

The two different methods of analysis, used to measure the concentration of Etanidazole in biological fluids, refer to two different parts of the molecule. The radiochemical synthesis introduced a label in the side chain: therefore radioactivity measurements refer either to the integer molecule or to a possible metabolite containing radioactive side-chain, while polarographic measurements concern the reduction of the NO_2 group, bound to the imidazole ring (Scheme 1).

Comparison of the results obtained by means of the two methods can offer suggestions for the possible metabolism pathway of the drug: a significant lack of concordance between the two sets of results would mean a degradation of the molecule in the biological environment. Due to its favorable partition coefficient (P = 61), Etanidazole is almost completely absorbed: in fact the amount of the drug recovered in faeces was in each case very low, less than 50 mg per patient after 48 h (Table 1).

In some cases polarography failed to produce concentration values of the active component either because of its low concentration or because of the presence of compounds hindering the measurements.

The Etanidazole excreted through the urine decreases with time: it was still present in the sample after the 5th day of the control. The measurement of radioactivity gives similar, almost parallel, results.

The highest concentrations were found after the second and fourth hours; after 24 h the concentration had decreased tenfold (Table 2). The concentration measured in the blood by radioactivity measurement was systematically higher than that measured by polarography (Table 3), but after 48 h after the administration, only 60% of the total radioactivity could be associated with the undegraded Etanidazole, as determined by quantitative thin layer radiochromatography. The presence of a metabolite was revealed in the blood sample 12 h after the administration. TLC-radiochromatography gave two peaks corresponding to the unchanged Etanidazole and to a more polar, but still radioactive, compound. This compound did not show the characteristic absorbance of the NO_2 group at 320 nm and therefore it could be formed either by the Etanidazole lacking a nitro group or of the Etanidazole portion carrying the radioactivity, i.e. the side chain. No attempt

was made to separate or to identify the product. The data collected by the two analytical methods fit first order kinetics: a half life of 3.9 days was thus calculated for the in vivo degradation of the Etanidazole. The in vivo degradation of Etanidazole has been previously reported; results obtained with hamster V79 cells in hypoxyc conditions revealed the presence of reduced Etanidazole inside the cells and it was suggested that the degraded nitro group was the consequence of the pharmacological activity of the drug.

The faster disappearance of Etanidazole in the blood, as measured by radioactivity, suggests that a part of the molecule is quickly metabolized, making the side-chain (the portion carrying the radioactivity) free to follow alternative metabolic pathways or to be more rapidly excreted. This can explain that the higher Etanidazole concentration values in urine, determined by radiometric measurements, are indeed due to radioactive metabolite.

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