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THE METABOLISM OF THE 5-7-DIBROMO-8-HYDROXYQUINOLINE (BROXYQUINOLINE) IN MAN

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Abstract—The urinary elimination in man of a single dose of 2 g of 5-7-dibromo-8-hydroxyquinoline (Broxyquinoline) amounts to 36 molar % after 24 hr and 48 molar % after 72 hr. The main metabolite consists on a glucuronide of the Broxyquinoline, probably an O.glucuronide, which represents about 98 per cent of the urinary metabolites found. Unchanged Broxyquinoline is excreted as a free compound but in very small quantities. Less than 1·3 molar % of the 8-hydroxyquinoline was found, showing that debromination by this way is an unimportant reaction. The elimination rate seems to be controlled by the conjugation process and limited to about 33 mg of Broxyquinoline/hr.

SEVERAL halogenated derivatives of the 8-hydroxyquinoline are widely used as amoebicides and intestinal bactericides. Experimental data about their metabolism are however very scarce.

This paper reports some characteristic features of the metabolism of the 5-7-dibromo-8-hydroxyquinoline (DBHQ) in man, based on a study of its urinary excretion.

Three topics have been specially studied: the rate of the urinary elimination of the drug and its metabolites, the nature of these metabolites and the importance of a possible dehalogenation reaction.

EXPERIMENTAL METHODS

I. Four male patients each received one single oral dose of 1·98 g of DBHQ (8 tablets), two of them at noon (12.30 p.m.) the other two in the evening (22.00 and 20.30 p.m.).

The 24-hr urines were separately collected in four fractions, from the beginning of the assay until 77 or 161 hr later. Each fraction was distinguished by its volume and its pH and stored at low temperature (−4°) before analysis.

II. The analyses were performed according to the following standard procedure.

(1) extraction and separation of the unconjugated products.

(a) an aliquot of 5 ml urine, whose pH has been adjusted to 7·2 was extracted three times with 5 ml chloroform and the aqueous fraction discarded;

(b) the organic layers were then pooled, evaporated at low temperature under vacuum to 5 ml and reextracted with 5 ml of an HCl/KCl pH 1·75 buffer solution in order to separate the unchanged DBHQ (organic phase) from the 8-hydroxyquinoline (HQ) which at this pH is transferred to the aqueous phase;

(2) extraction of the conjugated metabolites:

(a) a second aliquot of 10 ml urine was first buffered to pH 6·2 with 2 ml of a Na_2HPO_4/HAc buffer solution;

(b) the conjugated sulfates and glucuronides were then hydrolysed in presence of glusulase preparation at 37° during 24 hr.

Each glusulase fraction used contained 5,000 β-glucuronidase units (Fishman) and 40,000 sulfatase units (Roy);

(c) one half of the resulting solution is then treated as described above for the unconjugated derivatives (1a/b). The aqueous extract contained the free HQ and also the eventually deconjugated HQ;

(d) the second half of the solution is extracted at pH 1·75 with 5 ml heptane and the total DBHQ (free + deconjugated) titrated in the organic phase.

(3) The 8-hydroxyquinoline derivatives were spectrophotometrically assayed as their addition products with di.o.anisidine diazonium salt (Fast Blue salt B), according to the Robinson, Smith and Williams[1] method, which has been modified as follows.

The organic (1b and 2d) solutions were evaporated to dryness *in vacuo*. The aqueous solutions (2c) were similarly treated after addition of 0·2 ml isoamyl alcohol in order to prevent emulsion.

The dry residues were dissolved in 2·0 ml ethanol; one ml of the resulting solution was added to a mixture of 3·7 ml ethanol + 0·2 ml HCl 1 N and allowed to react with 0·1 ml of a 5% w/v ethanolic solution of the Fast Blue salt B (Merck).

The diazotation takes place immediately and the determination of the DBHQ or the HQ is made spectrophotometrically at 324 mμ and 367 mμ respectively in a PM Q II Zeiss spectrophotometer.

The measured molar extinction coefficients of the diazotated derivatives are respectively:

$\varepsilon = 2{\cdot}94\ 10^3$ mole^{-1} cm^2 at 324 mμ (DBHQ + Fast Blue salt B)

$\varepsilon = 1{\cdot}77\ 10^3$ mole^{-1} cm^2 at 367 mμ (HQ + Fast Blue salt B)*. Spectra of the DBHQ, HQ and of their respective reaction products are given in Fig. 1.

The Beer–Lambert law applies perfectly to the diazotated solutions between 0·5 and 7·0 10^{-4} mole/l. concentrations of the quinoline reactants.

The accuracy of this procedure depends on the efficiency of the glusulase action and of the separation by extraction. The activity of the enzymatic mixture was therefore carefully checked according to the method of Fishman,[3] using the phenolphthaleine glucuronide as synthetic substrate. Comparison of the rate of enzymatic hydrolysis with the glusulase preparation and pure β-glucuronidase was also performed. Experimental results are given in Table 4.

On the other hand, the partition coefficients of DBHQ and HQ between organic and aqueous phases were determined; they are listed in Table 1; it shows that the separation of these products may be safely considered as complete under our experimental conditions.

* Those ε are found higher than the coefficients of the DBHQ-Fe and HQ-Fe complexes used in the method of Haskins and Luttermoser.[2] In this last case $\varepsilon = 1{\cdot}67\ 10^3$ and $\varepsilon = 1{\cdot}3\ 10^3$ respectively for DBHQ and HQ at 630 mμ and 650 mμ.

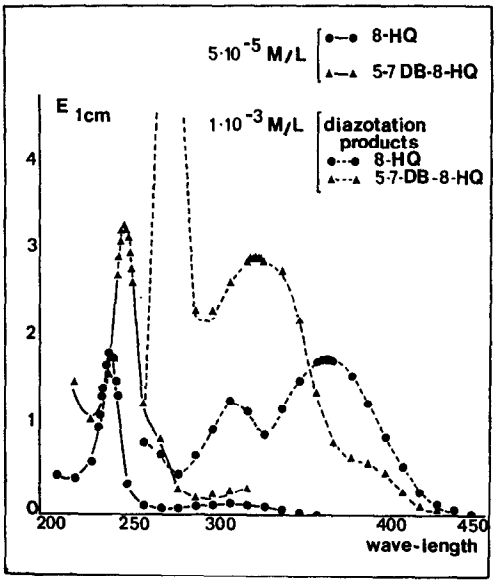

FIG. 1. Ultra-violet spectra of the 8-HQ and 5-7-DB-8-HQ and their diazotated derivatives.

TABLE 1.

Compounds	Nature of the phases		Partition coefficients
DBHQ	heptane/water	pH 1·75	18 ± 2
HQ	heptane/water	pH 1·75	0·02 ± 0·01
DBHQ	chloroform/water	pH 7·2	⩾ 50
HQ	chloroform/water	pH 7·2	4·4 ± 0·2
DBHQ	chloroform/water	pH 1·75	⩾ 50
HQ	chloroform/water	pH 1·75	⩽ 0·01

These values do not change significantly in the urine, even if, as was the case, isoamyl alcohol (up to 5 per cent) is added in order to prevent emulsion.

The nature of the metabolites was occasionally confirmed by thin-layer chromatography (TLC) using a specially purified Merck Kieselgel G and the eluents listed in Table 2.

TABLE 2.

Eluent	1	2	3	4	5	6	7
DBHQ R_f	0·43	0·72	0·43	0·06	0·30	0·20	0·35
HQ R_f	0·59	0·74	0·54	0·61	0·50	0·65	0·66

Eluent:

1	Butanol + NH_4OH 25% + water	7 + 2 + 1	organic layer
2	Propanol/NH_4OH 25%/water	7/2/1	
3	Butanol + NH_4OH 25% + water	50 + 13 + 3	organic layer
4	Ethylacetate/NH_4OH 25%	5/1	
5	Butanol + Ethylacetate + NH_4OH 28%	4 + 10 + 3	organic layer
6	Butanol + Ethylacetate + NH_4OH 28%	2 + 10 + 3	organic layer
7	Propanol + Ethylacetate + NH_4OH 28%	2 + 10 + 3	organic layer

After elution, u.v. light allows the detection of 0·8 μg of HQ and 0·05 μg of DBHQ.

EXPERIMENTAL RESULTS

TABLE 3. SUMMARY OF SOME TYPICAL EXPERIMENTAL RESULTS

Σt : total time (hr) after drug ingestion.
Σv : cumulated volume (ml) of the urine fractions collected.
Σ% DBHQ f : cumulated molar percentage of the free DBHQ recovered in the urine.
Σ% HQ f : cumulated molar percentage of free HQ recovered in the urine.
Σ% DBHQ c : cumulated molar percentage of DBHQ recovered in the urine as conjugated.
Σ% : total molar % of DBHQ recovered under the three forms.

Fraction n°	Σt (hr)	Σv (ml)	Σ% DBHQ f	Σ% HQ f	Σ% DBHQ c	Σ%
Patient A						
1	2·5	740	0·008	0·114	6·30	6·42
2	4·25	1035	0·012	0·157	9·80	10·08
3	10·5	1605	0·033	0·196	19·04	19·27
4	18·0	1855	0·042	0·224	25·52	25·80
5	22·5	2215	0·061	0·294	32·44	32·79
6	26·25	3035	0·069	0·509	35·96	36·54
7	27·75	3195	0·072	0·550	36·72	37·34
30	161·50	11720	0·330	0·335	56·42	58·09
Patient B						
1	4·5	600	0·016	0·097	8·07	8·19
2	10·5	1370	0·052	0·177	18·37	18·60
3	20·0	1670	0·084	0·267	24·89	25·24
4	23·5	1855	0·093	0·305	28·37	28·77
5	29·0	2025	0·109	0·333	32·62	33·06
6	34·5	2395	0·137	0·352	37·89	38·38
7	43·5	3035	0·150	0·465	42·97	43·58
13	77·0	4970	0·262	0·820	46·87	47·95
17	103·0	6550	0·317	0·910	⩾ 46·87	⩾ 47·95
Patient C						
1	10·05	420	0·032	0·089	12·63	12·75
2	13·0	615	0·044	0·127	18·59	18·76
3	22·0	1010	0·068	0·206	28·80	29·08
4	33·0	1780	0·110	0·301	35·24	35·65
5	37·0	1890	0·119	0·325	36·41	36·85
6	43·0	2225	0·134	0·452	38·76	39·34
7	51·5	3140	0·161	0·603	40·35	41·11
11	81·0	4585	0·254	0·845	43·47	44·57
24	177·0	9765	0·472	0·328	⩾ 43·47	⩾ 44·57
Patient D						
1	12·0	790	0·054	0·202	19·43	19·68
2	14·5	1120	0·078	0·271	25·95	26·28
3	21·0	1690	0·090	0·355	33·62	34·07
4	26·5	2140	0·107	0·378	38·60	39·08
5	35·5	2755	0·129	0·424	43·12	43·67
6	38·5	2930	0·134	0·477	43·93	44·54
7	45·0	3340	0·150	0·521	44·87	45·54
12	74·5	5955	0·281	0·812	46·74	47·83

The diagram in Fig. 2 shows that the rate of the urinary excretions, calculated on the sum of the unchanged DBHQ and its metabolites, looks very similar for the four patients.

The curves of Fig. 3 illustrate the relationship between the urinary excretion and the volume of urine. From these curves some difference appears to exist between patients

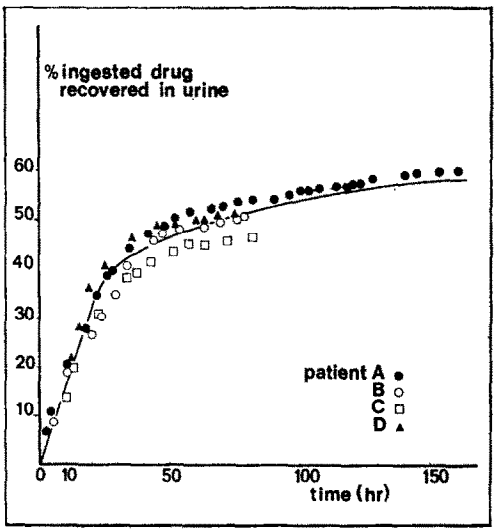

FIG. 2. Urinary excretion of the drug as function of time.

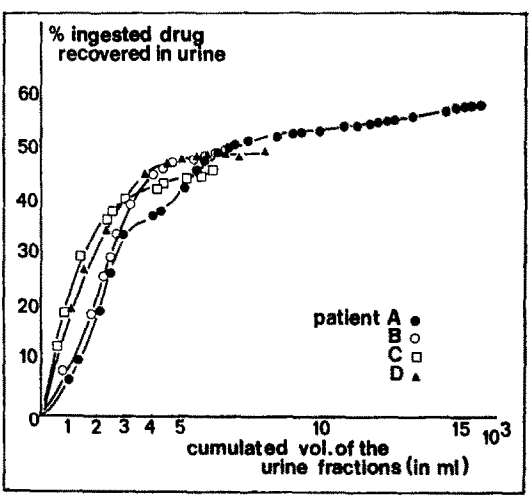

FIG. 3. Urinary excretion of the drug as function of urine volume.

A and B and patients C and D, according to the time of drug administration. Table 4 reports the results of some experiments comparing the effects of the glusulase and the β-glucuronidase, and showing that both enzymes do work similarly.

DISCUSSION AND CONCLUSIONS

1. *Metabolites*

The original drug was found in the urine in at least two forms, the free unchanged molecule and its glucuronic conjugate.

Both compounds were characterized in the urine by TLC before and after enzymatic hydrolysis; their chemical identity was confirmed by u.v. spectra of their diazotated derivatives which were shown to be identical to the standard diazotated DBHQ.

As the hydrolysis with β-glucuronidase and glusulase (enzymatic mixture of β-glucuronidase and arylsulfatase) gave the same results it is assumed that the DBHQ conjugate is the glucuronide. Furthermore this last metabolite could be the O.glucuronide. It is indeed well known that HQ behaves as a true phenol during its biotransformation[4] and as its halogenated derivatives give similar ^{14}N chemical shifts in their

TABLE 4. AMOUNTS OF DECONJUGATED DBHQ (IN mg)

Patient	Fraction n°	Enzyme used	
		Glusulase	β-glucuronidase
A	3	183	169
	4	127	117
B	1	161	186
	2	203	244
C	2	118	109
D	1	385	381
	2	129	135

NMR spectra[5] one can conclude that their 'enol' form is the exclusive one and assume that they will behave as HQ. This supports the idea of the O.conjugation. It also clearly appears from the forementioned experimental data that, although in very small quantity, free HQ could be found as a urinary metabolite.

Again its presence and identity were proved both by TLC and u.v. spectrophotometry.

Minor differences between the u.v. spectra of the urinary HQ fraction and standard pure HQ were however observed, suggesting that the urine extracted HQ could be slightly contaminated by a natural component absorbing at the same wavelength. The measured quantities of the HQ must therefore be considered as the maximal possible values.

It is thus concluded that the main pathway of the 5-7-dibromo-8-hydroxyquinoline metabolism is the formation of its glucuronic conjugate and that, to a very small extent, some dehalogenation occurs. We did not suceed in showing the presence of the mono-bromo derivative in the urine.

2. *Quantitative aspect*

The above conclusions about the nature of the urinary metabolites concern up to 58 per cent of the original drug, the total amount which was excreted during the 162 hr following administration. It is however worthwhile to emphasize that the total amount of free 8-hydroxyquinoline recovered remains very small all through the excretion period and never exceeds 1·3 molar % of the original drug, a value which must be considered as maximal for the reasons given above.

The debromination reaction is therefore not an important pathway in the DBHQ metabolism. Fig. 2 shows that the individual variations of the urinary elimination rate are very small, and that similarity is most obvious during the first 24-hr period after ingestion. After that period, excretion decreases gradually and almost reaches a plateau after 72 hr.

On the contrary Fig. 3 illustrates the fact that the rate of excretion has no direct relationship with the urine volume itself; two different behaviours were indeed recorded according to the time of day when the drug was taken. As the only important metabolite is the glucuronide of the DBHQ (± 56 per cent of the ingested drug after 162 hr) and as its excretion rate is strictly time dependent in the initial period, it seems logical to assume that the limiting step is either the transfer of DBHQ to the conjugation site or the conjugation process itself.

Whatever it is, the maximal rate of urinary elimination may be estimated as about 33 mg of DBHQ/hr.

The high rate of excretion through this channel and therefore the resorption of the drug is rather unexpected, as its aqueous solubility is definitely poor. However, our observations found confirmation in the experimental data obtained on the rabbit by other authors for some related compounds.[6-8]

Haskins *et al.*[7-8] reported the following percentages of excretion for the 5-7-diiodo-8-hydroxyquinoline (DIHQ), and the 5-chloro-7-iodo-8-hydroxyquinoline (CIHQ), after chronic p.o. administration to rabbits of 20 mg/kg/daily for 7 days:

– DIHQ = 29 per cent of the drug ingested
– CIHQ = 50 per cent of the drug ingested

The drug in the urine was estimated as organically bound iodine compounds.

As we have observed for DBHQ, no significant amounts of unchanged or dehalogenated drugs were found in the urines for the DIHQ and CIHQ administered to the rabbit. Likewise the main route of detoxication passes through a conjugation process giving glucuronides and sulfates in the case of rabbits.

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