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STUDY ON THE CINCHOPHEN DETOXICATION AND ITS RELATIONSHIP TO ULCEROGENESIS

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Abstract—A paperchromatographic method for analysing cinchophen and its derivatives in urine is presented. Cinchophen undergoes hydroxylation in dog, which applies mainly positions 4′ and 8. As the quantity of cinchophen and hydroxycinchophens found in the urine were less than 10 per cent of the cinchophen quantity ingested, cinchophen must become detoxicated by other means as well. The influence of some hydroxycinchophens on ulcerogenesis was examined.

CINCHOPHEN (2-phenyl-4-quinolinecarboxylic acid) has been found to cause ulcerogenesis in the pylorus and duodenum of dog and it is therefore often used to produce experimental ulcer.[1] In order to elucidate the mechanism of this cinchophen induced ulcerogenesis one must also consider the metabolism of cinchophen in the organism.

The fate of cinchophen in viable organisms was studied in 1912 by Dohrn.[2] According to him, cinchophen is excreted in the urine as 8-hydroxycinchophen, as 8-metaxycinchophen and as oxypyridinuric acid. Just recently it has been shown that cinchophen will be hydroxylated besides 8-hydroxycinchophen also to 4′-hydroxycinchophen in man.[3]

The purpose of the present work was to study the hydroxylation of cinchophen in dog *in vivo*, and the ulcerogenic effect of some hydroxycinchophens.

EXPERIMENTAL

Preparation of cinchophen derivatives

For identification we prepared 6-hydroxycinchophen (2-phenyl-6-hydroxy-4-quinoline-carboxylic acid), 7-hydroxycinchophen (2-phenyl-7-hydroxy-4-quinoline-carboxylic acid), 8-hydroxycinchophen (2-phenyl-8-hydroxy-4-quinolinecarboxylic acid), 4′-hydroxycinchophen (2- 4′-hydroxyphenyl-quinolinecarboxylic acid) and 8-metoxycinchophen (2-phenyl-8-metoxy-4-quinolinecarboxylic acid) as reference substances. The 6- and 8-hydroxycinchophens were prepared from *p*- and *o*-aminophenols, benzaldehyde and pyruvic acid.[4] The 6-hydroxycinchophen was recrystallized from aniline. 8-Metoxycinchophen was prepared from *o*-anisidine, benzaldehyde and pyruvic acid [5] 4-hydroxycinchophen from salicylaldehyd, pyruvic acid and aniline.[6] and 7-hydroxycinchophen from *m*-aminophenol, benzaldehyde and pyruvic acid.[6] The 3-hydroxycinchophen (2-phenyl-3-hydroxy-4-quinolinecarboxylic acid) was obtained from Carl Roth (Karlsruhe).

Feeding of cinchophen or hydroxycinchophens and collection of urine samples.

Dogs were used as test animals. They were daily given 0·2 g of cinchophen (in an 0·5 % tragacanth solution) per kg of body weight directly into the stomach and

24-hr urine was collected with toluol as preservative. For quantitative determination of the excretion of cinchophen and hydroxycinchophens one dog (10 kg) was fed with cinchophen for 6 days and the 24-hr urine samples were collected over a period of 8 days.

Experiments were also conducted to ascertain the role of hydroxycinchophens in the production of gastric ulcer. The 7- and 8-hydroxycinchophens were fed for a period of 14 days to 2 dogs weighing 10 kg. The daily dose was 0·2 g per kg of body weight. 3-Hydroxycinchophen was fed to 2 dogs and the daily dose was 0·1 g per kg of body weight. Urine samples were collected for paper chromatographic analysis.

Paper chromatographic analysis of the urine samples

Cinchophen and 3-hydroxycinchophen urine (50–100 μl) as such or after acid hydrolysis was pipetted into a Whatman No. 1 paper. For acid hydrolysis the urine was made 1 N with conc.HCl and hydrolysed in a boiling water bath for 30 min. For control purposes normal urine or urine obtained when the dog had been given only 0·5 per cent tragacanth solution was used. The runs were carried out by the descending technique at room temperature with propanol-2N NH_4OH (2 + 1) as the solvent and the running time was 18–20 hr. After the run the papers were dried and sprayed with 1–5% NaOH in methanol. This developes a fluorescence. To produce fluorescence the papers were also sprayed with a solution containing 0·5 g of boric acid and 0·5 g of citric acid in 20 ml of methanol and heated at 100° for 10 min.[7] The fluorescent compounds were detected with a Mineralight (SL 2537).

Ultraviolet analysis

To obtain additional criteria we ran ultraviolet absorption spectra for cinchophen and its derivatives and compared them with the absorption spectra of the corresponding derivatives isolated from cinchophen urine. These were obtained by elution from paper chromatograms. The measurements were carried out in 0·1 N HCl and 0·1 N NaOH.

Quantitative studies

For the quantitative determination of cinchophen and hydroxycinchophens from the urine, a paperchromatographic run was first performed. The spots obtained were cut out, eluated and measured spectrophotometrically. Both cinchophen and its derivatives have several absorption maxima. The maximum at the longer wavelengths (340–390 mμ) are most suitable for quantitative measurements as the background absorption decreases with the increase in wavelength.

Cinchophen and 8-hydroxycinchophen have their absorption maxima in acid solution at 345 mμ, which lends itself to quantitative determination. 4′-Hydroxycinchophen was also measured at 370 mμ in an acid solution. The cinchophen and hydroxycinchophen amounts corresponding to the reading obtained were taken from) the standard lines (1–10 μg/ml). In order to control the method, standards (5–50 μg) were run and the error of the method was established as about 10 per cent.

RESULTS AND DISCUSSION

Paper chromatography

The R_f-values and fluorescence properties of cinchophen and its derivatives are

given in Table 1. From these compounds only cinchophen is colourless on paper, the others give yellow spots on paper in daylight after staining with methanol-NaOH.

Cinchophen urine shows several intensely fluorescent spots which are not encountered in normal urine. Normal urine displays some spots emitting a blue or bluegreen fluorescence, which are usually fairly faint. Comparing these compounds

TABLE 1. THE R_f-VALUES AND FLUORESCENCE PROPERTIES OF CINCHOPHEN AND ITS DERIVATIVES

Compound	R_f value	Fluorescence produced by MeOH-NaOH	Fluorescence produced by Boric acid–citric acid
Cinchophen	0·89	Reddish (slight)	Blue (intense)
3-OH- Cinchophen	0·90	Bluegreen (intense)	Bluegreen
8-OCH_3-Cinchophen	0·90	Blue (intense)	None
8-OH- Cinchophen	0·85	Reddish (silght)	None
7-OH- Cinchophen	0·75	Yellow (intense)	Greenish (intense)
6-OH- Cinchophen	0·80	Yellow (intense)	Greenish (intense)
4′-OH Cinchophen	0·80	Yellow (intense)	Greenish (intense)

with paper chromatograms obtained from cinchophen derivatives, we identified besides cinchophen also the following metabolites: 8-hydroxycinchophen and a substance, which gave an intense yellow fluorescence and can be 6- or 4′-hydroxycinchophen. 3-hydroxycinchophen has not been encountered as a detoxication product of cinchophen. In addition to these compounds cinchophen urine contains other fluorescent substances. We did not try to identify these compounds.

Only cinchophen and 4′- (or 6-)hydroxycinchophen were visualized in the paper chromatograms before acid hydrolysis of cinchophen urine. 8-hydroxycinchophen appeared after hydrolysis.

3-Hydroxycinchophen urine samples were analysed by paper chromatography, too. Besides 3-hydroxycinchophen, a compound was encountered, which emitted intense yellow fluorescence and moved in the same spot as 3-hydroxycinchophen. In addition, some smaller fluorescent fractions were also observed. It is possible, that 3-hydroxycinchophen becomes detoxicated in the same manner as cinchophen and the metabolites detected may be dihydroxycinchophens. We made no more attempts to elucidate the detoxication of 3-hydroxycinchophen.

Ultraviolet analysis

The absorption maxima of cinchophen, its synthetic derivatives and derivatives isolated from urine are given in Table 2. The u.v.-spectra for cinchophen and 8-hydroxycinchophen isolated from urine were identical with the standards. The u.v.-spectra of the "yellow" metabolite was identical with the spectra of 4′-hydroxycinchophen.

Quantitative studies

The urinary excretion of cinchophen, 4′- and 8-hydroxycinchophen during cinchophen feeding (for 6 days) is presented in Tables 3 and 4.

The 8-hydroxycinchophen which appears after hydrolysis moves partly together with cinchophen in the same spot. We therefore determined cinchophen and 8-hydroxy

cinchophen together and calculated the result both as cinchophen and as 8-hydroxy-cinchophen because the molar absorption of 8-hydroxycinchophen is smaller than that of cinchophen at 345 mμ. Only a cinchophen spot was visible before hydrolysis.

Before hydrolysis cinchophen accounted for about 1·5 per cent of the amount

TABLE 2. THE ABSORPTION MAXIMA OF CINCHOPHEN AND ITS DERIVATIVES IN 0·1 N HCl

Synthetic compounds	λ max (mμ)
Cinchophen	242, 268, 345
8-OH- Cinchophen	287, 345
7-OH- Cinchophen	258, 375
6-OH- Cinchophen	280, 375
4′-OH- Cinchophen	245, 370
8-OCH_3 Cinchophen	287, 348
Compounds isolated from urine	λ max (mμ)
Cinchophen	242, 268, 345
8-OH- Cinchophen	287, 345
4′-OH-Cinchophen (= yellow metabolite)	245, 370

TABLE 3. URINARY EXCRETION OF 4-HYDROXY-CINCHOPHEN IN DOG AFTER FEEDING OF 0·2 G OF CINCHOPHEN/KG OF BODY WEIGHT.

Day	Before hydrolysis (mg/day)	After hydrolysis (mg/day)
1	7	18
2	8	9
3	14	48
4	24	52
5	17	27
6	16	32
7	10	18
8	3	5

The drug was given on the first 6 days.

TABLE 4. URINARY EXCRETION OF CINCHOPHEN AND 8-HYDROXYCINCHOPHEN IN DOG AFTER CINCHOPHEN FEEDING.

Day	Before hydrolysis (cinchophen) (mg/day)	After hydrolysis (cinchophen + 8-hydroxy-cinchophen) (mg/day)
1	1	57–74
2	0	52–69
3	45	156–208
4	50	206–275
5	38	82–109
6	32	103–137
7	11	56–75
8	0	14–19

The drug was given on the first 6 days, dose 200 mg/kg.

administered. After hydrolysis, cinchophen and 8-hydroxycinchophen was 6–10 per cent of the amount administered.

The total of cinchophen and hydroxycinchophens excreted was no more than 8–12 per cent of the total cinchophen dose, 12 g. About 3 per cent (cinchophen and 4′-hydroxycinchophen) was free. It seems probable that part of cinchophen is metabolized by other ways in dogs. The splitting of the quinoline ring of cinchophen is a possibility that can be considered.

The influence of hydroxycinchophens on ulcerogenesis

The feeding of 7- and 8-hydroxycinchophen showed that they did not cause ulcers in dogs examined. Attempts to make large amounts of 4′-hydroxycinchophen were not successful. Therefore the role of 4′-hydroxycinchophen in ulcerogenesis remains obscure.

3-Hydroxycinchophen is more toxic than cinchophen and other hydroxycinchophens examined and therefore the daily dose of 3-hydroxycinchophen was only a half of that of the others. The dogs, which were fed with 3-hydroxycinchophen, died after 7 days and both had several gastric ulcers.

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