GLC determination of betahistine in serum

J. F. Douglas[1] and T. L. Hohing[2]

Hermitage Farm, Front Royal (Virginia 22630, USA), 23 May 1977

Summary. A gas chromatographic method was developed for the determination of betahistine in serum in the range of 0.6–6.0 µg/ml.

Betahistine [2-(β-methylamino ethyl)-pyridine] is a known pharmacological agent that acts as a diamine oxidase inhibitor and has properties similar to histamine (H-receptor effects) when given orally. It has been used in the treatment of different pathological conditions (e.g. Menière's syndrome). Although there are a few methods mentioned in the literature for analysis of betahistine in biological fluids, none have the sensitivity or specificity for levels that would be encountered under therapeutic conditions[3]. This report describes a gas chromatographic method for the determination of betahistine that is simple, rapid, and reliable.

Experimental. GLC: A dual column gas chromatograph equipped with a hydrogen flame ionization detector and a 1-MV recorder (Packard model 804) was employed. The chromatographic columns used were 90 cm × 2 mm glass tubes packed with 28% Pennwalt 223 plus 4% KOH on 80/100 mesh Gas Chrom R. The instrument settings were: column temperature, 155 °C; injection port temperature, 225 °C; and detector block temperature, 245 °C. Gas flow rates were: hydrogen, 30 ml/min; nitrogen (carrier gas), 55 ml/min; and air, 300 ml/min. The sensitivity setting was 1×10^{-11} A. The retention times under these conditions were 8.5 min for betahistine, 7.0 min for its demethylated metabolite, and 21.0 min for 3-chloro-4-methylaniline (figure 1).

Reagents: The reagents were AR grade chloroform and concentrated ammonia.

Procedure: Fresh unhemolyzed serum, 2.0 ml, was made alkaline with 2 drops of concentrated ammonium hydroxide and mixed for 1 min in a mechanical shaker. To the sample was added 0.3 ml chloroform containing 3-chloro-4-methylaniline, and the mixer was shaken for 10 min followed by centrifugation for 10 min. The aqueous layer was removed by aspiration and 2.0 µl of the chloroform solution was injected into the gas chromatograph. If not assayed immediately, the organic solvent was kept at refrigerator temperature to prevent evaporation. The concentration of betahistine was determined by the relative peak height method using 3-chloro-4-methylaniline as the internal standard.

Results and discussion. The relationship between relative peak height and betahistine concentration in the range of 0.6–6.0 µg/ml of serum is shown in figure 2. The reproduci-

Recovery of betahistine from dog serum

Betahistine added (µg/ml)	Betahistine found (µg/ml)	Recovery* (%)	Mean±SD
0.61	0.24	39.3	
	0.29	47.5	
	0.29	47.5	
	0.24	39.3	
			43.4±4.8
1.22	0.62	50.8	
	0.57	46.7	
	0.57	46.7	
	0.57	46.7	
			47.7±2.1
3.06	1.33	43.5	
	1.33	43.5	
	1.38	45.1	
	1.33	43.5	
			43.9±1.4
6.13	2.75	44.9	
	2.66	43.4	
	2.85	46.5	
	2.75	44.9	
			44.9±2.4
		Overall mean±SD	45.2±2.4

*Recoveries are based on a comparison to GC response of pure solutions of betahistine.

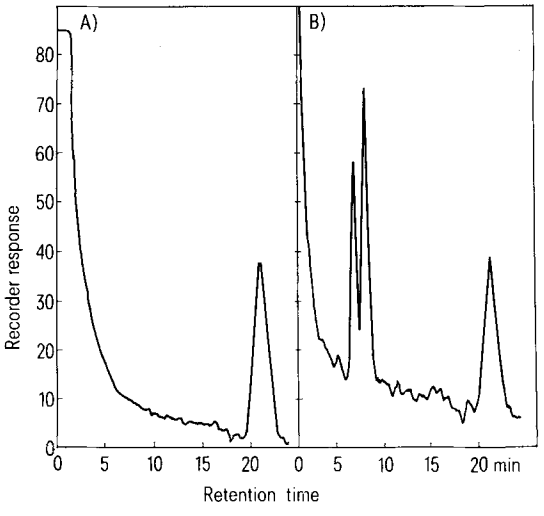

Fig. 1. Gas chromatograms of *A* normal serum with internal standard 3-chloro-4-methylaniline and *B* normal serum with betahistine, its demethylated metabolite, 2-(2-aminoethyl) pyridine, and 3-chloro-4-methylaniline added.

Fig. 2. Relationship between relative peak height and betahistine concentration in dog serum.

bility of the procedure, as indicated by the standard error of quadruplicate determinations, is also shown. The extraction technique effectively separates betahistine from normally interfering serum constituents since determinations with normal serum give little or no response. The major metabolite of betahistine, 2-(2 amino ethyl)pyridine[4,5], which has similar pharmacologic activity[3,6], was also detected by this technique. About 45% of the serum betahistine is reproducibly extracted regardless of drug concentration (table) over a range of 0.6–6.0 µg/ml.

1 Present address: National Cancer Institute, Bethesda, Maryland 20014, USA.
2 Litton Bionetics Inc., 5516 Nicholson Lane, Kensington, Maryland 20795, USA.
3 C. H. Holmen, Proc. 31st FIP Congress, Washington 1971.
4 H. Konzett, R. G. Bost, F. J. Bowman, E. R. Bowman and H. McKennis, Jr, J. Pharmac. exp. Ther. *178*, 122 (1971).
5 F. J. Bowman, E. R. Bowman and H. McKennis, Jr, Virg. J. Sci. *21*, 143 (1970).
6 W. H. Hunt and R. J. Fosbinder, J. Pharmac. exp. Ther. *75*, 299 (1942).

Mammalian spot test with moxnidazole, a 5-nitroimidazole[1]

R. Lang

Research Laboratories, Schering AG, D-1000 Berlin 65, West, 9 September 1977

Summary. Moxnidazole [3-(5-nitro-1-methyl-2-imidazolyl)-methyleneamino-5-morpholinomethyl-2-oxazolidinone, HCl], known to be mutagenic in microbial tests and *Drosophila,* induced genetic alterations in somatic cells of mice. Ethyl methanesulfonate (EMS) served as positive control.

The mammalian spot test, an in vivo method for the detection of genetic alterations - especially point mutations - in somatic cells of mice[2], has been shown to be sensitive to mutagens with different modes of action[2–6]. According to this method, embryos which are heterozygous to 4 different recessive coat-color genes are treated in utero during the 7th to the 10th day of fetal development. If the treatment leads to an alteration or to a loss of the wild type allele of one of the genes under study in a pigment precursor cell, a colour spot in the adult coat will result.

A number of 5-nitroimidazoles have mutagenic properties[7–12], and additionally metronidazole was shown to be carcinogenic in mice[13]. While moxnidazole was found to be mutagenic for *Salmonella typhimurium* TA 1538, *Escherichia coli* WP_2, WP_2 uvrA$^-$ (unpublished results), 343/113[14], *Neurospora crassa* heterokaryon H 12[14] and *Drosophila* (inducing recessive sex-linked lethals)[15], it was negative in the dominant lethal assay in male mice (unpublished results). The purpose of the present experiment was to investigate whether moxnidazole can induce point mutations in mammals in vivo.

Materials and methods. Embryos of the genotype *a/a; b/+; $c^{ch}p$/++; d se/++; s/+* (black coat, dark eyes) were produced by mating 9-week-old virgin females of the inbred C57BL/6J/BOM-spf strain (*a/a,* otherwise wild type) to fertile males of the rotation bred T-stock (*a/a* = nonagouti; *b/b* = brown; *$c^{ch}p/c^{ch}p$* = chinchilla and pink-eyed dilution; *d se/d se* = dilute and short ear; *s/s* = piebald spotting). The randomly selected pregnant females were given 1000 mg moxnidazole/kg b. wt (Schering AG, Berlin/Bergkamen) 3 times as a microcristalline suspension in physiological saline by gavage 8, 9 and 10 days after observation of the vaginal plug (day 1). Control females were given physiological saline at 10 ml/kg in the same manner. EMS (Ferak, Berlin, batch 7306) (100 mg/kg) dissolved in physiological saline served as positive control and was injected at the same days i.p. Litters were checked for colour spots once a week between 2 and 5 weeks of age. Examination of spots was performed with the naked eye. Differentiation between midventral white, white-gray and light-gray spots was possible by fluorescence-microscopy[2]. According to Fahrig[2], white and white-gray spots result from pigment cell death rather than from genetic alterations, and therefore only non-white spots were considered to be of genetic origin.

Results and discussion. The frequency of colour spots in negative and positive (EMS) control animals, as well as in moxnidazole treated mice, is summarized in the table. Compared to the negative control, EMS and moxnidazole induced a significant increase ($p < 0.01$) in brownish and grayish spots. Out of 235 offspring, 13 offspring (5.5%) with non-white spots were derived from 10 EMS treated females. In comparison, out of a total number of 255 offspring, non-white spots occurred in 9 (3.5%) derived from 8 females given moxnidazole, whereas none of the 275 offspring of the control animals showed similar spots. In the moxnidazole group, 1 animal showed 2 clearly separated spots. In addition to the non-white spots, 4 of the

The effect of moxnidazole and EMS in the mammalian spot test

Treatment	Dosage (mg/kg) (route and number of doses)	Females Treated	With litters surviving to observation	Offspring Surviving to observation (average litter size)	With mid-ventral white spots	With white-gray spots	With spots of brownish or grayish color
Negative control	0 (p.o. 3)	65	42	275 (6.6)	1	0	0
Moxnidazole	1,000 (p.o. 3)	70	38	255 (6.7)	4	0	9*
Positive control (EMS)	100 (i.p. 3)	67	38	235 (6.2)	17	1	13*

*$p < 0.01$ vs control. Fisher's exact test (one-tailed).