In Vitro Antibacterial Activity of Drugs against Human Intestinal Anaerobic Bacteria

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Abstract □ The *in vitro* antibacterial activity, against the major groups of anaerobic fecal bacteria, of a series of drugs was examined by an agar diffusion test and the minimum inhibitory concentration (MIC) test. Only the phenothiazines and amitriptyline showed any marked antibacterial activity, the MIC's being in the 40–640-μg/ml range. It is suggested that the detergent nature of the molecules of these drugs in aqueous solution is responsible for the observed antibacterial activity.

Keyphrases □ Antibacterial activity—commonly used drugs tested against human intestinal anaerobic bacteria *in vitro* □ Drugs—commonly used drugs tested for antibacterial activity against human intestinal anaerobic bacteria *in vitro* □ Phenothiazines—antibacterial activity against human intestinal anaerobic bacteria determined *in vitro*

Many drugs introduced into the human body are excreted *via* the feces, either as the parent molecule or conjugated with glycine or glucuronic acid (1). Porter and Beresford (2) showed that chlorpromazine was excreted in the urine, while Walkenstein and Seifter (3) reported the presence of phenothiazine compounds in the feces of dogs given the drugs orally. In both cases the drug was present as the free base and as metabolites, *e.g.*, chlorpromazine sulfoxide. Porter and Beresford (2) also claimed that only 4% of an oral dose of chlorpromazine (100 mg) was excreted in the urine within 7 days. Therefore, the possibility exists that the intestinal flora will come into contact with either the drugs or their metabolites, since it has also been shown that intestinal and fecal bacteria can deconjugate glycine and glucuronic acid conjugates to release the free drug. Substances were identified in human feces that had the spectrophotometric and polarographic characteristics of chlorpromazine and chlorpromazine sulfoxide 24 hr after an oral dose of chlorpromazine (100 mg) (4, 5). These compounds were not isolated and identified, but a concomitant decrease from 6×10^{10} to 2×10^{10} viable anaerobes/g of wet feces was noted and some GI distress occurred.

EXPERIMENTAL

Anaerobic bacteria were isolated from fresh human feces using a rumen fluid roll tube (medium) (6) and the method described by Brown (5). The isolates were broadly classified into three groups (5):

Group I—Anaerobic, Gram-negative asporogenous rods exhibiting very pleomorphic forms. The organisms in this group belong to the genera *Bacteroides, Sphaerophorus, Ristella, Capsularis, Fusobacterium,* and *Eggerthella.*

Group II—Anaerobic, Gram-positive, nonmotile, asporogenous rods. These organisms belong to the genera *Bifidobacterium, Catenebacterium, Eubacterium, Propionibacterium,* and *Actinomyces* (7).

Group III—Anaerobic, Gram-positive cocci of the genera *Peptococcus* and *Peptostreptococcus* (7).

These groups included all anaerobic bacteria occurring in normal human feces at the level of 10^9–10^{10}/g of wet feces. A preliminary test for drug sensitivity was performed on the isolates, using an antibiotic disk sensitivity test method modified for the testing of obligate anaerobes (5). The isolates were streaked onto rumen fluid glucose agar slants and incubated at 37° for 48 hr to ensure that there were no contaminants. Growth was washed from the slant with dilution solution (6), and the absorbance was adjusted to 0.2 (420 nm). This suspension (0.2 ml) was used to inoculate molten agar (50 ml) in a 120-ml (4-oz.) medical flat bottle which, after mixing, was allowed to set on one large face of the bottle. Filter paper disks containing the drug (50 μg) were placed on the agar surface and left for 1 hr at room temperature to allow the drug to diffuse into the agar.

The bottles were incubated at 37° for 48 hr before the zones of inhibition were measured. To ensure that the noninhibitory drugs in the disk test were actually desorbing from the disk and diffusing through the agar, the following test was performed. Disks of the drug were placed on uncontaminated agar and the drug was allowed to diffuse into the agar for 1 hr at room temperature. The disk was removed and the residual drug on the disk was assayed polarographically. The drugs were also detected in small pieces of agar cut from the plate at different distances from the disk. The minimum inhibitory concentration (MIC) was investigated using

Table I—Inhibition of Anaerobic Fecal Bacteria by Commonly Used Drugs

		Drug[a] and Zone[b] of Inhibition										
Culture Group	Number of Isolates Tested	Chlorpromazine	Promazine	Trifluoperazine	Fluphenazine	Promethazine	Amitriptyline	Chlordiazepoxide	Diazepam	Phenobarbital Sodium	Metformin	Tolbutamide
I	40	+++	+	++	+	++	+++	−	−	−	−	−
II	10	−	−	−	−	−	−	−	−	−	−	−
III	8	−	−	−	−	−	−	−	−	−	−	−

[a] Drug concentration = 50 μg. disk. [b] Zone diameters: − = no zone, + = 10 mm, ++ = 10–20 mm, and +++ = 20–30 mm.

Table II Drugs[a] Tested for Antibacterial Activity[b]

Culture Group	Number of Isolates Tested	Chlorpromazine				Promazine				Trifluoperazine				Fluphenazine				Promethazine				Amitriptyline			
		a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
I	40	18	22	—	—	—	20	20	—	30	10	—	—	—	20	20	—	—	30	10	—	20	20	—	—
II	10	—	4	6	—	—	7	3	—	—	3	7	—	—	10	—	—	—	7	3	—	—	8	2	—
III	8	—	2	6	—	—	8	—	—	—	—	8	—	—	—	8	—	—	—	8	—	—	—	8	—

Culture Group	Number of Isolates Tested	Chlordiazepoxide				Diazepam				Phenobarbital Sodium				Metformin				Tolbutamide			
		a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
I	40	—	—	—	40	—	—	—	40	—	—	—	40	—	—	—	40	—	—	—	40
II	10	—	—	—	10	—	—	—	10	—	—	—	10	—	—	—	10	—	—	—	10
III	8	—	—	—	8	—	—	—	8	—	—	—	8	—	—	—	8	—	—	—	8

[a] Concentrations of drugs in micrograms per milliliter: a = 40–80, b = 80–160, c = 160–320, and d = 320–640. [b] Figures indicate the number of isolates with that MIC.

rumen fluid glucose broth. Those drugs not showing any inhibition in the disk test were also examined by this method to exclude further the possibility mentioned previously.

Inocula for the broth cultures were produced as in the disk test. Sterile solutions of the drugs were added to sterile broth to give concentrations of 10, 20, 40, 80, 160, 320, and 640 μg/ml. Concentrations in excess of 640 μg/ml were found to precipitate the broth constituents.

RESULTS AND DISCUSSION

Of the drugs examined, only the phenothiazine derivatives and amitriptyline had any marked antimicrobial activity in the sensitivity disk test. This activity was limited to the Group I bacteria (Table I). However, in the MIC determinations where higher concentrations of drugs were used, these compounds were again the only active ones, but Group II and III organisms were also inhibited (Table II). The antimicrobial activity of the phenothiazines has been known for some time; Raffel *et al.* (8) reported that these compounds inhibited *Mycobacterium tuberculosum*, and Agarwal and Ignatur (9) found that chlorpromazine had a lytic effect on *Escherichia coli* G cells.

Phenothiazine was originally used as an anthelmintic (10) but was discarded because of toxic side effects. It has been shown to cause GI disturbances, *e.g.*, nausea, vomiting, and diarrhea. Haden (11) reported that patients treated with chlorpromazine complained of GI pain and nausea after 2 days. All phenothiazines tested exhibited a marked antibacterial action against the Group I (*Bacteroides*) bacteria. Since these organisms constitute the majority of flora in the latter segment of the small intestine, large intestine, and rectum (10^{10}–5×10^{11}/g of wet feces), any drug inhibiting them will permit the outgrowth of other commensals and possibly pathogens, *e.g.*, yeasts, candida, proteus, and clostridia. This alteration in the floral balance could result in large amounts of gas being produced and/or changes in pH and redox potential (Eh) of the gut contents (4). Thus, the distress suffered by persons ingesting the phenothiazines could be due to changes in gut flora.

The MIC results (Table II) indicate that the phenothiazines were all inhibitory at a similar concentration, suggesting some nonspecific activity. Several reports (12, 13) showed that the phenothiazine compounds and amitriptyline were amphipathic molecules in aqueous solution. It is possible that the inhibitory activity of these compounds was due to a surface-active effect on the bacterial cell membrane. It is unlikely, however, that the inhibitory action was solely due to a surface phenomenon since both chlordiazepoxide and diazepam, which are known to have surface-active properties, showed no antibacterial activity *in vitro* against the organisms tested (Table II). Furthermore, there is little evidence to suggest that chlordiazepoxide and diazepam cause intestinal distress.

The disk test has been criticized because it was considered that some drugs would not desorb from the disk and diffuse in the agar. Results obtained by placing disks on uncontaminated agar showed that only small amounts of drug were left on the disk after 1 hr and the drug could also be detected in sections of agar away from disk. Moreover, there was extremely good correlation between the disk tests (Table I) and MIC results (Table II).

REFERENCES

(1) "The Pharmacological Basis of Therapeutics," 2nd ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1958, p. 10.

(2) G. S. Porter and J. Beresford, *J. Pharm. Pharmacol.*, **18**, 223(1966).

(3) S. S. Walkenstein and J. Seifter, *J. Pharmacol. Exp. Ther.*, **125**, 283(1959).

(4) A. R. Scheline, *J. Pharm. Sci.*, **57**, 2021(1968).

(5) M. W. Brown, M. Phil. thesis, London University, London, England, 1972.

(6) M. P. Bryant and I. M. Robinson, *J. Dairy Sci.*, **44**, 1446(1961).

(7) L. D. S. Smith and L. V. Holdman, "The Pathogenic Anaerobic Bacteria," Charles C Thomas, Springfield, Ill., 1968, p. 423.

(8) S. Raffel, I. Kochan, N. Poland, and L. E. Hollister, *Amer. Rev. Resp. Dis.*, **81**, 555(1960).

(9) R. P. Agarwal and A. Ignatur, *Brit. J. Pharmacol.*, **24**, 466(1965).
(10) W. Ernst, *Trop. Dis. Bull.*, **48**, 185(1951).
(11) P. Haden, *Can. Med. Ass. J.*, **91**, 974(1964).
(12) G. Zografi and D. E. Auslander, *J. Pharm. Sci.*, **54**, 1313(1965).
(13) L. L. M. Van Deenen and R. A. Demel, *Biochim. Biophys. Acta*, **94**, 314(1965).

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Excretion of the Active Principle of *Catha edulis* (Miraa) in Human Urine

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Abstract □ *d*-Norpseudoephedrine, a central stimulant present in *Catha edulis*, is excreted unchanged in human urine. The alkaloid was detected in urine 30–50 min after ingestion of synthetic *d*-norpseudoephedrine, and trace amounts of the drug could still be detected 24 hr later. Approximately 40% of ingested *d*-norpseudoephedrine was recovered in urine in the first 6 hr.

Keyphrases □ *Catha edulis*—urinary excretion of *d*-norpseudoephedrine after mastication, humans □ *d*-Norpseudoephedrine—urinary excretion after mastication of *Catha edulis*, humans □ CNS stimulants—urinary excretion of *d*-norpseudoephedrine after mastication of *Catha edulis*, humans

Catha edulis Forsk. (Celastraceae) is an evergreen shrub, which usually is about 3–7 m tall but can grow to a height of 18 m under favorable climatic and soil conditions. It is found in the Eastern part of Africa, from Ethiopia to South Africa, and in Arabia. In Kenya, the plant is cultivated on a commercial scale on the slopes of Mount Kenya in the Nyambeni Division of the Meru District. The shoots of this plant are masticated while fresh to combat mental fatigue, allay hunger, and generally induce euphoria (1). The earliest recorded direct reference to the use of *Catha* was in a 1237 (2) prescription for relief of depression. Alles *et al.* (3) showed that the central stimulant activity of *Catha* can be attributed wholly to its *d*-norpseudoephedrine content.

Information gathered from those who masticate *Catha* indicated that the effect on the central nervous system is biphasic, *i.e.* initial stimulation followed by a compensatory phase of depression (4). It was reported that it is necessary to chew the material continuously for sustained mental stimulation, an effect that could be interpreted to mean that the material is rapidly eliminated from the body. A literature survey has not revealed any study of the elimination of *d*-norpseudoephedrine from the human body and, accordingly, this aspect was investigated. Since most phenylalkylamine derivatives are eliminated through the renal route (5), the present study was restricted to investigation of the presence of *d*-norpseudoephedrine and metabolites in urine.

EXPERIMENTAL

Each of four human volunteers was requested to masticate three bundles of *Catha* material at a rate of about two bundles per hour and to submit samples of urine at predetermined intervals. The average weight of a fresh bundle of *Catha* material was 80.6 g, of which 70–80% was ingested; the rest, a fibrous residue, was discarded. The volunteers had only drunk milk in the morning and were requested to give samples of urine just before the experiment and thereafter at the following intervals: 0.5–1, 1–2, 2–4, 6–8, 8–12, 12–15, and 15–24 hr. No food or beverage was consumed in the first 6 hr of the experiment, but the volunteers were encouraged to drink water freely so as to promote frequent voiding of urine.

Qualitative examination of urine for *d*-norpseudoephedrine and metabolites was carried out as follows. To each urine sample (usually 20–50 ml) was added 1–2 ml of saturated lead acetate solution and, after thorough mixing, centrifugation was performed to remove any precipitate. The urine was acidified with 0.1 *N* sulfuric acid, and any precipitate of lead sulfate was removed by centrifugation. The acidic supernatant liquid was extracted with an equal volume of ether three times to remove organic acids and neutrals.

The aqueous phase (urine) was made alkaline with 2 *N* sodium hydroxide and then saturated with sodium chloride and extracted with an equal volume of ether for 8 hr. Preliminary work had shown that the "salting-out" process with sodium chloride improves the percentage recovery of *d*-norpseudoephedrine from urine considerably. This extraction was repeated three times, and the combined ether extract was washed twice with 2 ml of 2.5% sodium bicarbonate solution and dried with anhydrous sodium sulfate. The ether was then distilled off, and the basic residue was examined by TLC and GLC techniques.

This experiment was repeated using synthetic *d*-norpseudoephedrine. Approximately 30 mg was accurately weighed, dissolved in a small volume of water, and given to each of four human volunteers with urine samples collected as described previously.

Examination of residues for *d*-norpseudoephedrine and metabolites using TLC was carried out as follows. Residues were taken up in 2 ml of ether, and approximately 2–5 μl was spotted on a TLC plate coated with silica gel. Approximately 5 μl of the following reference compounds was also spotted on the same plate as the residue: *l*-ephedrine, *d*-pseudoephedrine, and *d*-norpseudoephedrine. Plates were developed in one of the following solvent systems: A, butanol–acetic acid–water (60:15:25); B, butanol saturated with water (upper phase); C, methanol–ammonia (100:1.5); or D, isopropanol–water–ammonia (80:15:5). The spots were revealed by ex-