Action of Antibiotics on Respiratory Tract II: Dicloxacillin

G. BENZI*, F. BERTE, E. BERMUDEZ, and E. ARRIGONI

Abstract □ Dicloxacillin antagonizes *in vitro* the acetylcholine-, histamine-, and barium chloride-induced spasms on the dog bronchial chain. Against the histamine or acetylcholine contraction, dicloxacillin is about 2–3 times less active than ephedrine, about 2000–4000 times less active than isoprenaline, and about 5–6 times more active than ampicillin. *In situ*, the intravenous injection of dicloxacillin induces a decrease of the respiratory frequency with a moderate increase in ventilation.

Keyphrases □ Dicloxacillin—respiratory tract, effects □ Antibiotics, dicloxacillin—effect, respiratory tract □ Bronchospasm—comparison antibiotics, effects

Studies on the ampicillin action on the respiratory tract showed that *in vitro* the antibiotic antagonizes the barium chloride-, histamine-, and acetylcholine-induced spasms on the dog bronchial chains. *In situ*, the antibiotic induces at first a prompt but short increase in ventilation; subsequently, a lesser but persistent activity remains for 2 or 3 hr. (1). The plan of the present research was to study the dicloxacillin action on the airways *in vitro* and *in vivo*. The isoxazolyl group of semisynthetic penicillins has been useful in the therapy of infections caused by penicillase-producing staphylococci (2–7) and, collaterally, possesses a myolytic action on the rabbit-isolated duodenum, jejunum, and ileum; on the guinea-pig-isolated bronchial muscle; and on the rat-isolated intestine (8). Furthermore, dicloxacillin induces a relaxation *in vitro* and *in vivo* of both the terminal bile duct and ureter, normal or hypertonized by various spasmogenic agents (9, 10). The intrabiliary perfusion with dicloxacillin inhibits *in situ* the stimulation of the peripheral end of the right supradiaphragmatic vagus (9), while the perfusion into the ureter with the antibiotic induces no effect on the electrical stimulation of the hypogastric and pelvic nerves (10).

METHODS

The experiments were performed in the dog both *in vitro* and *in situ*. The following were used: dicloxacillin sodium monohydrate, of which amounts are expressed in terms of 3-(2,6-dichlorophenyl)-5-methyl-4-isoxazolyl-penicillanic acid; ampicillin sodium salt, of which amounts are expressed in terms of D(−)-6-(α-amino-α-phenylacetamido)-penicillanic acid; *dl*-isoprenaline hydrochloride; and *l*-ephedrine hydrochloride, the amounts of which are expressed in terms of the base.

Experiments *In Vitro*—Bronchi of 38 mongrel dogs of either sex (weighing 6.6–11.4 kg.) were removed immediately after death. All tissues were carefully dissected and cut into rings, which were tied together in chains with loops of thread. The mucosa was removed to allow for greater freedom of movement of the muscle; the cartilage in each ring was cut so that only the smooth muscle bands were left joining each ring together (11). Four to six rings were suspended in a 50-ml. organ bath containing Tyrode solution gassed with 95% oxygen and 5% carbon dioxide; the temperature was 36.5–37.3°. The tonus level of the preparations was continuously recorded by a strain-gauge lever, giving a magnification of ×15 to 20, tension 200 mg., writing on a kymograph drum. The preparations were left 2 hr. before any drugs were given. Three submaximal doses of acetylcholine were administered, until regular responses were obtained, before any doses of acetylcholine itself or other agonists were tested. The action of dicloxacillin (25–400 mcg./ml.) was evaluated against the stimulation by barium chloride (100–400 mcg./ml.); for the construction of the dose–response curve, dicloxacillin activity was taken as the percent inhibition of the recorded response area by barium chloride stimulation during a 20-min. period of contact.

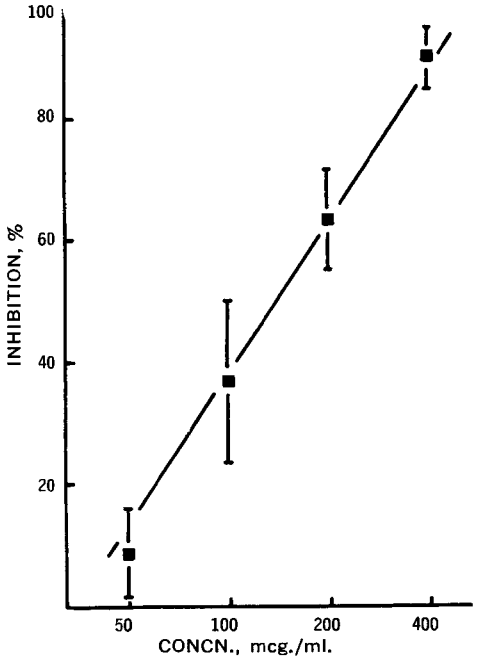

Figure 1—*Log dose–response curve of dicloxacillin action against the stimulation by barium chloride. The ordinate shows the inhibition percent of the contracting action by barium. The concentrations of the antibiotic (mcg./ml.) are plotted on the abscissa in logarithmic scale. The vertical lines indicate standard errors of the means. Six preparations at each dose level.*

For comparative assay with other *in vitro* bronchodilators, the activity of dicloxacillin (200–3200 mcg./ml.), ampicillin (2000–16,000 mcg./ml.), *l*-ephedrine (200–1600 mcg./ml.), and *dl*-isoprenaline (0.125–2.0 mcg./ml.) was evaluated against the stimulation by acetylcholine chloride (0.2–2.0 mcg./ml.) and histamine acid phosphate (1.0–10.0 mcg./ml.). Two dose levels of agonist and antagonist were usually used; the agonist doses were left in contact with the preparation for 0.5–1.0 min. Dicloxacillin and ampicillin were introduced into the bath 10 min., and ephedrine and isoprenaline 30 sec., before the acetylcholine or histamine doses. The ED_{50}, the slope of the curve, the potency ratio, and their 95% confidence limits were calculated (12).

Experiments *In Situ*—The experiments were carried out on 28 mongrel dogs of either sex (weighing 12.4–16.8 kg.) preanesthetized with urethan (0.4 g./kg. i.p.). Anesthesia was induced and maintained by chloralose (80 mg./kg. i.v.); the arterial blood pressure was measured from a cannula inserted into a femoral artery; the intestinal movement and tone were recorded by a rubber balloon inserted into the jejunum. During the succinylcholine chloride (1 mg./kg. i.v.) action, an intratracheal Warne tube was set in place. Through this tube, a little rubber balloon was pushed into a primary or, if possible, secondary bronchus under X-ray examination. Arterial blood pressure and intestinal and respiratory activity were recorded by a Physioscript EE12 Schwarzer polygraph.

Table I—Dog Bronchial Chain *In Vitro:* ED_{50}, Slope Function of Line (S) and Estimated Relative Potency (ERP), with 95% Confidence Limits, of *dl*-Isoprenaline, *l*-Ephedrine, Ampicillin, and Dicloxacillin against Stimulation by Acetylcholine Chloride (0.2–2 mcg./ml.) and Histamine Acid Phosphate (1.0–10.0 mcg./ml.)

Antagonist	Agonist		
	ED_{50}[a]	S[b]	ERP[c]
	Acetylcholine		
Isoprenaline	0.38 (0.20–0.72)	2.51 (1.04–6.02)	26,315 (9500–72,892) D
Ephedrine	520 (247–1902)	2.81 (0.97–8.16)	19 (6.44–56.05) D
Ampicillin	10,000 (4545–22,000)	2.49 (0.69–8.96)	1
Dicloxacillin	1500 (600–3750)	3.58 (0.79–10.13)	6.6 (1.79–20.10) D
	Histamine		
Isoprenaline	0.56 (0.30–1.03)	2.01 (1.12–3.63)	8214 (3042–22,178) D
Ephedrine	450 (214–945)	2.83 (0.97–8.20)	10 (3.4–29.5) D
Ampicillin	4600 (2090–10,120)	2.53 (0.70–9.10)	1
Dicloxacillin	1000 (416–2400)	3.47 (0.77–15.61)	4.6 (1.41–14.95) D

[a] ED_{50} = dose (mcg./ml.) of bronchodilator necessary to reduce by 50% the contraction produced by acetylcholine or histamine. [b] S = fold change in dose required to produce a unit standard deviation change in response along the line; thus S = antilog s = antilog $1/b$, where b and s are, respectively, the slope constant and standard deviation of a line relating log dose of antagonist and probit percent reduction of agonist activity. [c] D = ampicillin and compared drugs differ significantly in potency ($p < 0.05$).

Dicloxacillin (0.25–8.0 mg./kg.) was administered intravenously by a polystan tube inserted into the femoral vein. Two submaximal doses of *dl*-isoprenaline or *l*-adrenaline were given intravenously until regular responses were obtained before any doses of dicloxacillin were tested. To analyze the mechanism of action, dicloxacillin was tested also after: (*a*) cutting the vagi, and (*b*) treatment with atropine sulfate (2–3 mg./kg. s.c.), dibenamine hydrochloride (3–6 mg./kg. i.v.), D(−)INPEA[1] (4–8 mg./kg. i.v.), methysergide maleate (20–40 mcg./kg. s.c. + 10 mcg./kg. i.v.), chlorpheniramine maleate (2–4 mg./kg. s.c.), cyproheptadine hydrochloride (200–400 mcg./kg. i.v.), hexamethonium bromide (200–400 mcg./kg. i.v.), or morphine hydrochloride (4–8 mg./kg. i.v.).

RESULTS AND DISCUSSION

Dicloxacillin (25–400 mcg./ml.) reduces or inhibits the stimulation by barium chloride on the dog bronchial chains *in vitro;* the log dose–response curve is indicated in Fig. 1.

The comparative assay of dicloxacillin, ampicillin, isoprenaline, and ephedrine action against the stimulation by acetylcholine or histamine is indicated in Table I and shows that dicloxacillin is more active than ampicillin in reducing the agonist-induced spasm. It is possible to observe that against histamine- or acetylcholine-induced contraction, dicloxacillin is about 2–3 times less active than ephedrine, about 2000–4000 times less active than isoprenaline, and about 5–6 times more active than ampicillin.

Dicloxacillin, as ampicillin (1) and aminophylline (11), is more effective in preventing a histamine than an acetylcholine bronchospasm. Obviously, it is impossible to correlate directly the present results with clinical conditions, the *in vitro* studies being limited to denervated portions of the airways while many bronchoconstrictive stimuli act, at least in part, *via* reflex mechanisms.

[1] D(−)-1-(4-Nitrophenyl)-2-isopropylaminoethanol HCl.

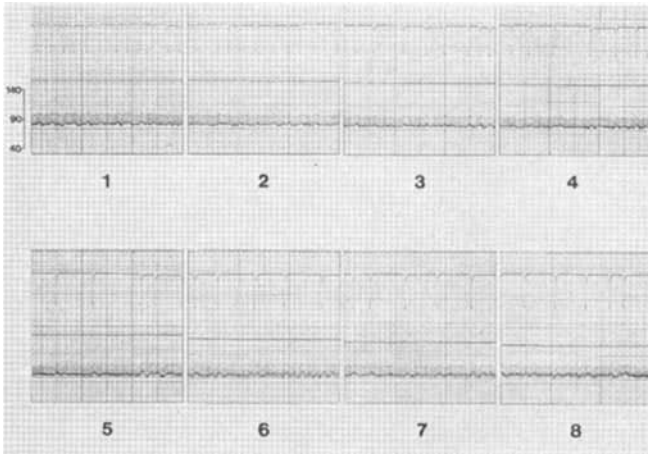

Figure 2—*Action of dicloxacillin intravenously on the ventilation of a dog* in situ. *From top to bottom: tracing recorded by a balloon inserted into the bronchus; blood pressure (mm. Hg). 1 = control condition; 2 = 5 min. after injection intravenously of 2 mg./kg. of dicloxacillin; 3 = 15 min. later; 4 = 30 min. later; 5 = 60 min. later; 6 = 75 min. later; 7 = 90 min. later; and 8 = 120 min. later.*

In situ, the intravenous injection of 0.25–8 mg./kg. of dicloxacillin induces a decrease of the respiratory frequency with a moderate increase in ventilation, as illustrated in Fig. 2. No change in systemic blood pressure occurs during the 2–3 hr. of the antibiotic action on the airways. The comparative evaluation of the action of dicloxacillin and ampicillin upon the dog ventilation is summarized in Fig. 3; ampicillin acts mainly on the depth of respiration, while dicloxacillin acts on the rate.

To analyze the mechanism of action of dicloxacillin *in situ*, it should be noted that: (*a*) the pressure recorded by the bronchial balloon technique reflects merely the effects of lung volume changes

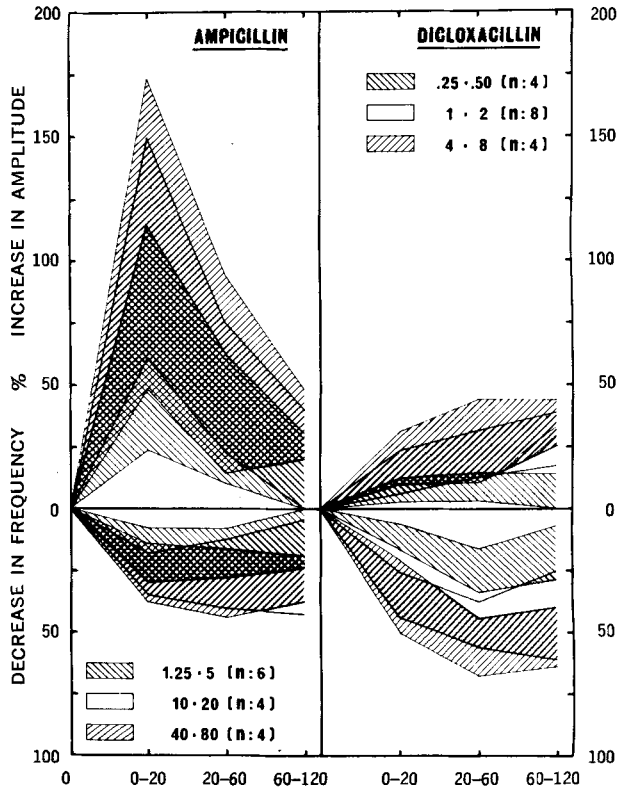

Figure 3—*Dog ventilation* in situ: *range of the percent changes (on ordinate) both in amplitude of the recorded tracing and in frequency of the respiratory rate at various periods of observation (on abscissa, in min.) after the intravenous injection of dicloxacillin (0.25–8 mg./kg.) and ampicillin (1.25–80 mg./kg.).*

on the intrathoracic airways, primarily reflecting changes in tidal volume; (*b*) the pretreatment with morphine antagonizes the action of the antibiotic; (*c*) the dicloxacillin activity is unaffected by the pretreatment with atropine, dibenamine, INPEA, methysergide, chlorpheniramine, cyproheptadine, and hexamethonium; and (*d*) the action of the antibiotic persists after cutting of the vagi.

Consequently, only *in vitro* is it possible to note the myolytic activity of the antibiotic on the bronchial musculature, while *in vivo* it is more likely that dicloxacillin, as ampicillin, induces at least in part an action on the medullary respiratory center.

REFERENCES

(1) G. Benzi, E. Bermudez, and E. Arrigoni, *J. Pharm. Sci.*, **59,** 556(1970).

(2) E. T. Knudsen, D. M. Brown, and G. N. Rolinson, *Lancet*, **2,** 632(1962).

(3) S. Sidell, R. E. Burdick, J. Brodie, R. J. Bulger, and W. M. M. Kirby, *Arch. Intern. Med.*, **112,** 21(1963).

(4) C. F. Gravenkemper, J. V. Bennett, J. L. Brodie, and W. M. M. Kirby, *ibid.*, **116,** 340(1965).

(5) C. M. Kunin, *Clin. Pharmacol. Ther.*, **7,** 166(1966)

(6) C. J. O'Connell and M. B. Lewis, *Amer. J. Clin. Pathol.*, **46,** 55(1966).

(7) G. J. Miraglia and H. I. Basch, *Appl. Microbiol.*, **15,** 566 (1967).

(8) A. Matsuzaki, *Jap. J. Antibiot.*, **21,** 274(1968).

(9) G. Benzi and E. Arrigoni, to be published.

(10) G. Benzi and E. Arrigoni, *Arch. Int. Pharmacodyn.*, **184,** 68 (1970).

(11) M. D. Mc Dougal and G. B. West, *Brit. J. Pharmacol.*, **8,** 26(1953).

(12) J. T. Litchfield, Jr., and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96,** 99(1949).

ACKNOWLEDGMENTS AND ADDRESSES

Received January 5, 1970, from the *Department of Medical Pharmacology, University of Pavia, Pavia, Italy.*

Accepted for publication February 16, 1970.

The authors thank Mr. A. Grandini and Miss C. Provilli for technical assistance.

* To whom correspondence should be addressed.

COMMUNICATIONS

Spectrographic Determination of Traces of Cobalt in Vitamin B_{12} Preparations

Keyphrases □ Cobalt determination—vitamin B_{12} dosage forms □ Emission spectrograph, direct—analysis

Sir:

The assay methods available for determination of vitamin B_{12} fall into four groups: biological, microbiological, chemical, and radioactive (1–3). Most of these methods are time consuming or difficult to perform, and often large samples must be taken for analysis.

A quick and simple analytical assay was needed for evaluation of active ingredients in pharmaceutical preparations containing vitamin B_{12} or its analogs, using only a few centigrams of the test substance.

We have developed a direct emission spectrographic method for rapid analysis of vitamin B_{12} in biological materials, where the amount of nonvitamin B_{12} cobalt is negligible. The method is based upon the cobalt content of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$, 4.35% Co in dry form). Mineralization, fusion, or ashing of organic substances is superfluous in this determination; separation or extraction of traces of Co is similarly unnecessary.

One milliliter of a liquor containing 25–125 mcg. vitamin B_{12}/ml. (corresponding to 1.1–5.4 mcg. Co/ml.) is mixed with 0.1 g. spectroscopically pure graphite powder and evaporated to dryness at 105°. Or, approximately 0.05 g. powdered solid sample is thoroughly mixed with 0.1 g. graphite powder. Then 20 mg. of this mixture or the residue is vaporized in a cupped carbon electrode, using a 14-amp. d.c. arc, and the spectrographic plate is measured as usual (4).

The entire procedure takes about 30 min. for a single determination. In batch analyses the time required is considerably reduced.

The results of this determination are expressed in terms of anhydrous cyanocobalamin. When less stable but more naturally occurring forms of vitamin B_{12}, like hydroxocobalamin and metabolically active coenzyme B_{12}, are also present in the test solution, total vitamin B_{12} activity is directly obtained in this method (calculated as cyanocobalamin equivalents).

Fraudulent addition of inorganic cobalt interferes with this assay.

The most sensitive cobalt line in the UV region, at 3435.5 Å, is chosen as the analytical line. Its intensity is suitable for the range 1–5 mcg. Co/ml. which is usually encountered in pharmaceutical preparations.

There are few ions that interfere with this determination, and it may be termed as specific. However, the intensity of the cobalt line is increased several times in the presence of organic matter existing usually in vitamin preparations. A similar increase of line intensity is observed in flame photometry when using solutions made with combustible organic solvents like methanol or methyl isobutyl ketone (5). For this reason, standard powders are made according to the "addition method," in which increasing amounts of Co are added to the same solution that is being analyzed (4). This "standard addition method" is equivalent, by its nature, to a recov-