EFFECTS OF SEVERAL MACROLIDE ANTIBIOTICS ON BLOOD PRESSURE OF DOGS

Kazuo WAKABAYASHI and Shigeo YAMADA
Department of Pharmacology, School of Pharmaceutical Sciences, Showa University, Shinagawa-ku, Tokyo, Japan

Received for publication June 8, 1972

Among research reports on antibiotics, clinical reports on the treatment of infection occupy an overwhelming majority. In basic animal experiments, studies of antibiotics on antibacterial activity, concentration of antibiotics in the blood and distribution in the organs have received almost all of the attention, while studies on the pharmacological actions have apparently been neglected. One of the main reasons for this has been that the toxicity of antibiotics is far milder than that of most other synthetic drugs, and also because there is seldom any cardiovascular action at the usual therapeutic dosages.

Recently the side effects resulting from large doses of antibiotics have presented various problems. The toxicity and pharmacological actions of the antibiotics, however, still remain to be clarified. In 1955, Bushby et al. (1) reported the participation of a histamine liberating action in the side effects of polymixin B, pointing out for the first time the presence of histamine-like releasers among antibiotics. In 1970, Matsumoto (2) reported the histamine liberating action in colistin derivertives of the same series.

In the present study, the effects of four representative macrolide antibiotics: erythromycin, oleandomycin, spiramycin and leucomycin were studied to examine depressor effects on the blood pressure of dogs.

MATERIALS AND METHODS

1. Experimental drugs

Erythromycin lactobionate (EM: Dainippon Pharmaceutical Co., Ltd.), oleandomycin (OM: Yamanouchi Pharmaceutical Co., Ltd.), leucomycin (LM: Toyo Jozo Co., Ltd.), spiramycin base (SPM: Kyowa Hakko Kogyo Co., Ltd.), (Fig. 1), histamine dihydrochloride (Merck) and atropine sulfate (Merck) were used.

2. Experimental animals

Healthy dogs were used for measurement of the blood pressure. Guinea pigs were used for the bioassay of histamine in blood.

3. Measurement of blood pressure in dogs

Healthy male and female dogs weighing 6–11 kg were anesthetized with 30 mg/kg sodium pentobarbital. The femoral artery and vein were exposed and an arterial cannula was inserted into the femoral artery to measure the femoral arterial pressure with a mercury manometer. Recording was done on smoked paper with a kymograph. Drugs were
FIG. 1. Structure of four macrolide antibiotics.

4. Extraction of histamine from blood

Fig. 2 shows the method of extraction according to the procedure of Code (3).

5. Bioassay of histamine in blood

Healthy male guinea pigs weighing 200-250 g were fasted for 24 hr and the carotid artery cut for blood letting. The ileum was immediately isolated, and a piece of small
intestine measuring about 1 cm was suspended in Tyrode's solution at 36-37°C under 95% O₂ and 5% CO₂. After administration of $1 \times 10^{-3}$ g/ml atropine, a standard histamine solution and the sample solution were administered at 2 min intervals. Contraction height of the intestine was recorded on a kymograph smoked paper via isotonic lever. The amount of blood histamine was calculated from the dose-response curve based on the use of standard histamine solutions.

RESULTS

1. Effect of macrolide antibiotics on the blood pressure of dogs

   1) Effect of erythromycin

   Administration of 5 mg/kg EM showed no effect on blood pressure. Injections of 10 mg/kg and 20 mg/kg resulted in decreases of blood pressure of 33% and 56.6%, lasting for 20 min and 30 min respectively. Administration of 30 mg/kg EM resulted in a conspicuous drop in blood pressure by 83% one min after administration, lasting for approx. 70 min (Fig. 3a).

   2) Effect of oleandomycin

   Administration of 5 mg/kg OM showed no effect on blood pressure. Injections of 20 mg/kg and 30 mg/kg resulted in decreases of blood pressure of 6% and 15%, lasting for

   ![Fig. 3. Effect of macrolide antibiotics on the blood pressure in dogs.](image)

   a : EM 30 mg/kg (i.v.)
   b : OM 50 mg/kg (i.v.)
   c : SPM 30 mg/kg (i.v.)
   d : LM 30 mg/kg (i.v.)
2 min and 15 min respectively. Administration of 50 mg/kg OM resulted in a reduction of blood pressure by 70-80% of the normal range lasting for 55 min (Fig. 3b).

3) Effect of spiramycin

Administration of 5 mg/kg SPM showed no effect on blood pressure. Injections of 10 mg/kg and 20 mg/kg resulted in decreases of blood pressure of 20% and 45%, lasting for 2 min and 40 min respectively. Administration of 30 mg/kg SPM resulted in a remarkable decrease in blood pressure of 77% one min later which lasted approx. 30 min (Fig. 3c).

4) Effect of leucomycin

Administration of 5, 10, 20 and 30 mg kg LM showed identical results as SPM (Fig. 3d).

2. Mechanism of depressor effect

1) Effect of bilateral cervical vagotomy, bilateral cervical sympathectomy and spinal cord transection on depressor effect of EM

After incising the neck of a dog, the bilateral cervical vagi and bilateral cervical sympathetic nerves were exposed, and the cervical spinal cord transected between C1-C2, immediately followed by artificial respiration. The cervical vagus and cervical sympathetic nerves were transected bilaterally followed by drug administration.

Administration of 30 mg/kg EM resulted in a conspicuous decrease in blood pressure. As in the experiments of drug administration alone, the decrease was 55%, one min after administration, lasting for approx. 70 min (Fig. 4). Administration of 50 mg/kg OM, 30 mg/kg SPM and 30 mg/kg LM by a similar method also resulted in a marked depressor effect.

2) Effect of parasympathetic nerves

After administration of 2.0 mg/kg atropine, the administration of 30 mg/kg EM resulted in a decrease in blood pressure of approx. 84%, one min later, lasting for 85 min (Fig. 5). Administration of 50 mg/kg OM, 30 mg/kg SPM and 30 mg/kg LM also resulted in a marked decrease in blood pressure.

Fig. 4. Effect of bilateral cervical vagotomy, bilateral cervical sympathectomy and spinal cord transection on the depressor effect of EM.
decrease in blood pressure. No effects were observed when pretreated with atropine.

3) Tachyphylaxis

Administration of 30 mg/kg EM resulted in a decrease in blood pressure by 86%, followed by recovery approx. 70 min later. Administration of the same dose of EM immediately after recovery resulted in a decrease in blood pressure by 42%. Depressor effect was approx. 50%, less than that of the previous experiment. Similar administration for a third time resulted in a decrease in blood pressure by only 10%. Depressor effect was approx. 90%, less than that in the first experiment (Fig. 6). Similar results were obtained with 50 mg/kg OM, 30 mg/kg SPM and 30 mg/kg LM.

4) Effect of diphenhydramine

Administration of 30 mg/kg EM following 20 mg/kg diphenhydramine resulted in a marked attenuation of the depressor effect (Fig. 7). Antagonism by diphenhydramine was also noted in 50 mg/kg OM, 30 mg/kg SPM and 30 mg/kg LM.

---

**Fig. 5.** Effect of erythromycin on the blood pressure of atropinized dog.

**Fig. 6.** Effect of repeated administrations of erythromycin on the blood pressure of dogs.
These results suggest that the depressor effect of these four macrolide antibiotics are due to the liberation of a histamine-like substance into the tissues. The histamine level in blood was biologically determined following the administration of these four macrolide antibiotics.

5) Bioassay of histamine in blood

i) Existence of histamine in macrolide antibiotics

Since histamine-like substances are frequently mixed in antibiotics, an attempt was made to determine whether or not such substances were present in these four macrolide antibiotics. As shown in Fig. 8, administration of $1 \times 10^{-8}$ g/ml standard histamine to isolated guinea pig ileum caused a marked contraction, while no such reaction was noted on application of $1 \times 10^{-2}$ g/ml EM and $1 \times 10^{-2}$ g/ml LM.
ii) Bioassay of histamine in blood

Extraction of histamine from blood was carried out by the method of Code (3), and quantification by the method of Guggenheim and Löffler (4) utilizing the contraction of guinea pig ileum.

**FIG. 9.** Concentration of histamine in the blood after administration of dogs utilizing bioassay method.

**FIG. 10.** Relationship between histamine concentration and isolated guinea pig ileum contraction.

**TABLE 1.** Concentration of histamine in blood.

| Drugs     | Before administration (mcg/ml) | After administration (mcg/ml) |
|-----------|-------------------------------|------------------------------|
| EM 30 mg/kg i.v. | 0.019                         | 0.136                        |
| OM 50 mg/kg i.v.  | 0.020                         | 0.260                        |
| SPM 30 mg/kg i.v. | 0.010                         | 0.330                        |
Blood samples were obtained for extraction and bioassay of histamine at the time of minimum blood pressure following administration of four macrolide antibiotics, 30 mg/kg EM, 50 mg/kg OM, 30 mg/kg SPM and 30 mg/kg LM (Figs. 9 and 10). As shown in Table 1, administration of 30 mg/kg EM, 50 mg/kg OM, 30 mg/kg SPM caused a 7 to 30 fold increase in the level of histamine in the blood as compared to pre-administration level.

DISCUSSION

Macrolide antibiotics have a macrocyclic lacton and dimethylaminosugar in the molecule and an antibacterial action mainly against gram positive bacilli. Oral administration is primarily employed clinically (5, 6). The toxicity is said to be quite low (5).

Pharmacological studies on macrolide antibiotics have been carried out for many years. According to Ogasawara and Matsumoto (7), EM participates in the excitation of sympathetic nerve endings and acts paralytically on vascular smooth muscle. According to the report of Nakagawa (8), Aratani et al. (9) and Nakatsuka (10), josamycin and spiramycin have a direct effect on smooth muscles, along with a cholinergic action.

In this experiment, all four macrolide antibiotics were found to cause a marked decrease in the blood pressure in dogs. Studies on the mechanism of this depressor effect revealed the absence of any influence from bilateral cervical sympathectomy, bilateral cervical vagotomy, transection of the cervical spinal cord, or pretreatment with atropine. Repeated administration caused the phenomenon of tachyphylaxis, which was noticeably attenuated by pretreatment with diphenhydramine. At the time of the maximum decrease in blood pressure after administration of the four macrolide antibiotics, the concentration of histamine in the blood, according to bioassay was found to be markedly increased. Consequently, the four macrolide antibiotics appear to be histamine releasers.

SUMMARY

The effect of four macrolide antibiotics on dog blood pressure was studied with the following conclusions.

1. Four macrolide antibiotics, EM, OM, SPM and LM, had a marked sustained depressor effect.

2. Histamine concentration in the blood following administration of the macrolide antibiotics, EM, OM, SPM and LM, rose to a level 7 to 30 times higher than that of the pre-administration level.

3. Four macrolide antibiotics, EM, OM, SPM and LM appear to release histamine in tissues, resulting in a depressor effect on the blood pressure.

REFERENCES

1) BUSHBY, S.B.M. AND GREEN, A.F.: Br. J. Pharmac. Chemother. 10, 215 (1955)
2) MATSUMOTO, T.: Folia pharmac. jap. 66, 284 (1970)
3) CODE, C.F.: J. Physiol. 89, 257 (1937)
4) GUGGENHEIM, M. AND LÖFFLER, W.: Biochem. Z. 72, 303 (1916)
5) Meguire, J.M., Buncti, R.L., Anderson, R.C., Boaz, H.Z., Flynn, E.H., Powell, H.M. and Smith, J.M.: *Antibiot. Chemth.* 2, 281 (1952)

6) Dowell, H.M., Bonience, W.S., Pittenger, R.C., Stone, R.L. and Cubberstone, C.G.: *Antibiot. Chemth.* 3, 167 (1953)

7) Ogasawara, Y. and Matsumoto, J.: *Medical Journal of Hiroshima University* 2, 327 (1954)

8) Nakagawa, H.: *Medical Journal of Hiroshima University* 12, 473 (1964)

9) Aratani, H., Yamanaka, Y., Onishi, R. and Kohno, S.: *Chemotherapy* 17, 597 (1969)

10) Nakatsuka, M.: *Igaku no Ayumi* 56, 285 (1966)