A FLUOMETRIC DETERMINATION OF HISTAMINE UPON THE ADMINISTRATION OF MACROLIDE ANTIBIOTICS

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(Received for publication January 25, 1974)

The administration of the macrolide antibiotics, oleandomycin, spiramycin and erythromycin, caused marked depressor effect and increased histamine content in the blood. Histamine in the blood was measured by colorimetry, fluorometry and bioassay. As method of measurement of minute quantities of histamine, fluorometry and bioassay were superior, and the technic of fluorometry was most simple.

The intravenous injection of the macrolide antibiotics such as spiramycin (30 mg/kg), oleandomycin (50 mg/kg), and erythromycin (30 mg/kg) in cats and dogs resulted in marked declines of blood pressure in our previous experiments[1]. The mechanism of action was shown to be due to the *in vivo* release of histamine (H) by a biological method.

Of the methods of bioassay for H, cat blood pressure[2] and isolated guinea pig intestine[3] have been utilized. These methods are subject to spontaneous errors. The influence of individual and sexual differences of animals, as well as skill in the experimental technic is recognized. As a consequence, many experiments are required and the results have to be analyzed statistically.

According to YAMADA[4] in the current concept of the presence of H releasers, the biological assay requires both the blood pressure method and the guinea-pig intestine method. In the present study H in the blood was determined chemically and by bioassay at the time of the blood pressure fall following the administration of macrolide antibiotics.

Experimental Materials

Experimental drugs

Erythromycin (EM, Dainihon Pharmaceutical Co.), oleandomycin (OM, Yamanouchi Pharmaceutical Co.), and spiramycin (SPM, Kyowa Hakko Kogyo Co.) were used.

Method of blood pressure measurement

Healthy dogs weighing 7~14 kg were anesthetized with sodium pentobarbital, 30 mg/kg. The femoral artery was exposed and an arterial cannula was inserted. A mercury manometer was connected to the cannula for the blood pressure measurement. Drugs were administered *via* a venous cannula inserted into the femoral vein.

Chemical determination of H

For the chemical determination of H, colorimetric and fluorometric methods were employed. A comparison was made between these two methods.

(1) Colorimetric determination: Fig 1 shows the procedure of the colorimetric determination of H in the blood. Fifteen ml of 5 % sodium nitrite was added to 15 ml of 0.9 % sulfanylic acid and mixed. After a few minutes, 6 ml of sodium nitrite was further added and mixed. After another 5 minutes, water was added to 2 ml of the sample or standard H solution and mixed. After 30 minutes, 10 ml of 1 % sodium carbonate was added and mixed. Three ml of the mixture was placed in a cuvette and the colorimetry was carried out at 420 mμ wave length with a photoelectric colorimeter (Hitachi EPW-4).

Fig. 1. Procedure for histamine determination of diazo-method

A solution 15 ml+B solution 15 ml
| mix after 5 min.
add B solution 6 ml
| mix after 5 min. add water
make total volume 50 ml (A·B solution)
sample 2 ml+A·B solution 4 ml
| mix after 30 min.
add C solution 10 ml
| mix
transfer 3 ml to a cuvette

A solution: 0.9% sulfanylic acid
B solution: 5% sodium nitrite
C solution: 1% sodium carbonate

(2) Fluorometry: According to the method of SHORE *et al.*[5], 5 ml of blood was deproteinized with perchloric acid, followed by the extraction of H with an alkaline butanol solution. Finally H was transferred to hydrochloric acid solution. The addition of phthalaldehyde to this solution yielded the fluorescence. This was measured in a Beckmann fluorometer (Fig. 2).

Experimental Results

1. Colorimetry of Histamine

H was extracted from the blood and degree of recovery was measured with paper chromatography, column chromatography and an ion-exchange resin.

Fig. 3 A shows the relationship between

Fig. 2. Procedure for extraction and determination of histamine in blood

Oxalated blood 5 ml+Water 4.5 ml+Conc. perchloric acid 0.5 ml
| shake 10 min. centrifuge 10 min. (2,500 r.p.m.)
Supernate 4 ml+5 N NaOH 0.5 ml+*n*-Butanol 10 ml
| shake 5 min. centrifuge 10 min.
Organic phase+Salt-saturated 0.1 N NaOH 5 ml
| shake 1 min. centrifuge 10 min.
Organic phase 8 ml+0.1 N HCl 5 ml+*n*-Heptane 15 ml
| shake 1 min. centrifuge 10 min.
Aqueous phase 2 ml+1 N NaOH 0.4 ml
| mix
+1 % OPT 0.1 ml
| mix after 4 min.
+3 N HCl
| mix
Sample

the recovery of H and the time of extraction with the use of paper chromatography which is developed by a medium composed of ethyl acetate, ethyl alcohol and distilled water for 24 hours. When the time was 10 minutes for the extraction of H from the blood, the recovery of H was about 60 %. Even if the time of extraction was prolonged to more than 20 minutes, the maximum recovery did not exceed 92.5 %.

Fig. 3B shows that relationship between the recovery of H and the elution rate of 0.1, 0.5 and 1.0 ml/min. from the ion exchange resin column. One hundred % of the H was recovered at an elution rate of 0.1 ml/min.

Fig. 4 A shows the relationship between the recovery of H and the time of extraction of H from the blood with the use of an ion-exchange resin. With the time of extraction for 30~40 minutes, the recovery of H reached 100 %.

Fig. 4 B shows the relationship between the recovery of H and the pH of the medium between 5 and 9. The recovery of H was 100 % when the pH of medium was 6.

The minimum concentration of H assayed by colorimetry was 1 μg/blood ml.

Fig. 3. Relationship between percent recovery and extraction time (A) and flow rate (B)

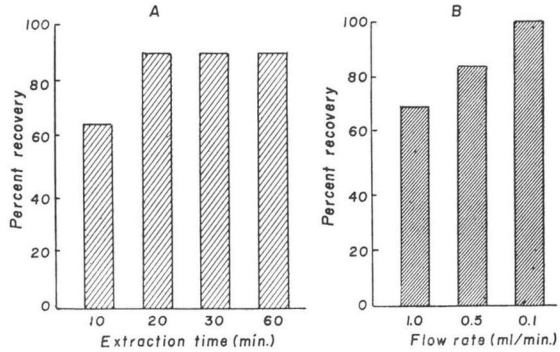
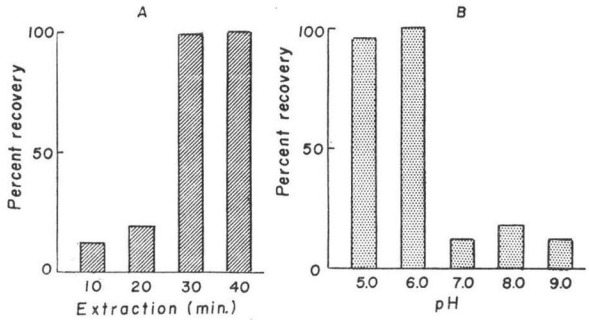


Fig. 4. Relationship between percent recovery and extraction time (A) and pH (B)

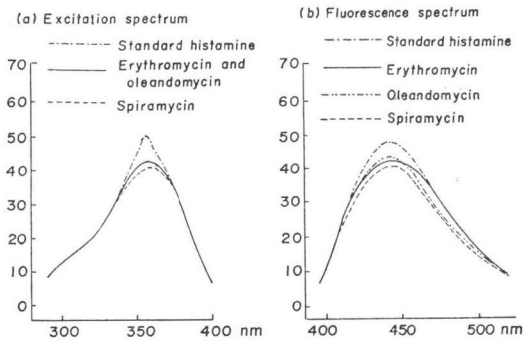


In summary of the above, colorimetry is time-consuming and it is impossible to determine very small quantities of H.

2. Fluorometry of Histamine

The procedure shown in Fig. 2 was followed for the measurement of H in the blood. It was possible to measure the fluorescence of H at 440 mμ using wavelength of 355 mμ. Authentic H was also measured at the same wavelengths and gave the same values (Fig. 5).

Fig. 5. Excited wave(a) and fluorescence wave(b) of authentic histamine and extracted histamine when injected erythromycin (30 mg/kg), oleandomycin (50 mg/kg) and spiramycin (30 mg/kg)



Colorimetry and fluorometry were compared during the measurement of H in the blood. Colorimetry was suitable for the measurement of H when concentration is more than 1 μg/ml, but it was difficult to measure H of less than 1 μg/ml. Colorimetry was also quite time-consuming. The method of SHORE *et al.*[5] was simple and suitable for detection of a minute amount of H. In the following experiment with the administration of antibiotics, H in the blood was measured by the method of SHORE *et al.*

Fig. 6. Effects of three macrolide antibiotics, oleandomycin (50 mg/kg), spiramycin (30 mg/kg) and erythromycin (30 mg/kg) on the blood pressure in dogs

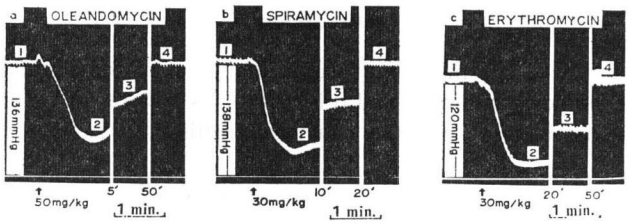


Table 1. Comparison of blood histamine content assayed by fluorometry and bioassay.

Drugs	Fluorometry (μg/ml)				Bioassay (μg/ml)			
	Control (normal)	Maximum falling of blood pressure	Falling of blood pressure	Recovery	Control (normal)	Maximum falling of blood pressure	Falling of blood pressure	Recovery
Oleandomycin 50 mg/kg i.v.	0.019 ±0.011 (140)	0.130 ±0.04 (78)	0.120 ±0.033 (98)	0.029 ±0.009 (140)	0.020 ±0.011	0.260 ±0.040	0.077 ±0.012	0.022 ±0.005
Spiramycin 30 mg/kg i.v.	0.012 ±0.006 (136)	0.193 ±0.035 (44)	0.045 ±0.041 (94)	0.013 ±0.008 (136)	0.018 ±0.002	0.183 ±0.024	0.051 ±0.016	0.014 ±0.005
Erythromycin 30 mg/kg i.v.	0.028 ±0.017 (138)	0.185 ±0.021 (30)	0.850 ±0.022 (88)	0.010 ±0.027 (138)	0.019 ±0.002	0.136 ±0.046	0.053 ±0.005	0.027 ±0.014

Blood pressure are indicated in parentheses. Each value is the mean of four experiments and standard error. These are the same samples.

3. H in the Blood after the Administration of Macrolide Antibiotics

In Fig. 6 the hypotensive effects of 50 mg/kg of oleandomycin, 30 mg/kg of spiramycin and 30 mg/kg of erythromycin in dogs are shown. At various intervals before and after the injection 5 ml of blood was withdrawn for H determination. One minute after the administration of 50 mg/kg of oleandomycin, the blood pressure gave a maximum fall of 69 % followed by recovery after 55 minutes. H in the blood at this time was 0.130±0.040 (S. E.) μg/ml, representing about a 7-fold increase over the control H value before the administration of oleandomycin (Blood pressure 136 mmHg) of 0.019±0.011 μg/ml. When 30 mg/kg of spiramycin was administered, H in the blood increased about 16-fold, while administration of 30 mg/kg of erythromycin led to an approximate 7-fold increase. H in the blood at the time of the maximum fall in blood pressure is shown in Table 1. This was compared with the value obtained by bioassay.

4. Comparison between Fluorometry and Bioassay

In the previous report, H in the blood at the time of a fall in the blood pressure in response to macrolide series antibiotics was measured by bioassay.

When a small amount of H was measured, the measurable range of H was not much different between fluorometric assay and bioassay. From the present result fluorometric assay

appeared to be easy to handle and required only a short time.

Discussion

SHORE *et al.*[5] introduced the method of fluorometry for microquantities of H contained in various tissues of the bodies of animals and this method is widely used. In the present study, the authors measured the release of H by macrolide antibiotics as in the previous report by fluorometry.

For the method of colorimetry, paper chromatography was used to extract H. When the time of extraction was 10 minutes the recovery of H was 61.5 %. Even if the time of extraction was prolonged the maximum recovery only reached 90 %. This was because a complete separation of H from the paper did not occur. When column chromatography was used and the speed of elution from the column was less than 0.1 ml/min., the recovery of H was excellent, and a pH of 5~6 appeared to be adequate. However, this procedure took a long time. When H was extracted with an ion-exchange resin, 30 minutes of extraction sufficed. Fluorometry was quite simple and excellent for quantification of H in the blood. As was pointed out by SHORE *et al.*[5], this method appeared to be adequate for the measurement of H contained in an organ.

H releasers had been cited from ancient times. Among the antibiotics, the polymyxin series antibiotics[6] were known as histamine releasers. This was demonstrated by a biological measurement of H in the blood.

Many difficulties were encountered in the measurement of minute amounts of H by colorimetry, because of the ready tendencies of histamine, tyrosine and indole to become diazo compounds.

These antibiotics did not contain H according to bioassay methods.

Conclusion

When blood pressure falls in response to the administration of macrolide antibiotics, oleandomycin, spiramycin and erythromycin, H in the blood was measured by a fluorometric assay and also by colorimetry. The following conclusions were drawn:

1. The H content in blood increased 7~16-fold in response to the administration of oleandomycin, spiramycin and erythromycin.

2. Colorimetry, fluorometry and bioassay were compared for their measurement of minute quantities of H; fluorometry and bioassay were superior, and the technic of fluorometry was most simple.

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