ENDURACIDIN, A NEW ANTIBIOTIC. IV

THE FATE OF ENDURACIDIN ADMINISTERED PARENTERALLY INTO RABBITS

SHIGEHARU TANAYAMA, TAKESHI FUGONO
and TOSHIYUKI YAMAZAKI

Biological Research Laboratories, Research and Development Division,
Takeda Chemical Industries, Ltd., Osaka, Japan

(Received for publication December 12, 1967)

A rapid mobilization of enduracidin, introduced into the circulation, to each tissue was suggested from the results of intravenous injection and constant infusion studies in which blood levels and excretion into urine and bile were observed. Following intramuscular injection of 2 mg/kg of enduracidin, a plateau in the blood level (approximately 4 mcg/ml) was reached at 2 hours after the injection and this persisted for the next 2 hours and then decreased gradually. Increase in the dose resulted in the prolongation of the plateau, rather than a further rise in the maximum blood level. Except for the brain, enduracidin was found to distribute widely in the tissues including heart, lung, liver, spleen, adrenal, kidney, testis and muscle. Among these tissues, lung, liver, spleen and kidney contained higher concentrations of enduracidin than others, and in the case of multiple daily intramuscular injections, a parallel was noted in tissue enduracidin levels with doses and number of injections, with the exception of kidney. Enduracidin, once transported to the tissues, was retained for rather long period of time in the liver, and except for the kidney, presumably in other tissues. Enduracidin may be excreted mainly *via* urinary excretion.

Enduracidin, a new antibiotic isolated from the fermentation broths of *Streptomyces fungicidicus* No. B-5477[1] was found to be highly effective *in vitro* against Gram-positive organisms and also active *in vivo* against experimental infections in mice[2].

In the present studies, the blood level, excretion into urine and bile, and tissue distribution in rabbits were observed in order to obtain some information regarding the fate of parenterally administered enduracidin.

Materials and Methods

Rabbits of both sexes weighing about 2.5~4.0 kg were used in these studies. Enduracidin was prepared as a 5 % aqueous solution containing 5 % HCO-50*, 1 % benzyl alcohol and 1 % inositol.

The assay of enduracidin in body fluids and tissues, including the extraction from tissue homogenates, has been described previously by one of the authors[3]. The concentrations of enduracidin in blood, urine and bile were assayed by a cylinder plate method.

* Hydrogenated castor oil, Nikkol® (Nikko Chemical, Ltd.)

Samples, after diluting with 0.1 M phosphate buffer (pH 8.0), were poured into stainless steel cylinders on thin-layer agar plates inoculated with *B. subtilis* PCI 219 as the test organism. The inhibition zones were measured after overnight incubation at 33°C. The composition of assay medium is as follows: 0.5 % Extract Ehlrich®*, 0.5 % Polypeptone, 3 % NaCl and 1.2 % agar (powder) in water. Before assay, blood and urine were diluted 4 times with the buffer, and bile was usually diluted 10 times. Diluted blood was centrifuged at 3,000 r.p.m. for 10 minutes to separate blood cells and the resulting supernatant was assayed for enduracidin. Levels of enduracidin in the supernatants were designated as "blood level" in the present paper, but plasma level in Table 4 was determined on plasma separated from non-diluted blood by centrifugation. The tissue levels of enduracidin were determined by a paper disc method after extracting from tissue homogenates with acidic acetone (60 %, pH 3~4).

Constant infusion was carried out *via* Vena jugularis externa under light Nembutal anesthesia, using the Harvard Infusion Pump, model No. 600-000. Biopsy of the liver and kidney was performed by an abdominal approach as follows: a small quantity of the liver and the kidney were excised at certain intervals during or after the infusion, and another sample of liver and kidney was obtained when the animal was killed, thus providing 2 to 4 samples of the liver and 2 samples of the kidneys from a rabbit. When animals were to be maintained alive for a few days or longer, they were housed in individual cages and given free access to water and stock diet.

Blood volume was calculated from hematocrit and plasma volume estimated by the Evans blue dilution method, as follows[4]:

$$\text{Blood volume} = 100 \times Vp/100 - Kht \times Ht$$

where Vp is plasma volume, Ht hematocrit reading, and Kht a constant to correct the hematocrit for plasma trapped between the packed cells (0.95 was used for Kht value in the present experiment).

Results

1. Blood level and excretion of enduracidin after a single intravenous injection and during constant infusion

Blood levels and excretion into urine and bile of enduracidin after a single intravenous injection and during constant infusion were studied to assess the diffusion of this drug when introduced into the circulation. Fig. 1 shows that the mean blood concentration of enduracidin rapidly fell during 2 hours after the intravenous injection (1 mg/kg) followed by a further decline below 1 mcg/ml at 24 hours, although urinary excretion was detectable for the

Fig. 1. Blood levels of enduracidin after a single intravenous injection.
Dose: 1 mg/kg (Rabbits 1, 2 and 3)

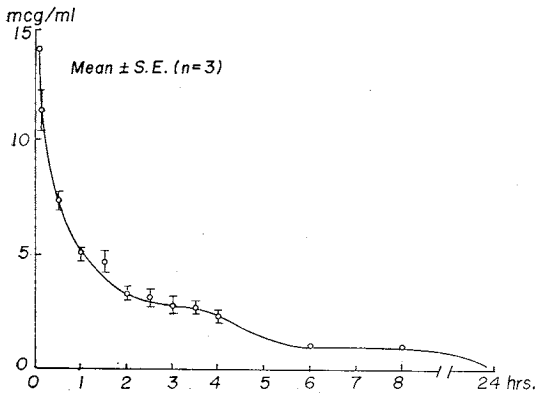
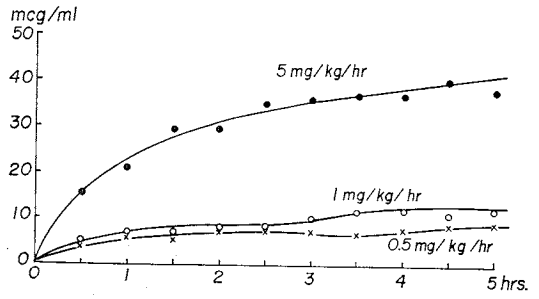


Fig. 2. Changes in blood enduracidin levels during 5-hour constant infusion.



* Meat extract (Wako Pure Chemical Industries, Ltd.)

Table 1. Enduracidin excretion into urine and bile of rabbits following a single intravenous injection (Dose ; 1 mg/kg)

	Time of collection and per cent excretion (Mean±standard error)							
	0~1	1~2	2~3	3~4 hours*	0~1	1~2	2~3	3~4days**
Urine	3.40±0.86	2.86±0.27	1.22±0.16	1.58±0.86	48.7±7.31	25.2±4.08	8.2±0.73	4.4±1.47
Bile	0.64	0.57	0.44	0.32				

* Rabbits 1, 2 and 3. ** Rabbits. 4, 5, 6 and 7.

next 3 days. The recovery in urine was 87 % of administered amount during 4 days after injection (Table 1, mean of 4 rabbits). This rapid fall of blood level observed during first 2 hours might indicate a rapid diffusion of enduracidin to various organs from the circulation, since the excretion of this drug into urine and bile was rather small as compared with the initial rapid decline of blood level, as noted in Table 1.

For example, about 83 % of the injected enduracidin disappeared from the blood in the first 2 hours after the administration (calculated as follows: the amount injected — blood level at 2 hours × blood volume of animals), while the excretion into urine and bile was about 7.5 % (Table 2).

Table 2. Absorption and excretion of enduracidin in 2 hours after a single intravenous injection into rabbits

	I	II	III	IV	V
Rabbit No.	Dose mg/kg	present in blood at 2 hours*	excreted into urine (in 2 hrs.)	excreted into bile (in 2 hrs.)	I−(II+III+IV)
1	1.0	15.7	4.82	0.93	78.6 %
2	1.0	20.1	5.49	1.36	73.1 %
3	1.0	15.3	8.42		76.3 %
Mean		17.0	6.25	1.15	76.0 %

* Blood concn. (mcg/ml) × Blood volume (ml).

This finding was further confirmed by the results shown in Fig. 2 and Table 3, in which the changes of blood level and the excretion into urine and bile of enduracidin were studied during constant infusion. Although the blood level increased slowly during the infusion period, the degree of increment was rather small as compared with the amount of enduracidin infused (Fig. 2). In addition, excretion into urine and bile accounted for only a small fraction of the dose administered, as noted in Table 3 (Column III and IV).

Table 3. Absorption and excretion of enduracidin during 5-hour constant infusion into rabbits

I		II	III	IV	V
Dose (mg/kg/hr.)	Total (mg)	present in blood at 5 hours*	excretion into urine (in 5 hrs.)	excretion into bile (in 5 hrs.)	I−(II+III+IV)
0.5	2.5	19.9	2.04	0.89	77.1 %
1.0	5.0	12.0	1.86	0.59	85.6 %
5.0	25.0	10.2	1.34	0.19	88.5 %

* Blood concn. × Blood volume

The quantity of enduracidin transported into the tissues was calculated according to the following equation:

The amount initially injected—(the amount present in blood at certain time+that excreted into urine and bile by this time), which was approximately 76 % for 2 hours after intravenous injection of 1 mg/kg (Table 2, Column V) and with 5-hour constant infusion, 77.1~88.5 % of the administered dose (Table 3, Column V).

2. Blood level and urinary excretion of enduracidin following a single intramuscular injection

Blood levels after various doses by intramuscular injection are presented in Fig. 3. When 2 mg/kg of enduracidin was injected intramuscularly, the mean blood level reached a plateau (approximately 4 mcg/ml) at 2 hours, this level was maintained for another 2 hours and gradually decreased thereafter. Increase in the dose resulted in prolongation of the plateau, rather than rise in the maximum blood level. Thus, maximum blood level of about 4 mcg/ml, obtained at 2 hours following the injection of 5 mg/kg of enduracidin, persisted for the next 6 hours, followed by a progressive decline. When 10 mg/kg was administered, the plateau of blood level was sustained for about 24 hours.

Fig. 3. Blood levels after a single intramuscular injection

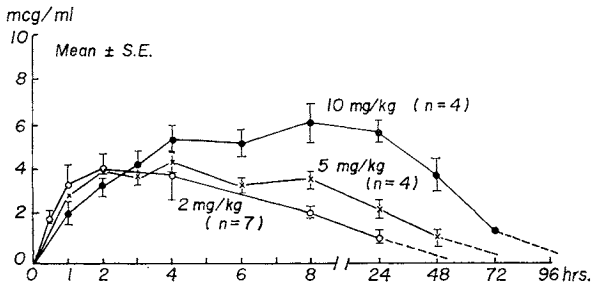
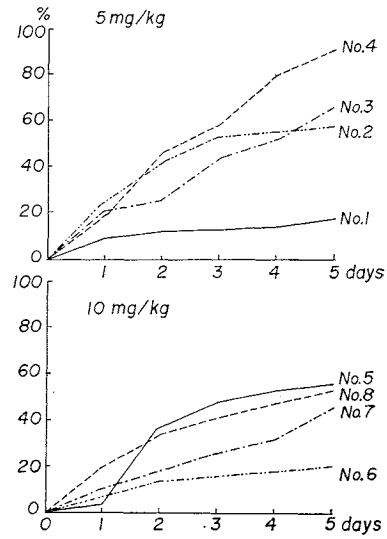


Fig. 4. Urinary excretion after a single intramuscular injection



As shown in Fig. 4, approximately 58 % of the administered amount was recovered in the urine in 5 days after the dose of 5 mg/kg and 44 % after the injection of 10 mg/kg (mean of 4 observations in each group), although considerable variation was encountered among animals.

3. Tissue distribution of enduracidin after constant infusion and intramuscular injection

Results of estimation of enduracidin in various tissues after constant infusion are presented in Fig. 5 which shows that, after 5-hour constant infusion of 5 mg/kg/hour, enduracidin was distributed in various tissues including the heart, lung, liver, spleen, kidney and skeletal muscle, but was not present in the brain. The enduracidin level of the kidney was consistently higher than other tissues, which was in contrast with the results obtained with daily intramuscular injections (Fig. 6).

Tissue distributions following multiple daily intramuscular injections are summarized in Fig. 6 and Fig. 7, in which enduracidin was found to be widely distributed in all tissues including the heart, lung, liver, spleen, adrenal, kidney and testis with an exception of brain. Especially high levels were found in the liver, adrenal and spleen. These data also indicate that the increase in dose and number of injections resulted in a rise in tissue levels with one exception that no elevation was found in the kidney, as shown in Fig. 7.

Fig. 5. Tissue distribution of enduracidin after 5-hour constant infusion (5 mg/kg/hr)

1) N.D.: non-detectable
2) +: trace

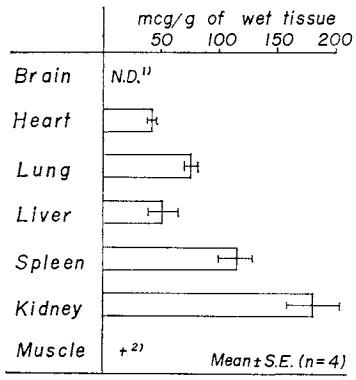


Fig. 6. Effect of dose on tissue enduracidin levels (No. of injection=55)
Data are means of two rabbits.

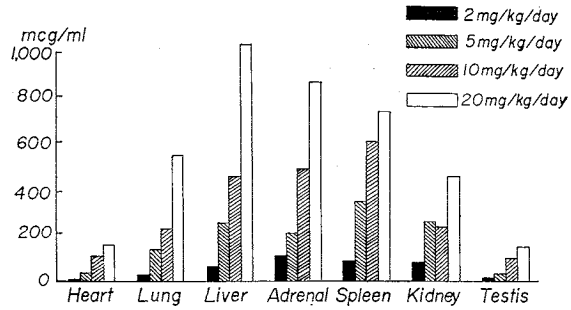


Table 4 represents the time course of enduracidin levels in the liver, kidney and plasma in 5 rabbits, during and after 5-hour constant infusions (5 mg/kg/hr). Each animal was infused with enduracidin for 5 hours under light Nembutal anesthesia and liver and kidney samples were obtained by biopsy at certain intervals. It is evident from the data shown in Table 4 that, although enduracidin levels in the liver, kidney and plasma all increased with time during infusion (Rabbits 1, 2 and 3), the rise in kidney level was extremely rapid as compared with liver, and continued to rise until 24 hours (Rabbits 1 and 3), followed by a gradual decline to below 10 mcg/g of wet tissue about 6 days after infusion (Rabbits 4 and 5). Plasma level was shown to fall immediately after infusion (Rabbits 2, 3 and 4), while liver level was maintained for at least 6 days after its maximum level was attained (Rabbits 2, 3, 4 and 5).

Changes in enduracidin tissue levels following single intramuscular injections (20 mg/kg) are shown in Table 5. Although enduracidin disappeared from the liver,

Table 4. Changes in enduracidin tissue levels during and after 5-hour constant infusion (5 mg/kg/hr)

Rabbit No.	Tissue	mcg/g wet tissue or mcg/ml plasma						
		2.5 hrs.	5 hrs.	8 hrs.	24 hrs.	2 days	3 days	6 days
1	Liver	54	106					
	Kidney	90	295					
	Plasma	96	140					
2	Liver	13	34	30	57			
	Plasma	41	81	30	6			
3	Liver	20	59	58	53			
	Kidney		125		220			
	Plasma	55	99	49	23			
4	Liver				70	58	61	
	Kidney				152		105	
	Plasma				4		—	
5	Liver						55	42
	Kidney						50	+
	Plasma						—	—

+: trace. —: non-detectable.

Fig. 7. Effect of number of injection on tissue enduracidin levels (Dose : 10 mg/kg/day intramuscularly)
1) + : trace

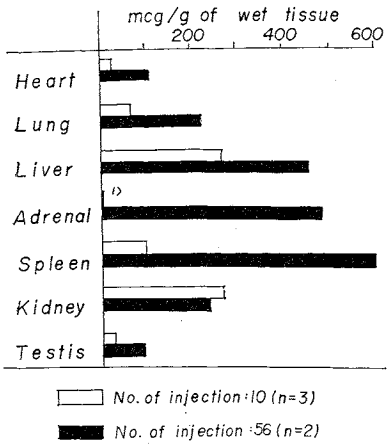


Table 5. Changes in enduracidin tissue levels following a single intramuscular injection (Dose : 20 mg/kg)

Tissue	Tissue level (mcg/g wet tissue)			
	1 day	5 days	10 days	20 days
Liver	13	28	+	—
	13	27	+	—
	12	22	15	—
	13±0.41*	26±1.87	(15)	—
Kidney	58	26	+	—
	53	22	+	—
	33	27	14	—
	48±7.64	25±1.53	(14)	—
Spleen	30	30	19	—
	31	29	14	—
	21	32	24	—
	27±3.19	30±0.91	19±2.89	—

* Standard error.
+ : Trace. — : Non-detectable.

spleen and kidney 20 days after the injection, it was still detectable in all the tissues examined, as long as 10 days after the administration.

Discussion

Following intravenous injection, the rate of disappearance of enduracidin from blood was rapid in the first 2 hours presumably due to distribution of enduracidin in various organs (Fig. 1), since excretions into urine and bile in this period of time were rather small, although the urinary excretion continued for 3 days after blood concentration decreased to levels under 1 mcg/ml (Table 1). A rapid diffusion of enduracidin from the blood circulation to each tissue was also suggested from the results of constant infusion studies, in which enduracidin blood level increased at a progressively slow rate during 5-hour infusion (Fig. 2), while excretion into urine in this period accounted for only 1.34~2.04 % of the dose administered, and into bile for 0.19~0.89 % (Table 2, Column III and IV).

A calculation was made of the amount of enduracidin transported into the tissues, on the assumption that this could be obtained by substracting the amount of enduracidin present in blood circulation at a certain time plus that excreted into the urine and bile by this time, from the amount which had been administered. Tables 2 and 3 show that these values were approximately 76 % of the administered amount during first 2 hours following a single intravenous injection (1 mg/kg) and 77.1~88.5 % with 5-hour constant infusions, depending on the dose administered (at a rate of 0.5 mg/kg/hr, 77.1 % ; 1.0 mg/kg/hr, 85.6 % ; and 5 mg/kg/hr, 88.5 %).

The mean blood level obtained after a single intramuscular injection of enduracidin reached a plateau, as noted in Fig. 3. The maximum blood level showed less increase as compared with the increase in dosage, while the increase in prolongation of the plateau was approximately proportional. As for the urinary excretion, 58 % of the dose injected was recovered in urine in 5 days after the administration of 5 mg/kg and 44 % after 10 mg/kg (mean of 4 observations in each group).

With constant infusion and daily intramuscular injection, it was found that, except for the brain, enduracidin was widely distributed in the tissues including the heart, lung,

liver, spleen, adrenal, kidney, testis and muscle (Figs. 5, 6 and 7).

The changes in enduracidin transported to the tissues are shown in Table 4, in which enduracidin levels in the liver, kidney and plasma during and after constant infusions (5 mg/kg/hr for 5 hours) were observed, indicating that liver enduracidin level was maintained for at least 6 days after its maximum level was attained, while the kidney level progressively decreased after a rapid rise during first 24 hours, presumably due to urinary excretion since it was detectable 4 days after infusions.

From the results obtained from multiple daily intramuscular injections (Figs. 6 and 7), it was found that increase in dose and number of injections resulted in the rise in tissue levels, with the exception of the kidney, in which no elevation of enduracidin level was noted with increase in number of injections, tentatively suggesting increased urinary excretion (Increase in urinary excretion of enduracidin was observed following daily intramuscular injection for 47 days in monkeys[5]). Among the tissues examined, the liver, adrenal, spleen and lung contained higher concentrations of enduracidin than others, which was in contrast with the result with 5-hour constant infusion (5 mg/kg/hr), in which enduracidin rapidly concentrated in the kidney, and to lesser extent in other tissues.

It was suggested by the following observations that enduracidin introduced into the circulation concentrated more rapidly in the kidney than in other tissues and then was progressively excreted into urine; First, after a single intravenous or intramuscular injection, enduracidin was found to be excreted largely in the urine (Table 1 and Fig. 4). Second, after 5-hour constant infusion, the kidney contained consistently higher concentration of enduracidin than other tissues (Fig. 5). Third, during infusion, in contrast with the liver, the kidney enduracidin level rapidly increased followed by a gradual decline (Table 4). Moreover, enduracidin was detectable in urine 4 days after the infusion when it was no longer detectable in plasma by the assay procedure employed. Finally, with daily intramuscular injections, increase in the number of injections resulted in no elevation of kidney enduracidin levels in contrast with other tissues, presumably due to the increased excretion into the urine (Fig. 6).

Enduracidin distributed in tissues other than the kidney might also be transported to the kidney after remaining for rather long periods of time, as suggested from the results shown in Tables 4 and 5 and then be excreted into urine.

References

1) Higashide, E.; K. Hatano, M. Shibata & K. Nakazawa: Enduracidin, a new antibiotic. I. *Streptomyces fungicidicus* No. B-5477, an enduracidin producing organism. J. Antibiotics, 21: 126~137, 1968
2) Tsuchiya, K.; M. Kondo, T. Oishi & T. Yamazaki: Enduracidin, a new antibiotic. III. *In vitro* and *in vivo* antimicrobial activity. J. Antibiotics 21: 147~153, 1968
3) Fugono, T.: On the assay methods of enduracidin in body fluids and tissues. Chemotherapy (Japan). In press.
4) Chinard, F. P.: Estimation of plasma volume by dye dilution method. Methods in Med. Res. 4: 38~48, 1951
5) Tanayama, S.: Personal communication