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Selective distribution of aclarubicin to regional lymph nodes with a new dosage form: aclarubicin adsorbed on activated carbon particles

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A new dosage form (ACR-CH) comprising aclarubicin adsorbed on activated carbon particles was designed to sustain release of aclarubicin. ACR-CH or aclarubicin aqueous solution (ACR-sol) was injected subcutaneously into the fore foot-pads of rats. ACR-CH distributed a statistically significantly higher level of aclarubicin to the axillary lymph nodes (detectable up to 7 days after injection) than aclarubicin distributed in an ACR-sol (not detectable after 48 h). To other tissues, ACR-CH distributed statistically significantly low levels of aclarubicin, as compared with ACR-sol.

Key words: Aclarubicin, activated carbon, drug delivery system, lymph nodes metastases, tissue distribution.

Introduction

The purpose of cancer chemotherapy is to concentrate anti-cancer drugs selectively in the cancer lesions, including lymph nodal metastasis for a long period of time. However, most anti-cancer drugs administered in aqueous solution consist of water-soluble small molecules which are hardly absorbed through the lymph capillary wall into the lymph flows.[1] So those drugs are not always selectively distributed into the regional lymph nodes.

To overcome this problem, we have developed a new dosage form comprising aclarubicin adsorbed on activated carbon particles in a suspension. This paper describes the new dosage form's selective distribution of aclarubicin into the regional lymph nodes when injected into rat tissues.

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Materials and methods

Agents

Activated carbon (Mitsubishi 1500AA^R, Mitsubishi Chemicals Co. Ltd, Tokyo) 20 nm in diameter for primary particles and 1480 m^2/g specific surface area, was used as the carrier. One of the anthracyclines. aclarubicin[2] (Aclacynon^R, Sanraku Co. Ltd, Tokyo), which had anti-cancer effects on breast cancer as well as other human cancers,[3] was used as the anti-cancer drug.

Adsorption of aclarubicin onto activated carbon

The adsorption isotherms of aclarubicin onto activated carbon were measured *in vitro*. Aclarubicin was dissolved in saline (0.1 mg/ml). Activated carbon (0.1–1.0 mg/ml) was added to the aclarubicin solution. The mixture was made into activated carbon suspension by ultrasound. The suspension was incubated for 1 h at 37°C in order to adsorb the aclarubicin onto the activated carbon in a state of equilibrium. The activated carbon was removed by centrifugation at 3000 rev/min for 10 min. The aclarubicin concentration in the supernatant was measured with the fluorometric

method using excitation and emission maxima at 440–445 nm and at 505 nm wavelength.[4] The equilibrium points were set on a log–log scale abscissa, and the adsorption isotherm line was drawn along the points.

Using the same procedures the adsorption isotherm was measured in phosphate buffer solution at pH 7.4–7.5.

Sustained release of aclarubicin from activated carbon *in vitro*

Desorption of aclarubicin from the activated carbon was measured in triplicate experiments. Aclarubicin of 0.4 mg and activated carbon of 1.0 mg were added to 4 ml of saline. The mixture was made into a suspension by ultrasound and incubated for 1 h at 37°C so that the adsorption should be at equilibrium. The activated carbon was removed by centrifugation at 3000 rev/min for 10 min. The aclarubicin concentration in the supernatant was measured. The sediment (activated carbon with adsorbed aclarubicin) was put into 4 ml of saline (without aclarubicin), and the mixture again made into a suspension by ultrasound. Washing out using the same process was repeated seven times for each sample.

Using these procedures the release from activated carbon was also measured in phosphate buffer solution at pH 7.4–7.5.

Preparation of dosage forms

Activated carbon at 50 mg/ml, and polyvinylpyrrolidone (polyvinylpyrrolidone K-30^{R}, Nakarai Chemicals Co. Ltd, Kyoto) at 20 mg/ml were mixed in saline and kneaded with three rollers to turn the carbon particles into a suspension; the size in the suspension was 157 nm on average, as measured with photon correlation spectroscopy.[5] The activated carbon suspension was sealed in a glass tube and sterilized at 120°C for 10 min. Aclarubicin at 5 mg/ml was added to the activated carbon suspension and the mixture was shaken at 120 cycles/min for 1 h at 37°C to bring the adsorption in equilibrium. Thus the new dosage form (ACR-CH) comprises 5 mg/ml of aclarubicin, 50 mg/ml of activated carbon and 20 mg/ml of polyvinylpyrrolidone in saline.

As a control drug, aclarubicin aqueous solution (ACR-sol) was prepared, comprising 5 mg/ml of aclarubicin in saline.

ACR-CH distribution

Forty-eight rats (Wistar strain, 250 g body weight, Shimizu Laboratory Animal Center) were divided into two groups (the ACR-CH group and the ACR-sol group) each consisting of 24 rats.

Aclarubicin at 5 mg/kg was injected subcutaneously into the foot-pad of the right forepaw in the form of ACR-CH or ACR-sol. The three rats in each group were killed by neck breaking at 1, 3, 6, 12, 24, 48, 96 or 168 h after injection. Blood was taken from the heart by heart paracentesis and was separated into plasma and blood cells by centrifugation at 6000 rev/min for 5 min. The kidneys, the spleen, the lungs, the heart and the right axillary lymph nodes were taken for aclarubicin concentration analysis. The samples were weighed with a microbalance and kept at −80°C.

Aclarubicin in the samples was measured by high-performance liquid chromatography (HPLC).[6] The assay limitation was 50 ng/ml or ng/g.

The Student's *t*-test was used in the statistical examinations of aclarubicin concentrations.

Results

Adsorption and desorption of aclarubicin

The adsorption isotherm was shown as $Q = 175C^{0.15}$ in saline at 37°C (where Q is the amount of aclarubicin adsorbed onto the activated carbon expressed in μg/mg, and C is the concentration of aclarubicin in a free state expressed in μg/ml) and as $Q = 207C^{0.24}$ in phosphate buffer solution at pH 7.4–7.5 at 37°C (Figure 1).

Desorption curves are shown in Figure 2. Aclarubicin was released from the activated carbon by washing out seven times, although the desorption concentrations of aclarubicin in the free state were decreased with washing-out times from 30% to 3% of the initial concentration in saline, and from 6% to 1.6% in phosphate buffer solution.

Drug distribution in rats

Axillary lymph nodes: Aclarubicin concentration in the axillary nodes is shown in Table 1. In the ACR-CH group, the concentration was about 100 μg/g within 3 h after injection (peaking to 119 μg/g at 3 h). Aclarubicin was detected 7 days after injection. By contrast, in the ACR-sol group the concentration was 77 μg/g at 1 h and decreased

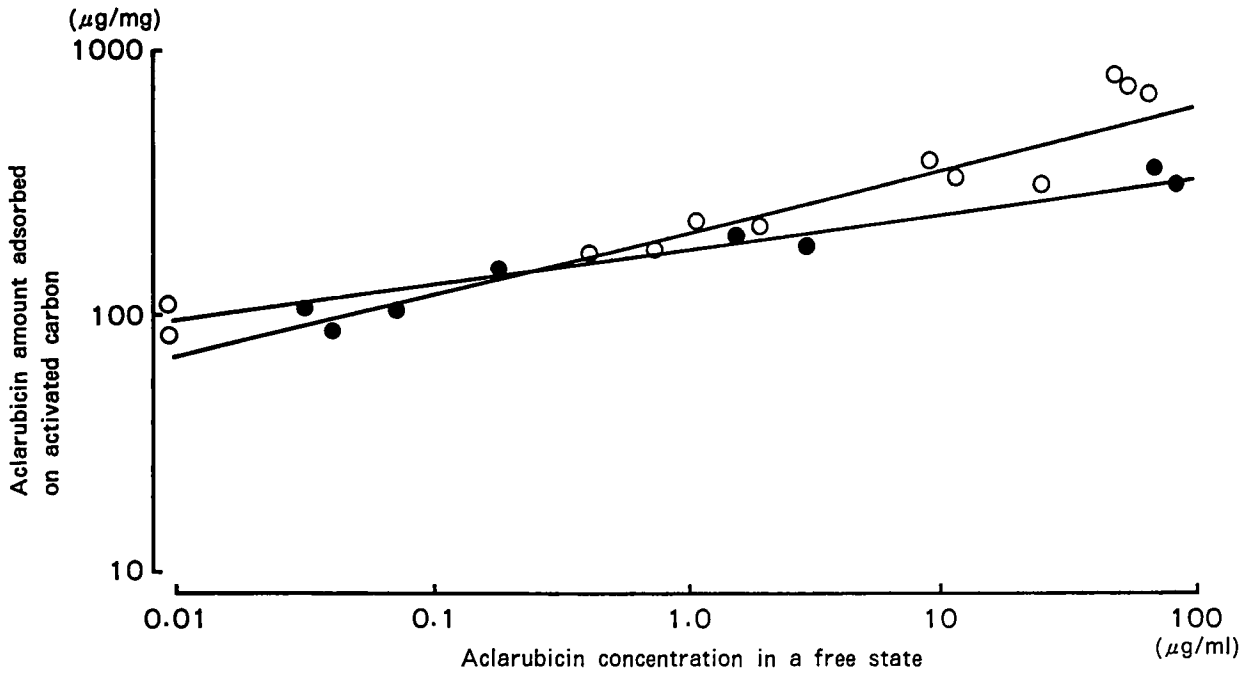

Figure 1. Adsorption isotherm of aclarubicin onto activated carbon. The adsorption isotherms of aclarubicin on activated carbon at 37°C in saline (●) and in phosphate buffer solution at pH 7.4–7.5 (○) are shown as $Q = 175C^{0.15}$ and $Q = 207C^{0.24}$ (where Q is the aclarubicin amount adsorbed on activated carbon in μg/mg, and C is the aclarubicin concentration in a free state in μg/ml), respectively.

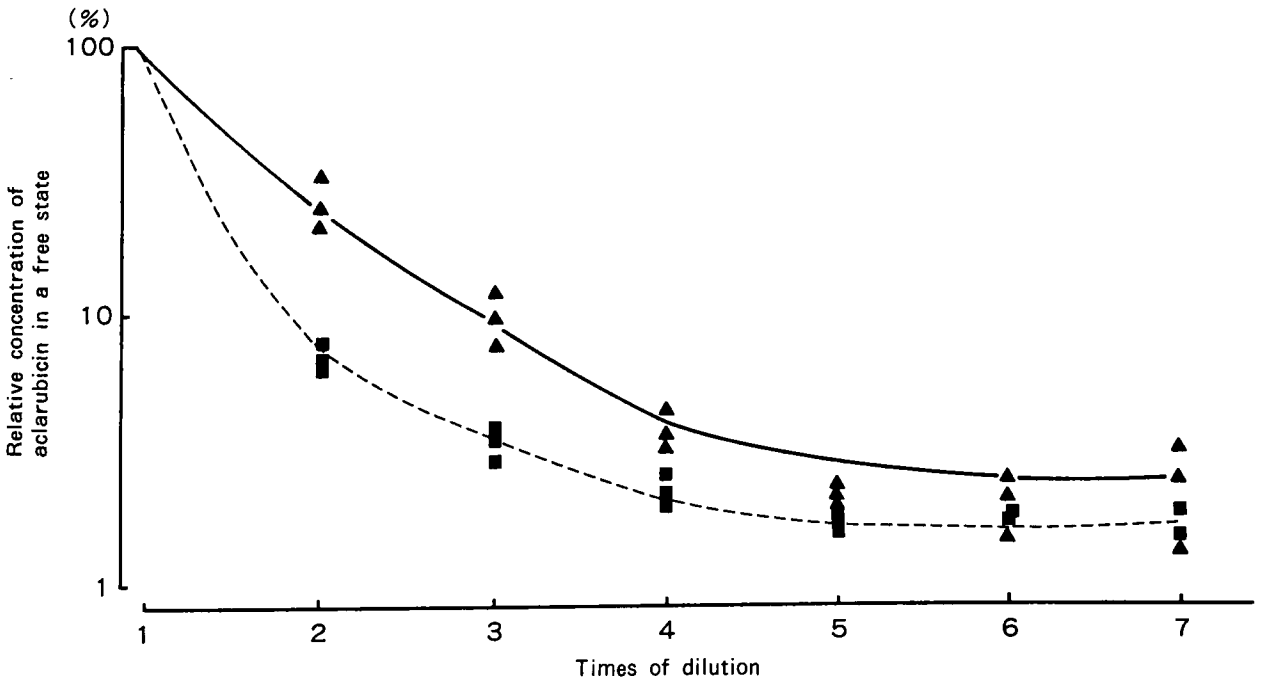

Figure 2. Desorption line of aclarubicin from activated carbon. Desorbed aclarubicin concentration in a free state from the activated carbon at 37°C in saline (▲) and in phosphate buffer solution at pH 7.4–7.5 (■) are shown. Aclarubicin was desorbed constantly at relatively small amounts (30% to 3% of the initial concentration in saline at 6% to 1.6% of the initial concentration in buffer solution).

Table 1. Aclarubicin concentration in axillary lymph nodes

Time after administration	Aclarubicin concentration (mean ± SD, μg/g)		Statistical significance
	Dosage form		
	ACR-CH	ACR-sol	
1 h	89 ± 44	77 ± 14	NS
3 h	119 ± 10	43 ± 14	$p < 0.01$
6 h	29 ± 10	9.3 ± 7.0	NS
12 h	15 ± 10	7.5 ± 3.0	NS
24 h	12 ± 5	2.3 ± 4.6	NS
48 h	5.8 ± 3.5	0.3	NS
7 days	4.6 ± 2.6	—	NS

NS: not significant.
—: not detectable.
Each value represents the mean ± standard deviation (SD) for 3–5 experiments.

rapidly thereafter. There was a statistically significant difference ($p < 0.01$) of aclarubicin concentration at 3 h after injection between the two dosage forms.

Blood plasma and erythrocytes: In the ACR-CH group, the aclarubicin concentration was less than the assay limitation, both in the blood plasma and the blood cells, during the experimental period. In the ACR-sol group, the concentration in plasma was 240 ng/ml at 1 h, decreasing to less than assay limitation at 48 h. The concentration in erythrocytes in the ACR-sol group was 150 ng/g (the maximal value) at 3 h, decreasing to less than assay limitation at 24 h. There was a statistically significant ($p < 0.01$) difference in the aclarubicin concentration in plasma and blood cells between the ACR-CH group and the ACR-sol group up to 12 h after injection (Tables 2 and 3).

Heart: Aclarubicin concentration in the heart was less than 0.12 μg/g (mean value) in the ACR-CH group. In the ACR-sol group, it peaked to 1.3 μg/g at 1 h and decreased to less than assay limitation within 48 h. There was a statistically significant ($p < 0.01$) difference in aclarubicin concentration between the ACR-CH group and the ACR-sol group during the initial 6 h (Table 4).

Lung: In the lung, aclarubicin concentration was less than 1.7 μg/g and was not detectable 6 h after injection in the ACR-CH group. In the ACR-sol group, it was 9.5 μg/g at 3 h and detectable up to

Table 2. Aclarubicin concentration in blood plasma

Time after administration	Aclarubicin concentration (mean ± SD, μg/ml)		Statistical significance
	Dosage form		
	ACR-CH	ACR-sol	
1 h	—	0.24 ± 0.04	$p < 0.01$
3 h	—	0.22 ± 0.06	$p < 0.01$
6 h	—	0.14 ± 0.03	$p < 0.01$
12 h	—	0.11 ± 0.02	$p < 0.01$
24 h	—	0.07 ± 0.04	NS
48 h	—	—	NS

NS: not significant.
—: not detectable.
Each value represents the mean ± standard deviation (SD) for 3–4 experiments.

Table 3. Aclarubicin concentration in erythrocytes

Time after administration	Aclarubicin concentration (mean ± SD, μg/g)		Statistical significance
	Dosage form		
	ACR-CH	ACR-sol	
1 h	—	0.13 ± 0.02	$p < 0.01$
3 h	—	0.15 ± 0.02	$p < 0.01$
6 h	—	0.10 ± 0.01	$p < 0.01$
12 h	—	0.12 ± 0.01	$p < 0.01$
24 h	—	—	NS
48 h	—	—	NS

NS: not significant.
—: not detectable.
Each value represents the mean ± standard deviation (SD) for 3–4 experiments.

Table 4. Aclarubicin concentration in heart

Time after administration	Aclarubicin concentration (mean ± SD, μg/g)		Statistical significance
	Dosage form		
	ACR-CH	ACR-sol	
1 h	0.11 ± 0.10	1.30 ± 0.23	$p < 0.01$
3 h	0.12 ± 0.12	0.69 ± 0.12	$p < 0.01$
6 h	0.02 ± 0.02	0.77 ± 0.39	$p < 0.01$
12 h	0.10 ± 0.08	0.36 ± 0.26	NS
24 h	—	0.12 ± 0.06	NS
48 h	—	—	NS

NS: not significant.
—: not detectable.
Each value represents the mean ± standard deviation (SD) for 3–4 experiments.

48 h after injection. There was a statistically significant ($p < 0.01$) difference during the initial 12 h between the two dosage forms (Table 5).

Kidney: Aclarubicin concentration in the kidney was less than 0.11 μg/g and decreased to less than assay limitation at 6 h in the ACR-CH group. In the ACR-sol group, it was 0.81 μg/g at 1 h and detectable up to 24 h. There were statistically significant differences ($p < 0.01$) between the two groups during the initial 6 h (Table 6).

Spleen: Aclarubicin concentration was not detectable during the experimental period in the ACR-CH group. In the ACR-sol group, it was 4.1 μg/g at 1 h, and detectable up to 48 h after injection. There were statistically significant ($p < 0.01$) differences between the two dosage forms for 6 h after administration (Table 7).

Table 5. Aclarubicin concentration in lung

Time after administration	Aclarubicin concentration (mean ± SD, μg/g)		Statistical significance
	Dosage form		
	ACR-CH	ACR-sol	
1 h	1.3 ± 1.2	5.3 ± 1.4	$p < 0.01$
3 h	1.7 ± 1.6	9.5 ± 0.8	$p < 0.01$
6 h	—	3.7 ± 0.9	$p < 0.01$
12 h	—	2.9 ± 0.4	$p < 0.01$
24 h	—	1.4 ± 0.3	NS
48 h	—	0.23 ± 0.02	NS

NS: not significant.
—: not detectable.
Each value represents the mean ± standard deviation (SD) for 3–4 experiments.

Table 6. Aclarubicin concentration in kidney

Time after administration	Aclarubicin concentration (mean ± SD, μg/g)		Statistical significance
	Dosage form		
	ACR-CH	ACR-sol	
1 h	0.10 ± 0.09	0.81 ± 0.09	$p < 0.01$
3 h	0.11 ± 0.11	0.57 ± 0.09	$p < 0.01$
6 h	—	0.22 ± 0.06	$p < 0.01$
12 h	—	0.13 ± 0.11	NS
24 h	—	0.06 ± 0.04	NS
48 h	—	—	NS

NS: not significant.
—: not detectable.
Each value represents the mean ± standard deviation (SD) for 3–4 experiments.

Table 7. Aclarubicin concentration in spleen

Time after administration	Aclarubicin concentration (mean ± SD, μg/g)		Statistical significance
	Dosage form		
	ACR-CH	ACR-sol	
1 h	—	4.1 ± 0.5	$p < 0.01$
3 h	—	2.0 ± 1.2	$p < 0.01$
6 h	—	3.6 ± 0.8	$p < 0.01$
12 h	—	1.2 ± 0.5	NS
24 h	—	0.29 ± 0.23	NS
48 h	—	0.06 ± 0.06	NS

NS: not significant.
—: not detectable.
Each value represents the mean ± standard deviation (SD) for 3–4 experiments.

Discussion

A large amount of aclarubicin is adsorbed onto the activated carbon and desorbed again from the activated carbon in a wide range of aclarubicin concentrations in the free state. This means that the adsorbed aclarubicin remains in a dynamic equilibrium with the aclarubicin concentration in the free state. When the aclarubicin concentration in the free state is decreased around the activated carbon, the activated carbon releases aclarubicin replacing the decreased concentration. Thus, the aclarubicin concentration in the free state is kept at a constant level around the carbon particles.

Small corpuscular particles are not absorbed through the blood capillary wall but are absorbed through the lymph capillary wall into the lymph flow, and smoothly distributed to the regional lymph nodes.[7–9] Utilizing this property of corpuscular particles, we designed a new dosage form consisting of small carbon particles adsorbing aclarubicin. Our experiments with rats revealed that ACR-CH distributed aclarubicin at large amounts to the regional lymph nodes for a long period of time; the drug was distributed at small amounts to the heart and the reticuloendothelial tissues as well as to other whole-body tissues, compared with ACR-sol. The main side effects of aclarubicin are the heart toxicity and the suppression of bone marrow. The anti-cancer effects of aclarubicin depend on its acting period as well as concentra-

tion.[10] The present results lead us to think that the therapeutic effects of aclarubicin on metastatic lesions in the regional lymph nodes will become larger and the side effects will become smaller, when ACR-CH is injected pre- and intra-operatively into the breast tissues with cancer lesion. We therefore conclude that subcutaneously injected ACR-CH selectively distributes a high level of aclarubicin to the regional lymph nodes, with low levels to the other whole body tissues, as compared with ACR-sol.

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