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Selective drug delivery to peri-tumoral region and regional lymphatics by local injection of aclarubicin adsorbed on activated carbon particles in patients with breast cancer—a pilot study

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ACR-CH, which consists of aclarubicin (ACR) adsorbed onto activated carbon particles, was developed for locoregional chemotherapy for breast cancer. Thirty patients with breast cancer received an ACR (10 mg) injection intra- and peri-tumorally, either as ACR-CH or as ACR aqueous solution (ACR-AQ) 5 min before the operation for breast cancer. The ACR concentrations were significantly higher in the peri-tumoral regions and regional lymph nodes, and were also significantly lower in the blood plasma in patients given ACR-CH versus patients given ACR-AQ.

Key words: Aclarubicin, activated carbon, breast cancer.

Introduction

Corpuscular particles such as fine activated carbon particles are retained around the injection site, and are absorbed gradually through lymphatic capillaries and distributed to the regional lymphatic system, while water-soluble small molecules are absorbed rapidly through the blood capillary wall into the circulation, and are neither retained around the injection site for a long period of time nor distributed selectively to the lymphatics.[1] We have developed a new dosage formulation (ACR-CH) of fine activated carbon particles adsorbing aclarubicin (ACR) for locoregional adjuvant chemotherapy in surgery for breast cancer. ACR-CH is designed to maintain highly concentrated ACR in a free state for a prolonged period of time around the particles of ACR-CH.[2] In animal experiments we have shown that ACR-CH selectively distributes high levels of free ACR to the regional lymphatic system and low levels to the rest of the body.[2] Animal experiments also have revealed that ACR-CH reduces toxicity to less than half of that seen with the ACR aqueous solution[3] and that ACR-CH achieves a superior therapeutic effect on metastases in the regional lympatics as compared to the ACR aqueous solution.[4]

In the first of the clinical pilot studies, we examined the drug distribution in comparison with a local injection of ACR aqueous solution in patients who underwent surgery for breast cancer.

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Materials and methods

Preparation of the drug

As an anticancer drug we used aclarubicin[5] (aclacynomycin A, Aclacinon®; Yamanouchi Pharmaceutical, Tokyo, Japan) which is one of the anthracycline antibiotics[5] and to which breast cancer has demonstrated sensitivity.[6] We used activated carbon (activated carbon 1500AA; prepared in our laboratory) with a diameter of 20 nm for primary particles and with a specific surface area of 1480 m^2/g as the drug carrier. Activated carbon at 50 mg/ml and polyvinylpyrrolidone (PVP K-30; Nakarai Chemicals, Kyoto, Japan) at 20 mg/ml were mixed in saline and kneaded with three rollers to produce a carbon particle suspension. The average size of the suspension particles was 157 nm^2 as measured by photon correlation spectroscopy. 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 carbon suspension and the mixture was shaken at 120 cycles/min for 1 h at 37°C to bring the adsorption of aclarubicin onto the carbon to equilibrium. Thus, ACR-CH composed of 5 mg/ml of aclarubicin, 50 mg/ml of activated carbon and

20 mg/ml of polyvinylpyrrolidone in saline was prepared. Aclarubicin aqueous solution (ACR-AQ) (5 mg/ml) was prepared in saline as a control.

Patient treatment

The present study included 30 patients who were to undergo surgical treatment for breast cancer at Kyoto Prefectural University of Medicine Hospital and Uji Hospital from 1992 to 1995. All patients met the following criteria: (i) younger than 70 years old, (ii) no findings of hematogenous metastases on chest X-ray films or X-ray CT, (iii) not treated with chemotherapy before the ACR-CH therapy, (iv) no abnormalities on electrocardiography and (v) not diagnosed with so-called locally advanced breast cancer. The surgical department gave ethical approval to the studies and all patients gave informed consent. No other preoperative or intraoperative chemotherapy was administered.

The 30 patients were randomized to two groups of 15 patients, i.e. the ACR-CH group and the ACR-AQ group. Immediately (5 min on average, range of 4–6 min) prior to the beginning of the operation, ACR (10 mg) was injected into the tumor and the peri-tumoral region in the form of either ACR-CH or ACR-AQ. At 10, 30 and 90 min after administration peripheral blood was taken and centrifuged at 5000 r.p.m. for 5 min. The blood plasma was used to assay the ACR concentration. Immediately after surgical removal of the primary tumor and the regional lymph nodes, the peri-tumoral region (the injection site) and the regional lymph nodes were separated from the adipose tissues and used in the ACR concentration assay. The samples were stored at −80°C in a deep freezer. The tissue sample of lymph nodes was minced in 5-fold distilled water and prepared into a suspension of tissue fractions by a tissue homogenizer. The suspension was centrifuged at 6000 r.p.m. for 5 min to remove the tissue fractions and the carbon particles adsorbing ACR. The supernatant was then subjected to an ACR concentration assay to measure the concentration of free ACR acting on the tissues. The ACR concentration was assayed by the high performance liquid chromatography method,[7] and the fluorometric method using excitation and emission maxima at 440–445 nm and 505 nm wavelengths.[8] The drug concentration was expressed in terms of the free ACR content in wet tissue or in plasma. The assay sensitivity was 1 ng/ml in blood plasma and 5 ng/g in tissues. We compared the two groups in terms of the clinicopathologic characteristics such as patient age, location and size of the primary tumor, histologic type and status of nodal metastases. The size of the tumor was expressed as (the longest diameter) × (the tumor diameter perpendicular to the longest diameter) in mm^2.

Analysis of variance and the χ^2 test were used in statistical analyses. A difference was considered statistically significant when the probability value (p) was less than 0.05.

Results

In the peri-tumoral region (the injection site), the mean ACR concentration was 235 μg/g [190–280 μg/g with a 95% confidence interval (CI)] in the ACR-CH group and 145 μg/g (91–199 μg/g with a 95% CI) in the ACR-AQ group (Figure 1). There was a significant difference of the ACR concentration ($p<0.05$) between the two groups. The mean ACR concentration in the regional lymph nodes was 41.0 μg/g (25.3–56.8 μg/g with a 95% CI) in the ACR-CH group. This was significantly higher ($p<0.05$) than the mean of 21.7 μg/g (8.05–35.3 μg/g with a 95% CI) in the ACR-AQ group (Figure 2).

In the ACR-CH group, the ACR concentration in blood plasma remained at a low level: 0.005 μg/ml (zero to 0.014 μg/ml with a 95% CI) at 10 min, 0.002 μg/ml (0–0.006 μg/ml with a 95% CI) at 30 min and below the level of sensitivity of the assay at 90 min after administration. In the ACR-AQ group,

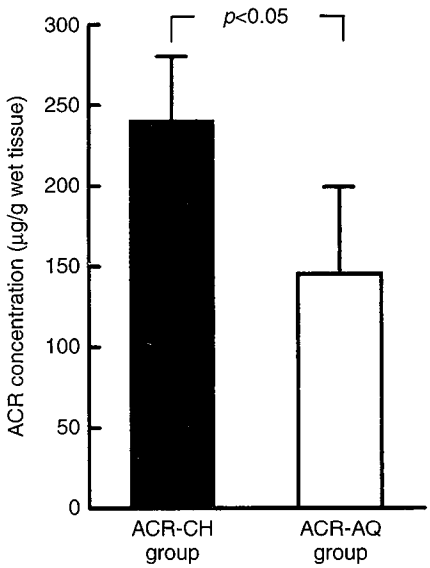

Figure 1. ACR concentration in the peri-tumoral region (the injection site). ACR concentration in the ACR-CH group was significantly ($p<0.05$) higher than that in the ACR-AQ group. A vertical bar represents the 95% CI. The black and white columns demonstrate the means in the ACR-CH and ACR-AQ groups, respectively.

the concentration in blood plasma was 0.017 μg/ml (0.007–0.027 μg/ml with a 95% CI) at 10 min, 0.010 μg/ml (0.005–0.015 μg/ml with a 95% CI) at 30 min and undetectable at 90 min after administration. There was a significant ($p<0.05$) difference in the blood plasma ACR concentration at 30 min after administration (Figure 3). Thus, ACR-CH resulted in a low concentration of ACR in the blood plasma, although a higher concentration was found in the regional nodes as compared to ACR-AQ.

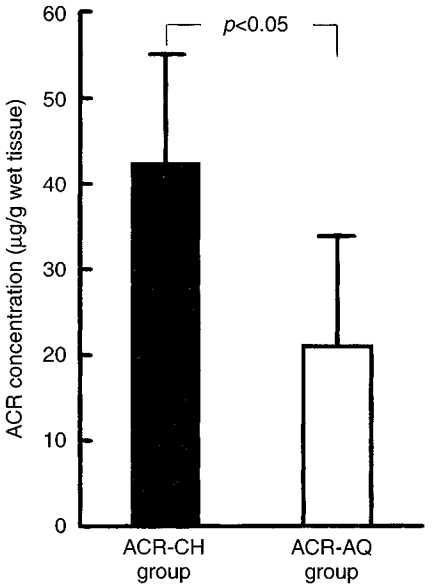

Figure 2. ACR concentration in the regional lymph nodes. ACR concentration in the ACR-CH group was significantly ($p<0.05$) higher than that in the ACR-AQ group. A vertical bar represents the 95% CI. The black and white columns demonstrate the means in the ACR-CH and ACR-AQ groups, respectively.

There were no statistically significant differences in clinico-pathologic characteristics between the two patient groups (Table 1).

Discussion

In breast cancer, post-operative locoregional recurrence is a serious problem,[9–11] not only in locally advanced cancers but also in 'early' cancers. Recently, the trend in surgery for breast cancer has tended to move towards 'smaller' operations. This means a narrower surgical margin from the primary tumor and a smaller visual field for the dissection of the regional nodes. This may result in an increase risk for residual microscopic disease and of disseminating cancer cells during the surgery. In order to prevent locoregional recurrences, the cancer cells potentially contained in the peri-tumoral regions and the regional lymphatic sysyem located in the locoregional tissues should be 'inactivated' before the surgery is performed. The purpose of ACR-CH was to inactivate these cancer cells potentially contained in the peri-tumoral regions and regional lymphatic system.

ACR, known as aclacinomycin A, is one of the anthracycline antibiotics.[6] We used ACR in the present study because (i) ACR has therapeutic effects on breast cancer[7–10] and (ii) preliminary experiments revealed that a greater amount of ACR is adsorbed onto activated carbon than other anthracyclic anticancer drugs which are known to be therapeutically effective against breast cancer. An *in vitro* study has shown that there is a dynamic equilibrium between adsorbed ACR and ACR in a free state.[2] When the free ACR concentration decreases around the carbon particles

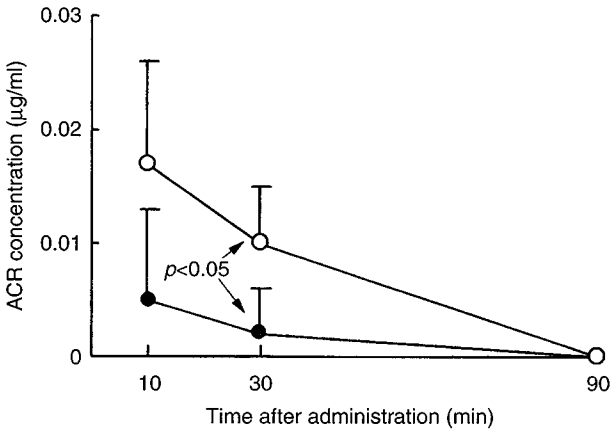

Figure 3. ACR concentration in blood plasma. ACR concentration in blood plasma was significantly higher ($p<0.05$) at 30 min after administration in the ACR-AQ group than in the ACR-CH group. A vertical bar represents the 95% CI. Closed and open circles demonstrate the means in the ACR-CH and ACR-AQ groups, respectively.

Table 1. Patient characterstics

	ACR-CH group	ACR-AQ group	
Mean age (years)	55.3 (49.0–61.7)	54.2 (47.9–60.6)	NS[a]
Tumor size (mm^2)	493 (0–1022)	695 (166–1224)	NS
Status of nodal metastasis[b]	1.07 (0–2.27)	1.53 (0.331–2.74)	NS
Location of the primary[c]			NS
A and AC	3	3	
B	2	1	
C and CD	7	8	
D	2	3	
E	1	0	
Histology[d]			NS
papillotubular carcinoma	7	6	
solid-tubular carcinoma	4	3	
scirrhous carcinoma	1	2	
unknown	3	4	

[a]NS, not significant.
[b]Number of regional lymph nodes with histologically proven metastasis.
[c]The locations A, B, C, D and E (and histologic type) were classified according to General Rules for Clinical and Pathological Recording of Breast Cancer:[13] A, upper-inner quadrant; B, lower-inner quadrant; C, upper-outer quadrant; D, lower-outer quadrant; E, central portion. 95% CI in parentheses.

due to absorption into the surrounding tissues and dilution into the tissue fluids, the carbon particles release the same amount of free ACR, thus raising the free ACR concentration. The free ACR concentration is therefore maintained at a constant level.[2]

Locally injected, small particles such as fine activated carbon particles infiltrate into the interstitial space around the injection site and are gradually absorbed from the lymphatic capillaries into the lymphatic system.[12] Thus, the carbon particles are retained selectively in the local tissues around the injection site and the regional lymphatic system for a long period of time.[13] In animal experiments, it has been demonstrated that a greater concentration of free ACR is distributed to the regional lymphatic system for a longer period of time via ACR-CH administration than via ACR-AQ administration[2] and that ACR-CH has a superior therapeutic effect against cancer lesions in the regional lymphatic system.[4] The present study in humans also showed that ACR-CH could induce high concentrations of ACR at the injection site (the peri-tumoral region) and the regional lymph nodes. The anticancer effects of ACR depend both on the concentration and the time of exposure.[14] The result of the present study suggests that the local injection of ACR-CH distributes a high concentration of ACR to the peri-tumoral regions and regional lymphatics, and that ACR-CH will have a selective therapeutic effect against microscopic cancer tissues located in the peri-tumoral regions and regional lymphatic system.

An adequate local or locoregional treatment,[15–18] such as radiation with or without systemic chemotherapy, or intra-arterial anticancer drugs, reduces the risk of locoregional recurrence, in early or locally advanced breast cancers. As compared with these locoregional treatments, ACR-CH therapy has several time-saving advantages, and ACR-CH therapy is economical and does not require special techniques or equipment.

In the present study, a therapeutically minimal dose of ACR of 10 mg/person was administered at 4–6 min prior to beginning the surgery, because the main purpose of this study was to examine the drug distribution rather than to evaluate its therapeutic effect. The prophylactic effects of ACR-CH against local recurrence should be evaluated by further examinations with a randomized controlled trial, and these studies should clarify the indications for the ACR-CH therapy and the optimal dose and timing of ACR administration.

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