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Report

Modification of adriamycin pharmacokinetics by direct electric current in rats

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Adriamycin (ADR, doxorubicin), a drug having cardiotoxicity, is electrically charged as a cation in blood. We therefore investigated whether iontophoresis caused by direct electric current (DC; 50 μA, 90 min) would cause systemic modification of ADR pharmacokinetics. Cathode and anode were placed into a right kidney and muscles of the abdominal wall, respectively, in six Donryu rats. Urinary excretion of ADR, as measured by catheterizing into the right kidney, was significantly higher in the DC group than in the controls ($p < 0.05$). Both plasmic and renal ADR clearances were significantly higher in the DC group ($p < 0.005$ and $p < 0.001$, respectively). Tissue ADR concentrations were significantly lower in the DC group (heart: $p < 0.003$; liver and lung: both $p < 0.05$). These results suggest that electric therapy might potentially induce modification of ADR pharmacokinetics by iontophoresis, and that the therapy might effectively change ADR concentration both locally and systemically. [© 2002 Lippincott Williams & Wilkins.]

Key words: Adriamycin, direct electric current, drug delivery system, electric therapy, pharmacokinetic modification.

Introduction

Adriamycin (ADR, doxorubicin) was discovered over 30 years ago, and is still one of the most widely used and effective anticancer drugs for the treatment of many tumors including breast and bladder cancers as well as Hodgkin's lymphomas.[1,2] However, despite widespread use, the clinical utility of ADR is severely compromised by dosage-dependent cardiotoxicity which poses a lifetime risk for patients. Chronic cardiotoxicity can develop many years after treatment. Children and younger adults treated with such anthracycline drugs are exposed to a lifetime risk of developing serious cardiomyopathy.[3] Because cancer patients are not usually monitored for more than 5–7 years, the number of these patients developing late-onset cardiomyopathies can be expected to increase substantially later on.[4,5]

For the purpose of minimizing side effects, including these cardiac toxicities induced by ADR, we utilize electric therapy in order to enhance the drug delivery system (DDS). Electric therapy for cancer patients has been described in various reports to date. A breakthrough was the report of disappearance of the tumor confirmed in 12 and a reduction in seven of 20 cases, induced by direct electric current (DC) in the range of 1.5–15 V, 400–1330 C in total.[6] This Swedish study was based on the original theory of biologically closed electric circuits (BCEC).[6] In a combined treatment of electric current and chemotherapy, bleomycin hydrochloride (BLM) was successfully gathered and accumulated around the cathode more than around the anode when electrodes were introduced into cancer tissues (10 V, 40 C).[7] Conventionally, two types of procedures have been attempted in electric therapy: one using two active electrodes inserted into target tissues; the other using one active electrode with the other one grounded. The efficacy of these protocols has been controversial, since the optimum amount of DC including voltage, amperage and therapy duration have not been established.

Although DDS theory has been developed for years, we have found no reports so far regarding the efficacy of electric therapy concomitant with chemotherapy on the basis of DDS theory. To confirm this efficacy by combining electric therapy and iontophoresis, it would be reasonable to use drugs that are ionized *in vivo* such as ADR (ionized as cation in blood) and methotrexate (MTX, ionized as

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anion). It seems reasonable to think that electric therapy would promote ionization of these drugs and so would gather more amount of ionized drug around to the other side of electrode which possesses the antipole.

Here, we investigated the pharmacokinetic modification of ADR induced by electric therapy in an experimental animal study.

Methods

Six male Donryu rats (age: 8 weeks; weight: 330–358 g) were divided into two groups: DC and controls (three rats each). The study protocol was based on the Guidelines for Animal Study (Ethics Committee of Nagoya University). The timeline of the experimental procedure is illustrated in Figure 1. All rats had to be anesthetized [pentobarbital (Nembutal) and ether] and restrained in supine position during current application. They were then catheterized in the right ureter 10–20 min after the anesthesia. The hilum of left kidney was ligated with silk thread for the purpose of excreting most of urine into the right kidney.

Current application

Current was applied for a full 90 min at 3.5. V, 50 μA (0.27 C in total). Both electrodes were of pure platinum wire (Kono Seisakusyo, Chiba, Japan), insulated with Teflon (polytetrafluoroethylene) coating except at both ends. The exposed ends were 5.0 mm long, had a diameter of 0.5 mm and an active surface area of 7.85 mm^2. Thus, the current density was calculated to be 6.37 A/cm^2. Each pair of platinum electrodes was inserted percutaneously through a small skin incision directly into the medullary portion of the right kidney (cathode) and muscles of the abdominal wall (anode). Proper electrode placement was verified by checking whether or not penetration of the top of the electrode was observed. The current source was a 3.5 V battery. The current level was monitored constantly by microammeter to maintain the intended current level. Control animals had similarly inserted electrodes, but they were not connected to the power source. Considering the frequent measurement, one pair of electrode outputs was used, since the experiment was limited to perform for one rat at a time; the same procedure was repeated a total of 6 times to measure six rats. The electrodes were replaced each time by new ones

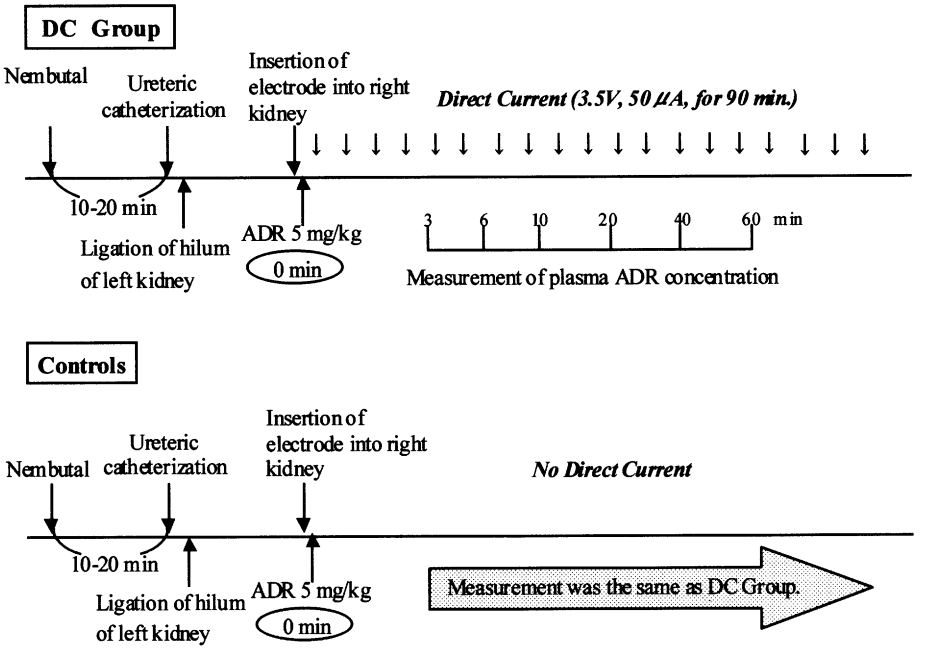

Figure 1. The timeline of the experimental procedure. Immediately after insertion of the electrodes, electric therapy (for the treated group) and ADR injection followed, and analyses were begun.

to prevent over-measurement of ADR resulting from possible drug residue adhering to the reused electrode.

Administration of ADR

A one-shot injection of ADR (Adriacin®; Kyowa Hakko, Tokyo, Japan) to the tail vein was given at a dose of 5 mg/kg dissolved in normal saline solution 30 min after initiation of electric therapy.

Measurement of ADR

Total excretion of urinary ADR was measured from total urine eliminated into the catheterized right ureter from 0 to 90 min post-injection. Blood samples were collected 6 times (3, 6, 10, 20, 40 and 60 min after ADR injection) for a total of 6×0.5 ml samples for each rat from the subclavian vein. After completion of electric therapy, tissue samples were resected from ablated organs including heart, liver, lung and kidney. Both plasma and tissue ADR concentrations were determined using high-performance liquid chromatography (HPLC) once per blood sample. The HPLC method followed that of a previous study.[8] ADR was separated on chromatograms by use of a reversed-phase column which yielded better determinations than a normal phase column.

Statistical analysis

Comparisons between the groups were statistically assessed by one-way analysis of variance (ANOVA). When statistically significant differences were found, pairwise comparisons were performed by Scheffe's multiple comparisons test, with $p<0.05$ as the minimum level of significance.

Results

Comparison of renal and plasma ADR

The total amount of urinary ADR excretion from the right kidney (cathode side) was significantly higher in the DC group than in the controls (mean $\pm$ SE: 64.2 ± 1.9 and 50.3 ± 1.3 μg, respectively, $n=3$, $p<0.05$). According to plasma ADR concentration–time analysis, the ADR concentration of the DC group was significantly lower than in the controls at 3, 6 and 10 min after injection (DC group mean $\pm$ SE: 2.34 ± 0.18, 0.92 ± 0.16 and 0.35 ± 0.06 μg/ml; controls: 3.43 ± 0.33, 1.71 ± 0.04 and 0.65 ± 0.02 μg/ml; $p<0.05$, $p<0.01$ and $p<0.01$, respectively; Figure 2). Plasma and renal ADR clearances (Figure 3) showed statistically significant differences between the DC group and the controls (DC group mean $\pm$ SE: 4.068 ± 0.027 and 0.148 ± 0.006 l/h · kg; controls: 2.779 ± 0.185 and 0.084 ± 0.004 l/h · kg; $p<0.005$ and $p<0.001$, respectively).

Tissue ADR contents

Tissue ADR concentration in kidney showed no difference between groups, whereas those in heart, liver and lung were significantly lower in the DC group than in the controls (mean $\pm$ SE: 7.1 ± 0.5 versus 10.3 ± 0.1 μg/g, $p<0.003$, 12.6 ± 0.3 versus 17.1 ± 1.1 μg/g, $p<0.05$, 8.3 ± 0.6 versus 14.8 ± 2.2 μg/g, $p<0.05$, respectively; Figure 4).

Discussion

Generally, ADR has a two-phase pharmacokinetic profile[9,10] in a time-dependent manner both in animals and humans. As for the pharmacokinetic parameter of ADR in human plasma, the half-life of each phase was approximately 10 min (α) and 30 h (β). A similar curve was observed in animal experiments,[11] though the authors divided the second phase into two (β and γ), which showed that the

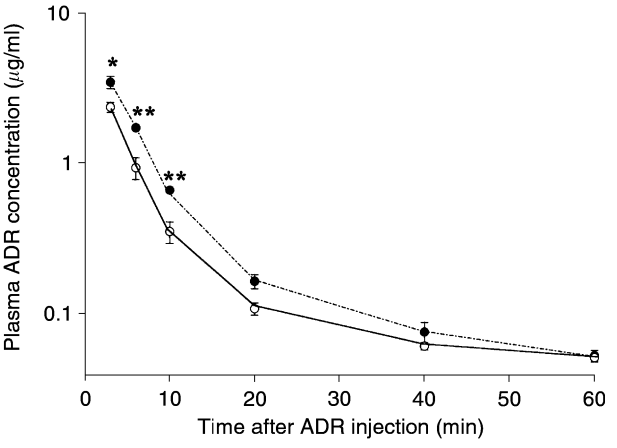

Figure 2. Plasma concentration–time curves of ADR: (○) DC group and (●) controls. Standard error (SE) was illustrated at each point of time. Significant differences were observed 3, 6 and 10 min after ADR injection. $^{*}p < 0.05$, $^{**}p < 0.01$.

peripheral compartment volume of distribution ($V_{2,3}$) was predominantly larger than the central compartment volume of distribution (V_1) (1.18, 27.34 versus 0.31, respectively). The concentration of unmetabolized ADR in tissue was markedly higher than adriamycinol or aglycone metabolite.[12–14] Based on these facts, a higher concentration of unmetabolized ADR is characteristically retained for a longer period of time in tissues. Therefore, after administration of ADR, a water-soluble agent into the bloodstream as cation, it is immediately taken up by tissues, i.e. the peripheral compartment. A part of ADR, thus, strongly binds to DNA with an intercalation of aglycone between the hydrophobic faces of a DNA base pair to form an ADR–DNA complex,[15,16] which inhibits DNA and RNA polymerase synthesis.[17]

On the other hand, ADR binds to flavin mononucleotide and activates the semi- quinone radical.[18] Previous reports have suggested that oxygen free radicals and a drug metabolite [C-13 hydroxy metabolite, adriamycinol (ADR-OH)] play a major role in the development of cardiotoxicity.[3,19–21] To counteract the toxicity of ADR, another anticancer agent, tamoxifen[22] or still other agents such as dexrazoxane,[23] a multidrug-resistance reversing agent[24] and mitochondrial enzyme[25] have been concomitantly applied.

ADR is predominantly metabolized in liver, and more of it is excreted into bile (approximately 40%; 42% as ADR, 22% as adriamycinol and 36% as other metabolites, in 7 days) than into urine (approximately 10–20%; 40% as ADR, 29% as adriamycinol and 31% as other metabolites, in 5 days).[14,26] Excretion within 24 h revealed only unmetabolized ADR.[27,28] Therefore, changes in renal function would appear to have little effect on drug elimination,[14] suggesting that individual differences in renal function in rats were of less importance in the study.

Though almost no one has ever reported pharmacokinetically modifying an electrically charged drug induced by electric therapy, we observed some results from which some implications might be drawn.

(1) Significantly more amounts of ADR, charged as cation *in vivo*, could be excreted from the cathode-side kidney ($p<0.05$). Although ligation of the

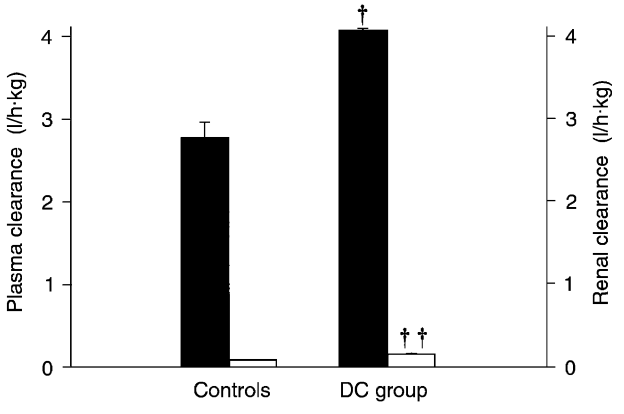

Figure 3. Plasma and renal clearance of ADR: (■) plasma clearance and (□) renal clearance. Standard error (SE) was presented in each bar. Both were significantly lower in the DC group than in the controls. $^{\dagger}p < 0.005$, $^{\dagger\dagger}p < 0.001$.

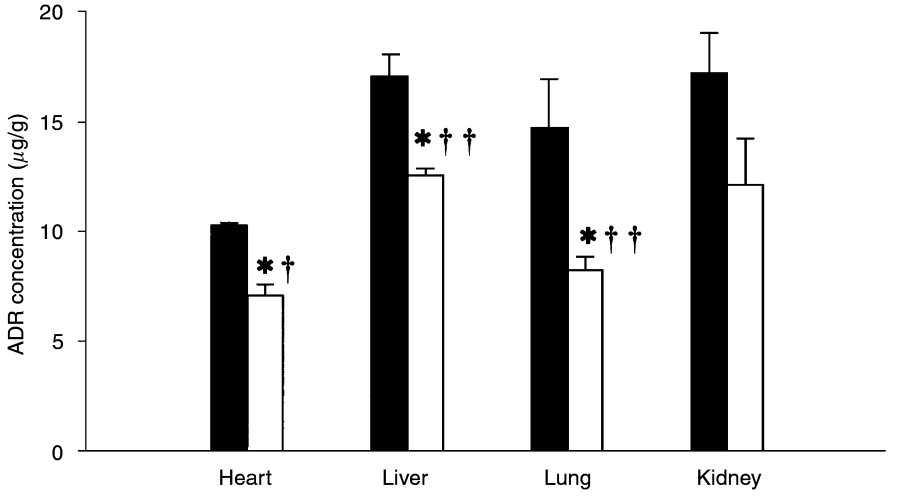

Figure 4. Tissue ADR concentration: (□) DC group and (■) controls. Standard error (SE) was presented in each bar. Lower ADR concentration was observed in the four organs. Significant differences were favored in heart, liver and lung. $^{*\dagger}p < 0.003$, $^{*\dagger\dagger}p < 0.05$.

hilum of the left kidney would be controversial in clinical application, it would be important should the result prove consistent with our previous outcome,[29] i.e. that significantly more MTX, charged as anion *in vivo*, was excreted from the anode-side kidney ($p < 0.01$), based on our iontophoretic hypothesis.

(2) Significant differences in plasma concentrations were observed at earlier stages such as 3, 6, and 10 min, and those were within the associated α compartment. Taking account of the fact that the principal circulating form is the unmetabolized drug,[16] the following time after 10 min, the plasma concentration would be of less importance from the point of cytotoxicity. The elimination rate constant, K_{12}, tended to be higher in the DC group than in the controls (mean $\pm$ SE: 19.088 $\pm$ 2.655 versus 15.022 $\pm$ 0.803 h^{-1}). Iontophoresis induced by DC might be somewhat involved in promoting ADR uptake from blood into tissue, which might further suggest that electric therapy could be involved to some degree in the inhibition of ADR–DNA binding in tissues. These suppositions must be investigated to ascertain the modification of ADR pharmacokinetics.

(3) The p value of renal clearance exceeded that of plasma clearance ($p < 0.001$ versus $p < 0.005$). In addition, the liver ADR concentration showed significant differences between the groups. Considering those results and the fact that ADR is characteristically metabolized mainly in liver, electric therapy might potentially modify ADR pharmacokinetics by accelerating renal ADR excretion and decelerating ADR metabolism in liver. If this is the case, cardiotoxicity induced by metabolized ADR (adriamycinol) would be decreased by electric therapy. Moreover, the result that there was statistically lower ADR concentration in heart tissue from the DC group than that from the controls might suggest a potential role of iontophoresis as an inhibitor of ADR from accumulating in heart. ADR pharmacokinetics were thus observed to be modified not only locally but also systemically. Further investigations using tumor-bearing models should be required to ascertain whether the ADR concentration would be increased in tumor tissue, but reduced in the cardiac tissue. In addition, it is of great consequence to elucidate that electric therapy, by placing the respective electrodes into normal and tumor tissue, would potentially gather a greater amount of ADR into the targeted tumor tissue than electrostatic therapy, would give rise to tumor regression. To ascertain the efficacy of the therapy one would have to investigate whether there is a pharmacodynamic difference between tumor and normal tissue.

The pharmacokinetic modification of ADR metabolites including adriamycinol and deoxyadriamycinol aglycone induced by electric therapy should be followed by molecular biological methods. Furthermore, the modification of ADR pharmacokinetics in other organs besides the kidney should be investigated, since this study was designed to focus partly on the analysis of urinary ADR excreted through the kidney, the only organ into which we directly inserted the electrodes.

In another experimental study[30] of the same series, we found modification of 5-fluorouracil (5-FU) pharmacokinetics, instead of ADR, in tumor-bearing mice treated with electric therapy. We also clinically obtained pharmacokinetic advantages in 5-FU-resistant patients with colorectal cancer. In fact, since 5-FU is a non-ionized drug, there might be a different mechanism involved in its pharmacokinetic modification from that of ADR.

For clinical application, further studies must be required for drug control by which the optimum concentration in target organ and minimum concentration in other organs as well as in blood could be maintained; in other words, cancer cells could be killed with doing as little damage as possible to normal cells.

Conclusion

This study suggested that electric therapy might potentially induce modification of ADR pharmacokinetics by iontophoresis, and that the therapy might effectively change ADR concentration both systemically and locally, especially in the heart, which might implicate the potential reduction of ADR cardiotoxicity.

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