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# Doxorubicin and local hyperthermia in the microcirculation of skeletal muscle

**Andreas S. Lübbe**

Freie Universität Berlin, Universitätsklinikum Rudolf Virchow, Medizinische Klinik und Poliklinik (Hämatologie, Onkologie, Poliklinik), Augustenburger Platz 1, D-1000 Berlin 65, Federal Republic of Germany

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**Summary.** Doxorubicin HCl (Doxo) is an established intercalating antitumor drug. Specific side effects of Doxo primarily affect the cardiac muscle tissue to cause cardiac arrhythmias and chronic cardiomyopathies. The mechanism of action of these side effects is incompletely understood. Thus, the first objective of the present study was to test whether Doxo might have a direct effect on the microcirculation of muscular tissue. We studied large and small arterioles and large venules in the cremaster muscle of rats before and after sequential infusion of 1 (low-dose) and 10 mg/kg (high-dose) Doxo. Large arterioles showed some constriction after low Doxo doses and pronounced constriction after high Doxo doses, whereas small arterioles showed a variable response to low Doxo doses. At high Doxo doses, small arterioles dilated almost maximally (80% of the maximal response to nitroprusside). The heart rate and the diameter of large venules did not change at high Doxo doses, although the blood pressure decreased. This indicates that Doxo directly affects skeletal muscle arterioles. The second purpose of this study was to determine whether local hyperthermia would influence the microcirculation of muscular tissue such that the systemic concentration of Doxo could be reduced. In this second series of experiments, we tested whether local hyperthermia would have an effect on the skeletal muscle microvasculature and whether Doxo would change that response. Local hyperthermia alone did not alter the diameter of small arterioles or large venules, but we observed constriction of large arterioles at temperatures above 37° C and during continued (60-min) hyperthermia at 40° C. The low dose of Doxo did not alter these microvascular diameters at 40° C. However, local hyperthermia at 40° C changed the response of small arterioles to low doxo doses (no vasodilation was observed). Large arterioles continued to constrict in response to Doxo during hyperthermia. These data suggest that large arteriolar responses could be partly responsible for the toxic effect of Doxo on cardiac muscle and that local hyperthermia potentiates that response.

*Correspondence to:* A. S. Lübbe, Reichssportfeldstrasse 16, Apt. 230, D-1000 Berlin 19, Federal Republic of Germany

## Introduction

Although the cytostatic anthracycline doxorubicin (Doxo) has a broad spectrum of clinical indications and a high degree of therapeutic efficacy, a fair amount of toxicity for the whole organism is associated with its application. Characteristic for doxorubicin are acute and chronic cardiotoxic side effects, which are dose-dependent and sometimes limit its application. The histopathological alterations in the heart produced by Doxo are well characterized [4, 10], but their pathophysiological basis is unknown. Direct effects of Doxo at the level of the myocyte have been demonstrated [2, 3]. The occurrence of abnormalities in the microcirculation of the heart in response to Doxo have not been examined, but vasospasms or other physiological microvascular effects may also contribute to the cardiac toxicity of the drug. Thus, the first aim of the present study was to find out whether microcirculatory responses of muscular tissue might also be a part of the effect of Doxo on the cardiac muscle.

Anthracyclines and hyperthermia potentiate each other's effects under clinical and experimental conditions [10–12]. Therefore, the effect of a low dose of Doxo might be potentiated by local hyperthermia in a particular area of interest (the tumor site). The administration of a low dose of Doxo to a patient would thus result in reduced toxicity for the same antitumor effect, and more effective therapy protocols could be envisioned that would take advantage of this synergistic effect. Thus, pathophysiological changes in the cardiac tissue could potentially be reduced. Therefore, our second objective was to explore whether hyperthermia would change the response of a given tissue (in this case, muscular tissue) to Doxo. In other words if hyperthermia changes the effect of Doxo on the microcirculation and thus increases the local efficacy of the drug, can the systemic concentration of Doxo (and, hence, its side effects) be reduced?

## Materials and methods

*Animal preparation.* Male Sprague-Dawley rats weighing between 160 and 175 g were used in the acute experiments of the present study. These animals were purchased at an age of 3–4 weeks (weight, between 50 and 100 g) and were acclimated for 3–4 weeks in an AAALAC-approved animal-care center, where they were allowed a limited amount of chow but were given access to water ad libitum. Food was withheld at 12 h prior to each acute experiment. This diet (12–15 g standard Purina lab chow per day) assured a consistent weight gain of approximately 25 g per week. All animals were handled according to the NIH Guidelines for Humane Care and Treatment of Animals.

For the acute experiments, rats were anesthetized with sodium pentobarbital (45 mg/kg i.p.), and tracheostomies (PE-240 tubing) were performed for airway control. Rectal temperatures were continuously monitored and were maintained between 36° and 37° C by a heating pad that was positioned under each animal. The back temperature of each rat was also continuously monitored by a surface temperature probe and was kept below 40° C, since a back temperature above 40° C can cause spinal cord reflexes and movements that give the false impression of a lightly anesthetized animal.

*Systemic hemodynamic measurements.* The left femoral artery was cannulated (with heat-stretched PE-90 tubing) for blood-pressure and heart-rate measurements, and the left femoral vein was cannulated (PE-50 tubing) for the infusion of Doxo or saline solution. Blood pressure and heart rate were continuously monitored by a Staham P23DB transducer, which was connected to a bridge amplifier and an electronic monitor.

*Cremaster-muscle preparation.* Following vessel cannulation, the animals received 2 ml isotonic saline s.c. to maintain vascular fluid volume throughout the protocol; the right cremaster muscle of the rat was then prepared using a significant surgical modification [5, 17] of the techniques originally described by Baez [1]. The scrotal sac was cut longitudinally and the cremaster-covered testicle was gently teased away from the sac. A thin white layer of connective tissue that covered the exposed cremaster-covered testicle was gently peeled away to expose the underlying cremaster muscle. After the scrotum had been cut and throughout the rest of the surgical procedure, the cremaster muscle was kept moist by frequent washes with a modified Krebs solution (25.5 mmol $NAHCO_3$/l, 112.9 mmol NaCl/l, 4.7 mmol KCl/l, 2.55 mmol $CaCl_2$-$2H_2O$/l, 1.19 mmol $KH_2PO$/l, 1.1 mmol $MgSO_4$-$7H_2O$/l, and 11.6 mmol dextrose/l) buffered to pH 7.4.

Next, the cremaster muscle was cut longitudinally from the distal tip to the external inguinal ring. This cut was positioned as far as possible from the major artery and vein that enter the cremaster muscle. Following this incision, three small vessels (diameter, <50 μm) between the testicle and the cremaster were cauterized, and the testicle was gently pulled away from the cremaster muscle and was pushed through the inguinal canal into the abdominal cavity.

The rat was placed on its back on a heating pad that had been positioned on a custom-made Plexiglas board fitted with a bath chamber. The neurovascularly intact (in terms of innervation and blood supply) cremaster muscle was secured in a flat position over a cover glass by five ties of 5-0 silk thread that were placed at approximately equal intervals around the margin of the cut cremaster. The 60-ml tissue bath was then filled with the modified Krebs solution. The animal and cremaster bath were next positioned on the stage of a trinocular microscope for transillumination. Nitrogen and carbon dioxide were continuously bubbled through the cremaster bath to maintain the $PO_2$ of the bath at 15–25 torr and the $PCO_2$ at 35–45 torr. The pH of the bath was maintained at 7.4 ± 0.05 by adjustment of the $CO_2$ gas-flow rate through the cremaster bath. Cremaster temperature was maintained at 34° ± 0.5° C by a custom-made heating coil in the bath; visible changes in muscle-fiber length that theoretically could have affected the vessel diameter did not occur.

The microcirculation of the cremaster muscle was directly observed with a trinocular intravital Nikon microscope, and the images were transmitted via a Sanyo video camera to a Sony television monitor and videocassette recorder by a lens system that gave an overall television-microscope magnification of × 1500. Intraluminal vessel diameters were measured retrospectively by videotape replay on a monitor screen that was calibrated with a microscope stage micrometer to give microvascular dimensions in micrometers. The microvascular anatomy of the cremaster muscle was defined [5, 17] according to vessel branch order. The major inflow arteriole was termed a first-order arteriole ($A_1$) and was accompanied by a parallel adjacent first-order venule ($V_1$). Smaller second-order arterioles ($A_2$) and accompanying venules ($V_2$) appeared as almost perpendicular branches from the parent first-order vessels. Successive arteriolar branches from the second-order vessels were designated as $A_3$ and $A_4$ vessels and did not have adjacent parallel venules; these $A_3$ and $A_4$ vessels correspond, respectively, to metarterioles and terminal arterioles in the nomenclatures of other investigators. In our protocols, diameter of $A_1$, $A_2$, $A_3$, $A_4$, $V_1$, and $V_2$ vessels were measured in each experiment.

*Doxo preparation.* Doxo (Adriamycin HCl) was obtained from Farmitalia Carlo Erba Inc. The preparation involved the dissolution of a lyophilized powder with isotonic saline. The final solution (of red color) usually had a pH of 4–5.5. Two doses of Doxo were given i.v. over 5 min (1 and 10 mg/kg).

*Hyperthermia protocol.* Animals were divided into four groups as described below. In two groups, the cremaster muscle was heated up via a heating element in the Krebs plexiglas bath in which the muscle was suspended. After an appropriate equilibration time, the bath temperature was increased in a stepwise fashion by 1° C/5 min beginning at 34° C (normal bath temperature and physiological cremaster-muscle temperature) and ending at 40° C. Each temperature step was precisely regulated by a custom-made heat-control system that assured temperatures within a 0.1° C range. Each temperature was maintained for 5 min before the next temperature value was set. Then, in group 2, the temperature was maintained at 40° C for 60 min before being slowly lowered to 34° C (within 30 min).

*Experimental protocols.* Four animal groups were used for observations of the microcirculation in this study. In all four groups, a 45-min baseline period was allowed after surgical preparation for equilibration of the cremaster muscle to the bath environment. After stabilization of mean arterial pressure (MAP), heart rate (HR), and microvascular tone (brisk vasomotion in the small arterioles), baseline hemodynamic measurements were made of MAP, HR, and microvascular diameters.

Group 1 animals ($n = 4$) received 1 ml isotonic saline i.v. over 5 min, and all hemodynamic measurements were repeated at 15-min intervals for 2 h. At the end of this 120-min period, nitroprusside (stored and prepared in a dark environment; bath concentration, $10^{-5}$ M) was topically applied to evoke maximal arteriolar dilation.

Group 2 animals ($n = 7$) received 1 ml isotonic saline i.v. over 5 min. The cremaster temperature was then increased stepwise as described above. Hemodynamic measurements were repeated at each temperature interval and at 15-min intervals for 60 min during the 40° C hyperthermia protocol. Nitroprusside was then added to the bath (bath concentration, $10^{-5}$ M) after the baseline temperature (34° C) had been reached.

After baseline measurements, group 3 animals ($n = 6$) were given Doxo (1 mg/kg) i.v. over 5 min, and cremaster microcirculatory and general hemodynamic measurements were repeated at 15-min intervals for 60 min. Then, a second dose of doxorubicin (10 mg/kg) was infused over 5 min into the animal, with subsequent microcirculatory and cardiovascular measurements being repeated at 15-min intervals over 60 min.

Group 4 animals ($n = 5$) received 1 ml isotonic saline i.v. over 5 min for fluid maintenance. Then, a hyperthermia protocol was carried out as described for group 2 animals. At the end of the hyperthermia protocol, Doxo (1 mg/kg) was infused i.v. over 5 min, and microcirculatory and systemic hemodynamic measurements were recorded at 15-min intervals for 60 min before the cremaster-muscle temperature was lowered and nitroprusside (bath concentration, $10^{-5}$ M) was topically applied to the cremaster bath.

*Statistical analysis.* Data were expressed as percentages of change from baseline values. Group means and standard errors of the mean were

**Table 1.** Baseline values before hyperthermia and/or saline or Doxo infusion in four experimental groups

| Variable | Group 1 (saline, $n = 4$) | Group 2 (hyperthermia, $n = 7$) | Group 3 (Doxo, $n = 6$) | Group 4 (HT + Doxo, $n = 5$) | Statistical analysis[a] |
|---|---|---|---|---|---|
| Weight (g) | 177 ± 4 | 179 ± 5 | 180 ± 8 | 182 ± 7 | *1234* |
| Mean arterial pressure (mmHG) | 112 ± 5 | 112 ± 5 | 120 ± 4 | 117 ± 5 | *1234* |
| Heart rate (beats/min) | 400 ± 10 | 436 ± 9 | 453 ± 11 | 447 ± 6 | *1234* |
| Vessel diameter (μm): | | | | | |
| $A_1$ | 120 ± 6 | 97 ± 4 | 102 ± 5 | 95 ± 6 | *1234* |
| $A_2$ | 77 ± 8 | 58 ± 6 | 66 ± 7 | 60 ± 8 | *1234* |
| $A_3$ | 20 ± 1 | 18 ± 2 | 16 ± 3 | 17 ± 2 | *1234* |
| $A_4$ | 10 ± 2 | 8 ± 1 | 7 ± 1 | 7.5 ± 1 | *1234* |
| $V_1$ | 172 ± 9 | 128 ± 6 | 133 ± 9 | 133 ± 6 | *1234* |
| $V_2$ | 98 ± 7 | 57 ± 8 | 70 ± 7 | 54 ± 9 | *1234* |

Data represent mean values ± SEM.
$A_1$, $A_2$, $A_3$, $A_4$, First-, second-, third-, and fourth-order arterioles, respectively; $V_1$, $V_2$, first- and second-order venules, respectively; HT, hyperthermia
[a] Data for group members within the same horizontal line do not significantly differ ($P < 0.05$, ANOVA)

**Table 2.** Microvascular diameters, blood pressure, and heart rate of rats over time after saline infusion at 34° C cremaster-muscle temperature

| | 15 min | 30 min | 45 min | 60 min | 75 min | 90 min | 105 min | 120 min |
|---|---|---|---|---|---|---|---|---|
| $A_1$ | 99 ± 1 | 99 ± 1 | 98 ± 1 | 99 ± 1 | 97 ± 2 | 99 ± 1 | 98 ± 1 | 98 ± 1 |
| $A_2$ | 99 ± 3 | 99 ± 3 | 97 ± 5 | 101 ± 1 | 101 ± 2 | 101 ± 2 | 104 ± 3 | 101 ± 3 |
| $A_3$ | 101 ± 4 | 97 ± 5 | 95 ± 7 | 96 ± 6 | 102 ± 7 | 104 ± 6 | 103 ± 4 | 106 ± 3 |
| $A_4$ | 99 ± 3 | 98 ± 2 | 99 ± 3 | 102 ± 4 | 98 ± 4 | 99 ± 4 | 107 ± 5 | 108 ± 6 |
| $V_1$ | 102 ± 3 | 102 ± 2 | 104 ± 2 | 102 ± 4 | 103 ± 3 | 104 ± 4 | 102 ± 3 | 103 ± 4 |
| $V_2$ | 101 ± 1 | 101 ± 1 | 104 ± 1 | 104 ± 3 | 104 ± 3 | 104 ± 3 | 103 ± 3 | 103 ± 3 |
| BP | 98 ± 2 | 95 ± 1 | 97 ± 1 | 97 ± 3 | 96 ± 2 | 94 ± 2 | 95 ± 2 | 98 ± 3 |
| HR | 105 ± 2 | 100 ± 3 | 99 ± 2 | 98 ± 1 | 99 ± 1 | 96 ± 3 | 94 ± 2 | 96 ± 3 |

Data represent mean values ± SEM for the percentage of change from baseline values, the latter being taken as 100%. BP, Blood pressure; HR, heart rate

**Table 3.** Microvascular diameters, blood pressure, and heart rate of rats at increasing cremaster-muscle temperatures

| | 34° C | 35° C | 36° C | 37° C | 38° C | 39° C | 40° C |
|---|---|---|---|---|---|---|---|
| $A_1$ | 100 | 101 ± 1 | 98 ± 2 | 96 ± 2* | 94 ± 2* | 91 ± 1* | 91 ± 2* |
| $A_2$ | 100 | 96 ± 3 | 97 ± 3 | 95 ± 4 | 93 ± 4 | 94 ± 3 | 90 ± 4 |
| $A_3$ | 100 | 102 ± 2 | 100 ± 5 | 102 ± 6 | 106 ± 6 | 102 ± 8 | 106 ± 10 |
| $A_4$ | 100 | 94 ± 3 | 102 ± 5 | 102 ± 8 | 99 ± 7 | 94 ± 5 | 104 ± 12 |
| $V_1$ | 100 | 100 ± 1 | 99 ± 1 | 99 ± 1 | 98 ± 1 | 99 ± 1 | 98 ± 2 |
| $V_2$ | 100 | 104 ± 4 | 104 ± 3 | 105 ± 4 | 101 ± 4 | 103 ± 4 | 103 ± 4 |
| BP | 100 | 100 ± 2 | 98 ± 4 | 96 ± 3 | 92 ± 2* | 93 ± 2* | 92 ± 3* |

Data represent mean values ± SEM for the percentage of change from baseline values, the latter being taken as 100%. BP, Blood pressure
* $P < 0.05$

calculated for baseline values and for changes over time. Differences with time *within a group* were evaluated (at the $P < 0.05$ level) by one-way analysis of variance (ANOVA). If this ANOVA revealed no time effect within one group, data points obtained at successive measurement times were averaged to give an overall pooled data point. These averaged time points were then used for comparisons (Student's two-sample, unpaired *t*-test) between the appropriate groups.

If the ANOVA indicated a time effect within one group, data obtained at individual time points were compared between the appropriate groups using Student's unpaired *t*-test. Nitroprusside data were analyzed by ANOVA to test for any changes (at the $P < 0.05$ level) among the groups at the various vessel levels. Differences among the baseline values for the general and hemodynamic variables were analyzed using Student's unpaired *t*-test ($P < 0.05$). If there was no time effect within a group, one-tailed paired *t*-test of the time-pooled data were performed to verify the presence or absence of statistically significant ($P < 0.05$) changes from the baseline values for that group. This was necessary since the ANOVA on percentage-expressed data cannot be used to test for changes from baseline values that have been normalized to 100% with no variance. If a time effect was indicated within a group, individual time points were compared with baseline values by paired one-sample *t*-tests ($P < 0.05$).

## Results

### *Systemic data*

Baseline values for general and hemodynamic variables (Table 1) were similar, with only minor intergroup differ-

**Table 4.** Microvascular diameters, blood pressure, and heart rate of rats after Doxo administration

| | 30 min (after 0.1 mg/100 g Doxo) | 60 ming (after 0.1 m/100 g Doxo) | 90 min (30 min after1 mg/100 g Doxo) | 120 min (60 min after1 mg/100 g Doxo) |
|---|---|---|---|---|
| $A_1$ | 96 ± 2 | 93 ± 1* | 91 ± 1* | 87 ± 2* |
| $A_2$ | 98 ± 1 | 109 ± 8 | 111 ± 12 | 106 ± 10 |
| $A_3$ | 96 ± 8 | 141 ± 30 | 169 ± 17* | 170 ± 19* |
| $A_4$ | 96 ± 12 | 124 ± 18 | 178 ± 28* | 184 ± 29* |
| $V_1$ | 101 ± 1 | 100 ± 1 | 101 ± 1 | 102 ± 2 |
| $V_2$ | 97 ± 6 | 93 ± 5 | 95 ± 4 | 95 ± 3 |
| BP | 98 ± 4 | 96 ± 6 | 86 ± 3* | 82 ± 6* |
| HR | 97 ± 3 | 99 ± 5 | 93 ± 5 | 96 ± 5 |

Data represent mean values ± SEM for the percentage of change from baseline values; the latter being taken as 100%. BP, Blood pressure; HR, heart rate
* $P < 0.05$

**Table 5.** Microvascular diameters, blood pressure, and heart rate of rats during hyperthermia and after Doxo administration in the posthyperthermia phase (all values in percentage change from baseline – being 100% – as mean ± SEM, legends see above)

| | During hyperthermia (40° C) | | After Doxo, posthyperthermia (40° C) | |
|---|---|---|---|---|
| | 30 min | 60 min | 30 min | 60 min |
| $A_1$ | 88 ± 4* | 87 ± 3* | 84 ± 3* | 84 ± 3* |
| $A_2$ | 90 ± 4* | 94 ± 3 | 86 ± 5* | 86 ± 4* |
| $A_3$ | 105 ± 15 | 110 ± 18 | 100 ± 15 | 99 ± 14 |
| $A_4$ | 118 ± 23 | 120 ± 21 | 102 ± 18 | 101 ± 16 |
| $V_1$ | 95 ± 1 | 98 ± 2 | 97 ± 2 | 96 ± 3 |
| $V_2$ | 102 ± 3 | 104 ± 6 | 100 ± 9 | 97 ± 4 |
| BP | 97 ± 3 | 98 ± 4 | 101 ± 4 | 104 ± 5 |
| HR | 99 ± 2 | 103 ± 3 | 104 ± 4 | 105 ± 3 |

Data represent mean values ± SEM for the percentage of change from baseline values, the latter being taken as 100%. BP, Blood pressure; HR, heart rate
* $P < 0.05$

**Table 6.** Diameters of microvessels at the time of maximal change after cremaster-muscle exposure to $10^{-5}$ M nitroprusside

| Microvessel branch order | Group 1 (saline, $n = 4$) | Group 2 (hyperthermia, $n = 7$) | Group 3 (Doxo, $n = 6$) | Group 4 (Doxo + HT, $n = 5$) | Statistical analysis[a] |
|---|---|---|---|---|---|
| $A_1$ | 99 ± 1 | 90 ± 6 | 96 ± 1 | 85 ± 7 | *1234* |
| $A_2$ | 103 ± 1 | 94 ± 4 | 114 ± 11 | 89 ± 7 | *1234* |
| $A_3$ | 147 ± 8 | 167 ± 23 | 208 ± 20 | 155 ± 21 | *1234* |
| $A_4$ | 202 ± 27 | 204 ± 19 | 202 ± 32 | 208 ± 19 | *1234* |
| $V_1$ | 103 ± 2 | 104 ± 3 | 100 ± 1 | 103 ± 4 | *1234* |
| $V_2$ | 104 ± 2 | 113 ± 8 | 103 ± 1 | 119 ± 10 | *1234* |

[a] Data for group members within the same horizontal line do not significantly differ

ences being observed in animal weights, blood pressures, and heart rates among the four groups. Blood pressure was maintained in group 1 (Table 2). Toward the end of the hyperthermia protocol, blood pressure decreased slightly in group 2 (Table 3). At 60 min after the infusion of 1 mg/kg Doxo, blood pressure was below the baseline level in group 3, and it continued to drop further after the infusion of 10 mg/kg Doxo (Table 4). The blood pressure in group 4 was not reduced during the 1-h hyperthermic interval at 40° C, regardless of Doxo (1 mg/kg) administration (Table 5). The heart rate did not change significantly from the baseline value in any group (Tables 2–5).

*Microcirculatory data*

In general, baseline diameters of large and small arterioles and large venules were similar, with few differences being noted among the groups (Table 1). For unknown reasons, baseline diameters of most arterioles and venules were larger in group 1 than in the other groups (Table 1).

Nitroprusside data show that small ($A_3$, $A_4$) arterioles in all groups had dilated maximally by the end of the experiment (Table 6). Local hyperthermia caused constriction of large ($A_1$) arterioles beginning at 37° C; this constriction correlated with the drop in blood pressure noted at that

time. All other microvessels showed no change during local hyperthermia (Table 3).

A low dose (1 mg/kg) of Doxo caused constriction of large ($A_1$) arterioles and some dilation (albeit not statistically significant) of small ($A_3$, $A_4$) arterioles at 60 min after the infusion. The high dose (10 mg/kg) of Doxo caused constriction of large ($A_1$) arterioles and profound (80% of maximal possible) dilation of small ($A_3$, $A_4$) arterioles immediately after the infusion. Prolonged hyperthermia (for 30 and 60 min at 40° C) caused large cremasteric arterioles to constrict, whereas some small ($A_3$, $A_4$) arterioles dilated in some animals (Table 5). At a later time point (30 and 60 min after Doxo administration in the posthyperthermic phase), large arterioles maintained that constriction, whereas small ($A_3$, $A_4$) arterioles did not dilate after the infusion of 1 mg/kg Doxo during the period of 40° C hyperthermia (Table 5).

## Discussion

Several animal models are used for studies of Doxo, of which the rat model has been fairly well characterized [10]. The closeness in many physiological aspects of the skeletal and the cardiac muscle tissue lends itself to the use of an established skeletal-muscle microcirculatory preparation in the rat to study the microvascular effects of Doxo and hyperthermia. Although the tumoricidal effect of Doxo formed the basis of this study, it is the side effects of this substance on normal tissues that frequently limit the clinical application of Doxo. Therefore, it is remarkable that only few physiological studies have examined the effects of Doxo on normal tissues [8, 9].

Doxo infused i.v. over 5 min lead to a time- and dose-dependent constriction of large ($A_1$) arterioles. However, dilation of small ($A_3$, $A_4$) arterioles also occurred, which (in the case of fourth-order arterioles) reached 80% of the maximal dilation induced by nitroprusside. Only subsequent to high doses of Doxo and after a fairly long delay phase would a drop in blood pressure via a passive (myogenic) mechanism explain these phenomena. Thus, the changes in diameter observed in the presence of normal blood pressure could be explained as a direct effect of Doxo on the endothelium or vascular smooth muscle.

It is well known that within the dose range used in this study, Doxo not only intercalates with intracellular DNA but also induces alterations in cell membranes. Hence, it has the potential to alter receptors located at the endothelium and thus to cause changes in endothelium-mediated microcirculatory mechanisms [16, 18]. These mechanisms may partly be the result of a calcium overload in the myocyte. Thus, data from other investigators, which demonstrate a cardioprotective effect for verapamil [12], may also indicate the occurrence of some microcirculatory events. Under hyperthermic conditions, much more Doxo is taken up intracellularly [15]. This is one explanation for the synergistic effect of these two modalities [3, 6, 14].

Our data show that the diameter of large and small arterioles and large venules did not vary much over time or within a temperature range of 34°–40° C. However, some constriction of large ($A_1$) arterioles occurred at higher temperatures (above 37° C) concomitantly with a slight drop in blood pressure at that time. Constriction of large arterioles was also observed during 60 min hyperthermia at 40° C, whereas both blood pressure and the diameter of small arterioles remained normal. For its anatomical position and its physiological role, a baseline (control) temperature of 34° C for the cremaster muscle is widely accepted [5, 17]; a temperature of 40° C for 60 min therefore represents a clinically relevant choice for this muscle that can be used for hyperthermia protocols in patients [6, 19]. Only at temperatures above 44° C do clearly visible pathological changes take place in the microcirculation of this tissue, characterized by red-blood-cell sludging, constriction of large and small arterioles, thrombus formation, and petechial bleeding (unpublished observations).

Our data also show that Doxo administration following hyperthermia at 40° C for 1 h did not change the arteriolar diameters obtained after 1 h at 40° C in the absence of Doxo (constriction of large arterioles, little change in small arterioles). However, in contrast to the effect of Doxo in the absence of hyperthermia, small arterioles did not dilate following infusion of the low dose during hyperthermia. On the other hand, vasoconstriction of large arterioles in response to Doxo occurred during hyperthermia at times during which the blood pressure remained normal. This suggests that hyperthermia at 40° C prevented the compensatory dilator response of small arterioles to Doxo but that the constriction of large arterioles was induced under these conditions.

Collectively, these data indicate that Doxo does not change microvascular responses caused by local hyperthermia. On the other hand, it appears that local hyperthermia changes the microvascular reactivity to Doxo. If these data from skeletal muscle apply to cardiac muscle as well, then vasospasms occurring via the constriction of large arterioles and small arteries by Doxo might explain part of the drug's cardiac toxicity. However, it is unknown whether small-arteriole dilation occurs as a consequence of the "upstream" constriction (partly as a compensatory mechanism) or, alternatively, whether the dilator response of small arterioles (via modulation of endothelial membrane receptors) precedes and causes the constriction of large arterioles. Until the sequence of these events has been resolved, the interpretation and clinical evaluation of these data in the context of hyperthermia should be made with caution.

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