

Doxorubicin in Isolation Limb Perfusion in the Treatment of Advanced Limb Soft Tissue Sarcoma

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Hyperthermic antineoplastic perfusion (HAP) has been proven to be an effective neoadjuvant treatment in the treatment of advanced soft tissue limb sarcoma. As a matter of fact high percentage of limb sparing surgery, local control and functional results have been obtained with this technique. Many antineoplastic drugs have been associated to hyperthermia by isolation limb perfusion, the aim of this paper was to describe the results obtained with doxorubicin in association to hyperthermia with or without Tumor Necrosis Factor (TNF) alpha in order to identify the most effective regimen in the multidisciplinary treatment of soft tissue limb sarcoma.

A total of 106 patients have been evaluated. Three different studies were performed: the first was a phase I study carried out in order to assess the maximum tolerable dose (MTD) of doxorubicin during HAP; the second was a phase II study with doxorubicin, and the third was a phase I – II study aimed at evaluating the MTD and tumor response of TNF alpha in association to doxorubicin and hyperthermia.

Grade IV limb toxicity was recorded in 11 patients (4 in trial A, 3 in trial B, and 4 in trial C). The grade of limb reaction was strictly related to TNF dosage (> 1 mg) and temperature level (> 41.5 °C), therefore the best regimen is represented by temperature level not exceeding 41.5 °C and 1 mg of TNFα.

The trimodality association (TNF, doxorubicin and hyperthermia) was proven to be the best regimen able to obtain a 77% of objective response (complete response, 22%) and a 77% of limb sparing in patients candidate to amputation.

The results above mentioned showed the HAP with doxorubicin and TNFα (1 mg) is a very effective neoadjuvant treatment in the multidisciplinary treatment of advanced soft tissue limb sarcoma.

Key Words: Limb soft tissue sarcoma, Hyperthermic perfusion, Doxorubicin, TNFα

Approximately 60% of all soft tissue sarcoma develop in the extremities, treatment must therefore consider three main objectives: functional results, loco-regional control, and survival. Many surgical procedures have been employed, and recurrence rates are strictly related to the type of surgery performed: 90% with marginal excision, 20-40% with wide excision, and 0-17% with demolitive surgery. (1-4)

Adequate surgery, as suggested by Simon and Enneking, improved loco-regional control (91%) but required amputation or disarticulation in 63% of the

patients, and the 5-year survival did not exceed 52%. (5)

Since the first experience of Krementz in 1957 at Tulane University, the technique of isolation limb perfusion (ILP) has been widely employed in the treatment of limb tumors including advanced soft tissue sarcoma. (6).

The rationale for employing this technique is based on the following:

- The tumor bearing limb is temporarily isolated from the systemic circulation, therefore high doses

of antineoplastic drugs (5-10 times the maximum tolerable systemic dosage) can be delivered in the perfusional circuit, without relevant local and systemic toxicity.

- Hyperthermia possesses a tumoricidal effect “per se” and in combination with antineoplastic drugs can provoke a synergistic effect.
- The tumor shrinkage after H.A.P. can permit a conservative instead of demolitive surgery

The most commonly employed antineoplastic drugs in association to hyperthermic antitumoral perfusion (HAP) have been Melphalan and ActinomycinD, in many patients radiotherapy has been administered after the surgical removal of the perfused tumor. In a collected series of 379 patients the results were very satisfactory with a conservative surgery carried in 97% of the patients, the mean local relapse and 5-year survival were 13% and 72% respectively.

Since doxorubicin is considered the most active drug against soft tissue sarcoma we decided to employ this drug during hyperthermic perfusion.

A dose escalation study was undertaken and when the maximum tolerable dose for upper and lower limb respectively was established. HAP with doxorubicin was employed in treatment of advanced soft tissue sarcoma. Finally doxorubicin was associated to Tumor Necrosis Factor (TNF α) and hyperthermia in order to increase the therapeutic efficacy of hyperthermic antitumoral perfusion.

The aim of this paper is to illustrate the results obtained with doxorubicin without or in combination with TNF α in the treatment of advanced soft tissue limb sarcoma.

Materials and Methods

Three clinical trials on hyperthermic antitumoral perfusion were carried out with 106 patients who had soft tissue sarcoma of the extremities, there were 60 males and 46 female; the mean age was 58 years; range 18-76 years.

Seventy-one patients had primary tumors, 25 had local recurrence, 7 had primaries with synchronous metastases, and the remaining 3 had local recurrences and metastases. The tumor was located in the lower limb in 90 cases, and in the upper in 16 cases. Tumor size varied from 2.5 to 28 cm. There were 35 malignant fibrous histiocytomas, 21 leiomyosarcomas, 13 peripheral nerve sheath tumors, 13 synovial sarcoma, 11 liposarcoma, and 13 other histologic type.

The first trial (“A”), a multicentric phase I study

was carried out with the aim to find the maximum tolerable dose (MTD) of doxorubicin in HAP. The second trial (“B”) was a phase II study aimed at evaluating the tumor response to doxorubicin, whereas the third trial (“C”) was a multicentric phase I – II study aimed at assessing the MTD of TNF alpha and tumor response to doxorubicin associated to TNF α .

The inclusion criteria for studies “A” and “B” were patients with primary or recurrent extracompartmental or large (>5 cm) G2/G3 tumors, whereas in trial “C” were enrolled only patients candidate for amputation because of extracompartmental or multicompartimental lesions, tumor with gross bone or sciatic nerve infiltration, or multiple lesions in the same limb.

Other eligibility criteria used in the 3 studies were the following:

1. Age 18-75 years;
2. Histologic confirmation of diagnosis
3. Eastern Cooperative Oncology Group Performance status up to 2
4. Fully informed consent

Patients were excluded if any of the following criteria existed:

1. Severe cardiovascular, hepatic, or renal disease;
2. Severe vascular disease of the involved limb;
3. Limb tumors located in the proximal third of the thigh, too close to the inguinal ligament or gluteus.

In each trial an accurate staging before HAP was performed with physical examination, complete chemical profile, chest and abdomen computed tomography (CT), as well as CT or magnetic resonance imaging (MRI) of the involved limb.

Patients enrolled in trial “A” were treated with a doxorubicin dose ranging between 1 mg/kg of body weight up to 1,6 mg for lower limb, for each dosage at least 3 patients were treated, with a slight increase of mean muscle temperature. For upper limb the starting dose was 0.7 mg/kg of body weight up to 0.8 mg. (table I).

The MTD for lower limb was 1.4 mg/kg of body weight since at 1.6 mg 2 grades IV limb reaction were observed.

For upper limb the MTD was 0.7 mg/kg of body weight because at 0.8 mg two grades IV limb reactions were recorded.

Patients included in trial “B” underwent doxorubicin perfusion for 60 minutes at 40.5 – 42°C employing the MTD doxorubicin dose.

In trial “C” a dose escalation study was carried out according to Fibonacci modified scheme. The TNF dosage ranged between 0.5 to 3.3 mg, the doxorubicin dose and temperature level remained constant (41.5°C).

**Tab. I - HYPERTHERMIC PERFUSION WITH DOXORUBICIN:
PHASE I STUDY IN PATIENTS WITH LIMB S.T.S.**

GROUP	LIMB REACTION*	PATIENTS	MEAN MUSCLE TEMPERATURE (°C)
LOWER LIMB			
Group I (1 mg/kg)	II - II - III	3	39 - 40 - 41.2
Group II (1.2 mg/kg)	II	4	40.7 - 41.2 - 41.2 - 42
Group III (1.4 mg/kg)	II - II - II - III	4	41.2 - 41.3 - 41.7 - 41.8
Group IV (1.6 mg/kg)	II - III - IV - IV	4	40.4 - 41 - 41.5 - 41.6
UPPER LIMB			
Group I (0.7 mg/kg)	III - III - III - II	4	40.8 - 41.4 - 41.4 - 41.5
Group II (0.8 mg/kg)	IV - IV	2	40 - 41.4

*according to Wieberdink classification

HAP technique

The technique of isolation perfusion was extensively reported in previous papers (7-10). Only few details will be described for TNF perfusion.

The axillary and iliac vessels were cannulated for tumors of the upper and lower limbs, respectively. Temperature monitoring was carried out by multiple muscle thermocouples inserted into the skin, thigh and leg muscles, and into the tumor. As soon as the tumor temperature reached 41°C, TNF α was injected into the extracorporeal circuit at the preestablished dose. Doxorubicin was administered after 30 minutes and the extracorporeal circulation continued for other 60 minutes. During regional perfusion technetium 99 mm-albumin was injected into the circuit, a scintillation probe was placed on the cardiac area in order to continuously monitor the systemic leakage.

A Swan-Ganz catheter was positioned in all the

patients before the HAP treatment to continuously record intra and postoperative cardiac index, heart rate, and pulmonary arterial blood pressure for 24 hours.

All the patients were admitted to the intensive care unit for at least 24 hours so that acute systemic and local side effects could be carefully monitored.

Systemic toxicity was evaluated according to World Health Organization (WHO) criteria, whereas Wieberdink classification has been adopted to evaluate loco-regional toxicity (11).

The tumor response to treatment was evaluated radiologically and pathologically.

A CT scan or MRI was carried out before the perfusion and again after 20-30 days. Tumor response was defined as percentage increase of the tumor necrosis rate before the perfusion (liquid component) applying the ellipsoid formula (diameter x diameter x diameter x 0.523). The pathologic evaluation was both macroscopic (longitudinal and transversal plane) and mis-

Tab. II - ANALYSIS OF CLINICAL OUTCOME OF 106 PATIENTS TREATED WITH DOXORUBICIN + TNF ACCORDING TO CLINICAL TRIAL

Trial	5 year outcome (%)		OS
	LRC	DFS	
A Doxorubicin (Suboptimal dose)	53	30	40
B Doxorubicin (Optimal dose)	70	40	50
C Doxirubicin and TNF alpha	p=0.02 94	0.05 61	0.04 69

croptic (the decreased percentage of viable nuclei in the tumor surrounding the necrotic area after HAP, compared with the pretreatment nuclear density).

Depending on their response to treatment, patients were categorized as responders (histologic necrosis >75%), partial responders (histologic reduction >25- <75%), or non responders (>25% histologic necrosis).

The post perfusional treatment, carried out 4-6 weeks after perfusion, consisted of marginal resection, wide resection, or amputation.

External beam radiotherapy was delivered only in case of intralesional or marginal resection.

After the completion of the treatment the patients were followed with a physical examination, chest and upper abdomen CT, and CT/MRI of the treated limb every 4 months for the first 2 years and then every 6 months for 3 more years after surgery.

Statistics

The following factors were considered for statistical analysis: age (>or<50 years); gender; site (upper or lower limb); trial (A, B or C); tumor size (>or<10 cm); grading (G1, G2 or G3); tumor necrosis (>50%, 51-75%, >75%) and local toxicity (I – II – III/IV – V).

The chi-square test was employed to evaluate the whole set of factors influencing local toxicity and tumor response (necrosis). Analysis of variance was used to confirm the results of the chi-square test.

Disease free survival and cumulative proportion survival curves were analyzed according to Kaplan-Meier method from the date of diagnosis to recurrence, metastases, or death, respectively. Multivariate analysis was performed using the cox model. All analyses were performed using the BMDP statistical package for PC (BMDP Statistical Software, Snc, Los Angeles, CA).

Results

The comparative analysis of the three trial will be described both in terms of toxicity and efficacy of the treatments (tumor response, loco-regional control, disease-free and overall survival).

Toxicity

There were no post-operative deaths from toxicity. Loco-regional toxicity was mild (I – II) in 55% of the patients, a grade III was observed in 23% of the patients, a grade IV was recorded in 11 patients (4 in trial A, 3 in trial B and 4 in trial C).

Systemic hematology toxicity was observed in 11% of the patients (1 of these enrolled in trial C had also a grade IV renal toxicity that recovered completely after dialysis. Hepatic toxicity was grade I in 2 patients, grade II in 5, and grade III in 3).

Tab. III - STATISTICAL ANALYSIS (p VALUE) IN 106 SOFT TISSUE LIMB SARCOMA PATIENTS TREATED WITH HAP DOXORUBICIN + TNF

Factor	Local Toxicity (P)	Necrosis (P)
Age (</>50)	0.74	0.96
Sex	0.63	0.93
Site (upper/lower)	0.28	0.04
Trial (A/B/C)	0.03	0.03
Grading (G1, G2, G3)	0.56	0.04
Necrosis (<50/51-75/>75)	0.02	-
Local toxicity (I-II-III/IV-V)	-	0.02

Another patient (trial B) developed grade IV renal toxicity that required temporary dialysis.

Treatment efficacy

Tumor response was assessed in all the patients submitted to surgery after HAP.

Not responders accounted only for 8%, in 65% of the patients a partial response was recorded, whereas 27% of the cases were classified as responders.

Complete pathologic response was observed only in 5 patients of the trial C.

Limb sparing surgery was possible in 93% of the patients, amputation was carried out only in the remaining 7%. It must be emphasized that limb sparing surgery was carried out in 77% of the patients belonging to trial C, all candidate to amputation.

The patient outcome stratified according to trial is showed table II.

The utilization of optimal doxorubicin dose obtained better results in terms 5 year loco-regional control (70% vs 53%) disease free survival (40% vs 30%) and overall survival (50% vs. 40%), than those achieved in patients treated with suboptimal doxorubicin dose.

It is readily apparent that in the trial C (hyperthermia-doxorubicin-TNF) the best results were obtained, therefore all the patients with advanced non resectable soft tissue limb sarcoma are now treated with this association.

A multivariate analysis was carried out in order to verify factors able to influence the toxicity and tumor necrosis (tab. III).

The only two factors able to influence the local toxicity were trial (C) and tumor necrosis with the chi-square test $p=0.03$ and $p=0.02$ respectively.

Regarding tumor necrosis, factors with independent predictive value were site ($p=0.04$); trial ($p=0.03$); grading ($p=0.04$) and local toxicity ($p=0.02$).

As far as local disease free and overall survival are concerned the results of multivariate analysis are reported in table IV.

Regarding local disease free survival the only factors with independent predictive value at multivariate analysis were sex ($p=0.044$); trial ($p=0.03$) and tumor necrosis ($p=0.01$); whereas the only factor able to influence the overall survival, at multivariate analysis, was the grading ($p=0.04$).

Discussion

The use of doxorubicin during hyperthermic perfusion has a strong rationale:

- Doxorubicin is the most active cytotoxic agent against soft tissue sarcoma, with objective response rates ranging between 15% and 35% (C.R. 6%).
- A dose-response relationship has been observed in no randomized studies.
- “in vivo” studies have demonstrated that the simul-

Tab IV - STATISTICAL ANALYSIS (p VALUE) IN 106 SOFT TISSUE LIMB SARCOMA PATIENTS TREATED WITH HAP DOXORUBICIN + TNF

Factor	Local dis.free surv.		Overall survival	
	Univariate (P)	multivar. (P)	Univariate (P)	multivar. (P)
Age (</>50)	0.098	N.S.	0.07	N.S.
Sex	0.15	0.044	0.26	N.S.
Site (upper/lower)	0.23	N.S.	0.47	N.S.
Trial (A/B/C)	0.02	0.03	0.06	N.S.
Grading (G1, G2, G3)	0.69	N.S.	0.01	0.04
Necrosis (<50/51-75/>75)	0.05	0.01	0.14	N.S.
Local toxicity (I-II-III/IV-V)	0.35	N.S.	0.52	N.S.

taneous application of heat and doxorubicin (41.5°C) increases the anti tumor effect of doxorubicin (12-13).

- 90% to 97% of the doxorubicin is bound to the tissue after hyperthermic perfusion
- doxorubicin tissue concentrations obtained with hyperthermic perfusion are 5, 25 and 45 times greater than those obtained with normothermic perfusion and with intra – arterial and systemic infusion respectively.

The first Italian cooperative study was aimed at assessing the maximum tolerable doxorubicin dose during hyperthermic perfusion.

This was the first important step because patients treated with optimal doxorubicin dose did better than patients treated with suboptimal dose in terms of loco-regional control, disease-free and overall survival. Moreover limb sparing surgery was carried out in more than 90% of the patients with satisfactory functional results.

The second Italian cooperative group was a phase I - II study aimed at evaluating the maximum TNF tolerable dose in association to doxorubicin and hyperthermia (9).

This trimodality association has a strong rationale:

- in “in vitro” study the effect of TNF combination with various drugs was tested, Alexander demonstrated that among all the drugs employed the association of TNF and doxorubicin resulted in the greatest increase of efficacy (14).

Recently, the association of doxorubicin and TNF was tested in animal models in ILP regimen. The

results of this study have demonstrated that the association of TNF and doxorubicin exhibits a synergistic anti tumor effect, resulting in regression of the tumor in 54% and 100% of the cases for BN175 fibrosarcoma and ROS-1 osteosarcoma.

In both tumor models increased accumulation of doxorubicin in tumor tissue was found: 3.1-fold in the BN175 and 1.8-fold in the ROS-1 sarcoma after ILP with doxorubicin combined with TNF alpha in comparison with ILP with doxorubicin alone. This increase in local drug concentration may explain the synergistic anti-tumor response after ILP with the combination (15).

In our opinion hyperthermia play an important role in exploiting the treatment efficacy. Hyperthermia not only potentiates the effectiveness of doxorubicin but also the action mechanism of TNF alpha.

“In vitro” and “in vivo” experiments have demonstrated that a direct correlation exists between TNF alpha efficacy and temperature levels. (16).

At high temperature (> 41°C) the TNF alpha-hyperthermia association results in a greater than additive effect. Moreover, the TNF alpha resistant tumor cells can be killed by hyperthermia-TNF association.

The clinical effectiveness of the trimodality association (true hyperthermia-doxorubicin-TNF alpha) has been proven by results obtained in 30 patients candidate to amputation treated according to this protocol.

A complete pathological tumor response was observed in 22% of the patients, a partial response was recorded in 55% with an objective response of 77%. Moreover, a limb sparing surgery was performed in 77% of the patients.

Many authors have argued that hyperthermia may provoke an unacceptable toxicity in the perfused limb.

In our phase II study we have realized that the limb toxicity is strictly correlated to TNF α dosage and temperature level. As a matter of fact all grade IV limb reactions have been observed in patients treated with a mean muscle temperature > 41.5°C, therefore is mandatory to pilot the hyperthermic perfusion in a range of 40.8°C and 41.5°C, because within this range only grade II and few grade III limb reactions were observed.

As previously demonstrated no correlation was found between TNF α dosage and type of tumor response and it is our firm opinion that 1 mg of TNF α is the best dosage to be applied in clinical setting.

This statement is supported by “in vivo” experiments in which the TNF α dosage able to provoke a synergistic effect with doxorubicin or Melphalan is equivalent to 1 mg. (17, 15)

Moreover, a recent communication of a randomized study in which TNF α was administered at different dosages has pointed out that tumor response is not correlated with the TNF α dosage (Bonvalot, unpublished data).

In conclusion hyperthermic perfusion with doxorubicin and TNF α has been proven to be an excellent neoadjuvant therapy for unresectable soft tissue limb sarcoma because permits a limb sparing surgery in a high percentage of the cases with good functional results.

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