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In Transit Metastases of Malignant Melanoma Treated by High Dose rTNFα in Combination with Interferon-γ and Melphalan in Isolation Perfusion

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To increase the therapeutic efficacy of recombinant tumor necrosis factor alpha (rTNFα) and reduce the systemic side effects, a protocol was designed using isolation perfusion of the limbs with hyperthermia for in transit metastases of melanoma. A triple combination of high dose rTNFα + recombinant interferon-gamma (rIFN-γ) + melphalan was chosen because of a synergistic anti-tumor effect of rTNFα with rIFN-γ and of rTNFα with alkylating agents reported in the literature. Twenty-nine patients of mean age 60 years (range 22–82 years) entered the study after informed consent and received a total of 31 isolation perfusions with the triple combination. There were 24 women and 5 men with multiple progressive *in transit* melanoma metastases of the lower limb (stage IIIa or IIIab). rTNFα at the unique dose of 4 mg was injected as a bolus in the arterial line, under mild hyperthermic conditions (40 to 40.5°C) for 90 minutes. rIFN-γ was given subcutaneously on days −2 and −1 and in the perfusate, with rTNFα, at the dose of 0.2 mg. Melphalan was administered in the perfusate at dose giving a concentration of 40 μg/ml.

In all the 31 isolation perfusions performed in the triple combination protocol, in order to prevent a septic shock-like syndrome which had been encountered in 2 patients treated outside this protocol for sarcoma and carcinoma, the patients received dopamine continuous infusion at 3 μg/kg/min from the start of isolation perfusion and for 48 hours, and only showed mild hypotension and very transient chills and temperature. Regional toxicity attributable to rTNFα was minimal. There have been 16 patients with hematologic toxicity consisting of neutropenia (11 cases, 1 case grade 4 and 1 case grade 3) and neutropenia with thrombocytopenia (12 cases, 1 case grade 4 and 4 cases grade 2). Eighteen of 29 patients had been previously treated with melphalan in isolation perfusion (n = 13) or with cisplatinum (n = 2), rTNFα-Melphalan (n = 1), or rTNFα alone (n = 2). Median follow-up has been 41 weeks. The 29 patients are evaluable: there have been 26 (90%) complete remissions (CR), 3 (10%) partial remissions (PR), and no failures. Actuarial disease-free survival and total survival have been 63% and 73%, respectively, at 12 months. In all cases, softening of the nodules was obvious within 3 days after isolation perfusion and time to definite response ranged between day 6 and 22.

This interim analysis of a phase II study suggests that high dose of rTNFα can be administered with acceptable toxicity by isolation perfusion with dopamine and hyperhydration. Tumor responses can be evidenced in all patients, with 90% CR. Furthermore, combination of rTNFα, rIFN-γ, and melphalan seems to achieve high efficacy with minimal toxicity, even after failure of prior therapy with melphalan alone.

Recombinant tumor necrosis factor alpha (rTNFα) has a potent anti-tumor activity on human tumor xenografts when administered intralesionally, intraperitoneally, or subcutaneously [1, 2]. In humans, the administration of rTNFα is hampered by the occurrence of severe systemic side effects. rTNFα has been a major mediator of septic shock [3, 4]. Phase I studies so far indicate that the maximal tolerated single dose in humans is ≤350 μg/m² intravenously and 522 μg/m² intra-tumorally [5, 6]. Under these conditions, a negligible clinical response rate has been reported in all diseases including melanoma [7–9].

In 1975, TNFα was described as a peptide in the serum of animals treated with (Bacillus Calmette-Guerin) BCG [10]. Intra-tumoral BCG in subcutaneous and cutaneous metastatic melanoma nodules produced temporary tumor regression of injected nodules in patients and in immunocompetent animals [11]. In these cases, the anti-tumor effect of BCG has been correlated with the endogenous production of TNFα by the activated macrophages [12].

The beneficial anti-tumor effects of TNFα seem to be related to endothelial cell activation and vascular damage resulting in hemorrhagic necrosis, which starts within 1 to 4 hours after rTNFα administration, principally in intradermal tumors [13]. This might be due to the neovascular supply of superficial neoplastic nodules and/or to some specific property of the capillary bed of tumors in the skin. Another potential consequence of local administration of rTNFα is the lysis of the extracellular matrix resulting from the release of elastase by activated polymorphonuclear neutrophils observed in patients treated with interleukin-2 (IL2) and lymphokine activated killer (LAK) cells [14] during septic shock. Neutrophil elastase is a major determinant of neutrophil-induced endothelial and tissue damage [15].

It has been demonstrated that the number of the TNFα receptors on malignant cells increases when they are incubated with IFN-γ [16, 17]. In addition, a synergistic anti-proliferative activity of rTNFα and IFN-γ and alpha has been demonstrated *in vitro* and *in vivo* using human melanoma xenografts in nude mice [18–20].

An enhancement of the cytolytic activity of rTNFα by hyperthermia (40°C) has been demonstrated in experimental tumors both *in vitro* [21] and *in vivo* [22]. Cytotoxic activity of

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Table 1. Characteristics of patients receiving isolation perfusion with rTNFα, rIFN-γ and melphalan for melanoma.

Age (yrs)	
Mean	60
Range	22–82
Sex	
Male	5
Female	24
Stage	
IIIa	25
IIIab	4
In transit metastases	
1	3
2–10	16
10–50	5
50–100	1
>100	4
Previous isolation perfusion	18

rTNFα is enhanced when chemotherapeutic drugs, especially alkylating agents, are added *in vitro* and *in vivo* [23, 24].

Isolated limb perfusion (ILP) is an established method for treatment of in transit melanoma metastases as it allows the delivery of high doses of drug in a closed system with acceptable toxicity and minimal systemic side effects [25]. It was therefore tempting to use rTNFα in this setting despite the limitation of ILP to 1 operation, or a maximum of 3 with an increased surgical risk [26].

Melphalan is the first line ILP chemotherapy for the treatment of melanoma. It is a bifunctional alkylating agent which induces an average response rate, partial or complete, of 80% [26]. Complete response in gross disease averages 35% with a range of 10% to 65% [27]. Five-year survival after ILP for patients with stage III disease range from 31% to 74% [26]. *In vitro* anti-tumor cytotoxicity of melphalan can be enhanced by hyperthermia [28].

Materials and Methods

Patients

Criteria for eligibility in this phase II study was in transit metastases of melanoma either alone (stage IIIa) or associated with regional lymph nodes involvement (stage IIIab). Twenty-nine consecutive patients entered the study. Their characteristics are listed in Table 1. Patients entered the study from December 21, 1988 to May 12, 1991, after extended work-up and informed consent.

There were 24 women and 5 men, whose mean age was 60 years (range 22–82 years). Weight ranged 37–92 kg and body surface 1.18–1.97 m^2. There were 25 stage IIIa and 4 stage IIIab melanoma patients, all of the lower limb. Most of them (24 of 29) had more than 5 tumor nodules (Table 1). Eighteen of these patients had been previously treated by ILP with melphalan (n = 13), TNF-melphalan (n = 1) or CisDDP (n = 2), or rTNFα alone (n = 2) and had proven recurrence when admitted in this protocol.

Isolation Perfusion

Twenty-eight ILPs were performed through the iliac vessels, 2 through the femoral vessels, and 1 through the popliteal vessels. The technique has been described elsewhere [29]. We used a membrane oxygenator (VPCML Cobe) and silicone tubing. Priming consisted of 19 ml bicarbonate, 0.4 ml heparin, and 1 liter Haemaccel (Behring Hoechst, Belgium). This was supplemented with 1 unit of autologous fresh blood in the last 10 perfusions. Perfusate flow was set as high as possible and was typically 700 ml/min for lower limb ILP.

Potential leakage of the drugs was measured with radioactive human albumin (RIHSA, IRE) injected into the circuit and the radioactivity assessed in the peripheral plasma at 5, 30, and 60 minutes. The high levels of leakage observed in 5 patients were attributed to the development of an important collateral circulation in the thigh and around the hip as a consequence of previous ILP. The arterial blood temperature of the perfused limb was maintained at 40°C during the whole ILP. Four thermistor probes were implanted in the subcutaneous tissues and into the muscles to monitor the tissue temperature which ranged from 38°C to 40°C.

Drugs

rTNFα (Genentech Boehringer Ingelheim, 0.2 mg per ampule) and rIFN-γ (Boehringer Ingelheim, 0.2 mg or 1.5×10^6 U per ampule) were a gift from Boehringer Ingleheim. Both drugs were supplied as a lyophilized powder which was aseptically reconstituted with 1 ml sterile saline. Melphalan (Alkeran®) was obtained from Wellcome Benelux SA as sterile powder (100 mg) which was solubilized aseptically using solvent and diluent provided by the firm.

A high dose of rTNFα with a total dose of 4 mg for lower limb ILP was selected as the previous experience with cytostatics shows a tenfold increase of the systemic dosage provides good efficacy without significant systemic toxicity [26, 27]. During ILP, 1.5×10^6 U IFN-γ was injected subcutaneously for 2 days before surgery according to preclinical data on the induction of TNF receptors. This dosage of IFN-γ was chosen as it received the National Cancer Institute clearance for biological response modifiers (BRM) protocols. Prophylaxis of chills was achieved by 500 mg of paracetamol (Dafalgan®, Upsa Medica) given orally on the 2 pre-operative days.

Melphalan dosage was calculated according to the liter-volume method [30], 10 mg/l of perfused limb, and the total dose ranged between 54 mg and 120 mg. Exchangeable limb blood volume (ELBV) was assessed with the hematocrit method and additional priming with Haemaccel solution was done in order to achieve a final concentration of 40 μg melphalan/ml at equilibrium [29].

rIFN-γ and rTNFα were injected successively as a bolus in the arterial line. Melphalan was administered 30 minutes later. The whole perfusion lasted 90 minutes. At the end of the ILP, the limb was washed twice with 1 liter of Haemaccel and 1 liter of Macrodex® (Travenol).

Prophylaxis of Shock

Since a pilot study [30] had shown in 2 patients a profound circulatory shock which responded well to fluid loading and dopamine, the patients include in the phase II study received a prophylactic treatment. It consisted in the use of continuous intravenous infusion of 3 μg/kg/min of dopamine, starting

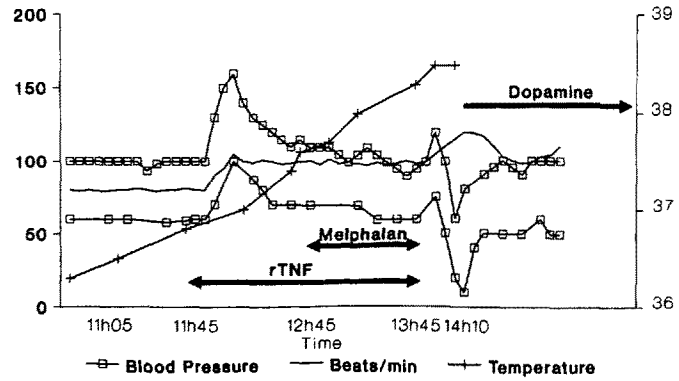

Fig. 1. Time course of blood pressure, cardiac frequency, and central temperature during isolation perfusion with 2 mg rTNFα, 0.2 mg rIFN-γ, and 80 mg melphalan. Dopamine 3 μg/kg/min was started when blood pressure dropped after releasing the tourniquet.

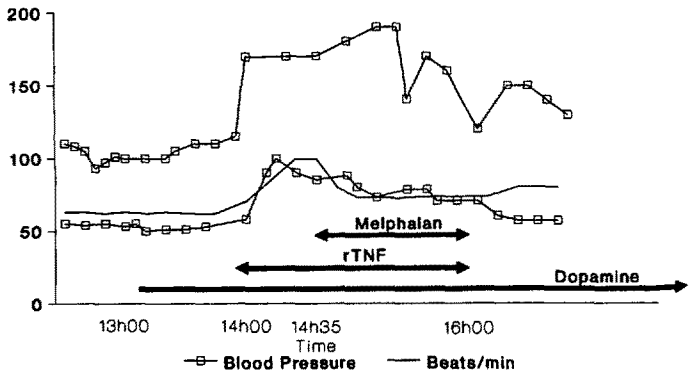

Fig. 2. Time course of blood pressure and cardiac frequency during ILP with 3 mg rTNFα, 0.2 mg rIFN-γ, and 79 mg melphalan. Dopamine 3 μg/kg/min was started before injecting rTNFα in the arterial line.

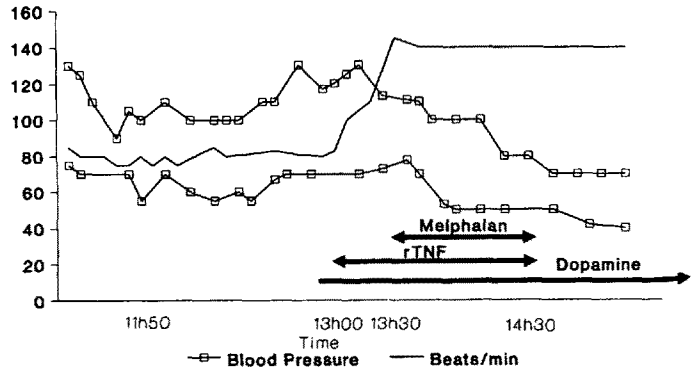

Fig. 3. Time course as for Figure 2. ILP with 4 mg rTNFα, 0.2 mg rIFN-γ, and 110 mg melphalan. Dopamine was started before TNF.

before the injection of rTNFα in the circuit and systematically during at least 48 hours postoperatively. Hyperhydration was applied before releasing the tourniquet after completion of the washout.

Monitoring and Assessment of Regional Toxicity

Regional tissue toxicities were recorded according to the classification described by Wieberdink and coworkers [31]. Electrocardiogram, urine output, blood pressure, venous and pulmonary pressures, and arterial wedge pressure were recorded and monitored by an arterial and a Swan-Ganz catheter from the beginning of the ILP until the second postoperative day.

Assessment of Response

Complete (CR) and partial responses (PR), no change (NC), and progressive disease (PD) were assessed according to standardized criteria [32].

Results

At the time of this report, 19 of the 29 patients are alive and a median follow-up of 41 weeks has been obtained, with a range of 8-112 weeks.

Hemodynamic Changes during ILP with rTNFα

Figure 1 shows the time course of blood pressure, pulse rate, and central temperature of a patient who received the triple combination therapy without dopamine prophylaxis (case from the pilot study). Because of the leakage, there was a rise in temperature from the beginning due to the hyperthermic perfusion and probably also to the pyrogenic effect of rTNFα and rIFN-γ. Injection of rTNFα increased both pulse and blood pressure. After washout and release of the tourniquet, the patient experienced a sudden shock which responded well to dopamine and fluid support.

In Figures 2 and 3, patients received shock prophylaxis. Figure 2 is a typical example of what was observed, i.e., a hyperdynamic phase without shock. In Figure 3, the patient had a blood pressure drop and tachycardia which reversed slowly.

Systemic Side Effects

As shown in Table 2, 7 patients experienced hypotension either during, or and more often, after releasing the tourniquet. This was not accompanied by shock in any of the patients and all quickly recovered under dopamine and fluid support. All the patients had fever and chills an average of 6 hours (4–15 hours) after the end of the perfusion. During that time, studies to document any potential infection were negative. There have been 5 cases of ARDS incipiens (acute respiratory distress syndrome) with low arterial pO_2 and positive chest x-ray. With adequate therapy, recovery was obtained within 12 hours postoperatively. Although 1 patient had experienced a reversible WHO grade 3 kidney toxicity after rTNFα alone [30], she did not develop any kidney toxicity after triple combination. No other patients developed kidney toxicity. There have been 10 cases of isolated moderate (2 to 3 fold) bilirubin elevation without increase of liver enzymes. Hematological toxicity was encountered in 16 patients. Only 1 case of WHO grade 4 for leukocytes and platelets was found. As the cumulative systemic leakage during perfusion reached 31%, this toxicity can be attributed to melphalan. All hematological toxicities were reversible.

Table 2. Toxicity following isolation perfusion with rTNFα, rIFN-γ and melphalan for melanoma.

Systems	Symptoms	Frequency
Blood pressure	Hypotension	<80/40:4/31 mild:3/31
Chills, temperature	38°C–40°2C	31/31 lag time 6 hours
Respiratory	ARDS-like	5/31
Kidney		0/31
Liver	Bilirubin elevation	10/31
Hematological	Leukocytes 11/31	WHO 4:1/31 WHO 3 1/31 WHO 2:7/31 WHO 1:2/31
	Platelets 12/31	WHO 4:1/31 WHO 2:4/31 WHO 1:7/31
Perfused limb	Skin toxicity	Grade II:24/31 Grade III:7/31

ARDS: acute respiratory distress syndrome; number of patients with symptom/total number of patients.

Regional Toxicity

Regional toxicity attributed to rTNFα was minimal (Table 2). In the 31 ILPs, grade II skin toxicity was observed in the majority of patients but no grade IV skin toxicity was observed.

rTNFα Levels in Perfusate

Perfusate samples from 3 ILPs using 2 mg and 3 mg of rTNFα showed plateau levels of rTNFα varying from 970 ng/ml to >2000 ng/ml as measured by enzyme linked immuno-sorbant antibody (ELISA) method.

Melphalan Levels

The presence of rTNFα, rIFN-γ, and hyperthermia at 40°C did not alter melphalan pharmacokinetics which were similar to previously reported data [33] with a typical biexponential disappearance curve (data not shown).

Surgical Complications

No peri-operative death has occurred. One infection of a retroperitoneal hematoma had to be drained by laparotomy. The 2 oldest patients underwent partial amputation. The first patient underwent amputation to the mid-thigh 1 week after ILP for repeated arterial thrombosis at the cannulation level of the iliac artery. This complication must be attributed to the advanced atheromatosis and to the 2 previous ILPs with melphalan and not to the direct toxic effect of rTNFα on the vessels. One month later, after a wound infection was treated with antibiotics and local irrigation, this patient presented with an acute hemorrhage secondary to the rupture of the external iliac artery. The artery was ligated and the patient recovered completely. The second patient presented with major distal arteriopathy of the leg. Eleven days later, amputation was undertaken at mid leg because the proximal arteries were normal. In both cases, the major part of the tumor bed was not amputated as an objective response had already been detected. Two cases of iliac venous thrombosis were recorded and successfully treated with heparin for 3 weeks.

Table 3. Tumor response following isolation perfusion with rTNFα, rIFN-γ and melphalan for melanoma.

Number of patients entered	29
Number of ILPs	31
Follow-up time (range)	median 41 weeks (3–112)
Number of evaluable patients	29
Tumor softening time (range)	mean 2.8 days (1–4)
Time to definite response (range)	mean 9 days (6–22)
Complete response (CR)	26 (90%)
Partial response (PR)	3 (10%)
Stable disease or progression	0
Response duration (range)	mean 33^+ weeks (8^+–112)
Histologic documentation of responses	15 (51%)

Tumor Response

In all cases, an early and spectacular softening of the tumors was seen within the 3 first days after TNF ILP, as the first sign of tumor response (Table 3). Objective regression rapidly appeared. In the 29 patients, there have been 26 (90%) CR and 3 PR, with no failures after the triple drug regimen. Mean time to objective response was 9 days (range 5–22 days) for CR and 16 days (range 13–21 days) for PR. A striking observation is that most CR occurred with bulky tumors and that the 3 PR were at least with 75% tumor shrinkage.

Median follow-up time has been 41 weeks and mean response time 33^+ weeks with a range of 8^+ weeks to 112 weeks (Table 3). Complete remission was further documented histologically in 15 patients. Four CR patients developed new nodules on the hip and the iliac region. Two patients were treated with intralesional rTNFα 0.2 mg + rIFN-γ 0.2 mg with mixed response. Those patients developed distant metastases without recurrence in the perfused limb. Overall, 10 patients died from distant metastases but only 2 of these patients developed recurrence in the perfused limb.

Survival

Figure 4 shows the actuarial disease free survival and Figure 5 the actuarial overall survival of 63% and 73%, respectively, at 12 months (Kaplan-Meier).

Discussion

The preliminary results of this phase II study that high doses of rTNFα can be administered safely by ILP provided patients are prophylactically treated with dopamine and hyperhydration. When hypotension occurs, fluid loading and increase of dopamine dosage results in early recovery. Tissue toxicity of the perfused limb seems to be less severe in the patients treated with the combination rTNFα/rIFN-γ/melphalan than in those treated with melphalan alone as the current rate of grade III toxicity has been 36% with an average dosage of 10 mg melphalan per liter of limb volume or 40 μg/ml perfusate [34].

The combination rTNFα/rIFN-γ/melphalan seems to achieve high efficiency with acceptable toxicity and prolonged response

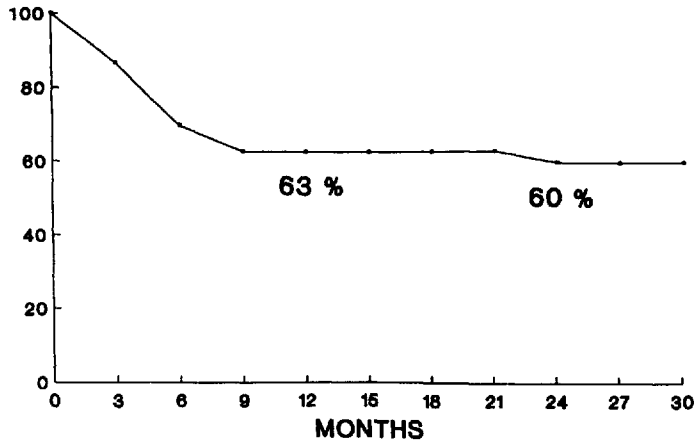

Fig. 4. Overall survival of 29 patients treated by 31 ILPs of the lower limb for in transit melanoma metastases (stage IIIa and IIIab) with high dose rTNFα, rIFN-γ and melphalan.

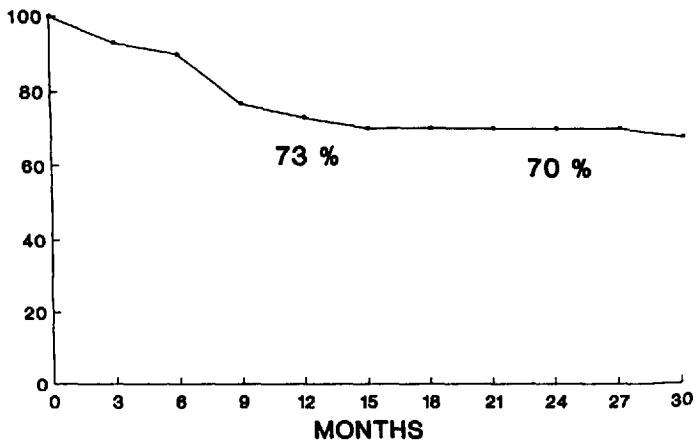

Fig. 5. Disease free interval of 29 patients treated by 31 ILPs of the lower limb for in transit melanoma metastases (stage IIIa and IIIab) with high dose rTNFα, rIFN-γ and melphalan.

time. However, it should be stressed that the prevention of septic shock-like syndrome is of paramount importance. Recurrent sarcomas and carcinomas of the extremities have recently been treated at the Jules Bordet Institute with the same regimen (in preparation) and 2 peri-operative deaths have been observed. In 1 patient, a severe pre-existing lymphedema resulted in a high release of rTNFα into the systemic circulation after perfusion, which caused a septic shock-like syndrome. The second patient presented with a deep shock which was associated with an iatrogenic pneumothorax followed by cardiac arrest for 20 minutes. The 2 patients died on day 21 and 18 respectively of genuine septic shock and decerebration. To the authors' knowledge, an additional death was reported in another institution abroad (confidential communication) the day after TNF isolation perfusion, from septic shock-like syndrome. This had not been diagnosed because the patient seemed well after perfusion and was not sent to the intensive care unit. These records indicate the necessity of carefully monitoring the patients in intensive units at least for 48 hours. In addition, patients with lymphedema have to be excluded.

The delay of tumor response, especially softening, was much shorter after rTNFα than that usually observed after ILP with high dose melphalan [35]. An early softening reaction had been observed in 2 of 3 patients who received rTNFα alone in a pilot study [30] suggesting that the early softening can be attributed to rTNFα. A partial hemorrhagic necrosis was reported 24 hours after systemic TNF administration of 200 μg in a sarcoma patient [36].

In the present study, time to response was 14 days and 13.5 days for CR and PR, respectively, as compared to 49.3 days and 50.1 days in a previous series of ILPs with melphalan alone also in our institute [35].

The response rate of 100% observed in this series of 29 evaluable patients with 90% CR appears higher than is obtained with melphalan in patients who had not been previously treated with melphalan [26, 27], and it should be emphasized that 18 of 29 patients had recurrence or persistence of tumor after ILP. Moreover, most patients included in this study had very bulky tumors which responded as rapidly, and to an equal extent, as smaller tumors. It should be stressed that despite the poor prognostic factors such as the tumor bulk and the fact that most cases were recurrent, the overall survival and disease-free interval have been unexpectedly high.

rTNFα pharmacokinetics showed high plateaus of 1.5 to 2 μg/ml with no decay attributable to degradation. Thus rTNFα at high concentration appears to be more stable than the reported < 60 minutes half life when administered systemically [37]. This stability may also be due to the lack of by-pass in the liver during ILP.

These results demonstrate for the first time, a rapid, impressive and sustained response of melanoma metastases to high dose rTNFα when administered by ILP, in combination with rIFN-γ and chemotherapy. This triple drug regimen appears to be an attractive therapeutic approach in the treatment of regionally advanced melanoma [30], especially in case of melphalan failure. Because of the unacceptable systemic toxicity of such a regimen, it should be limited to isolation perfusion of the limbs. Moreover, great care must be taken to avoid septic shock-like syndrome. Additional multicentric randomized studies are underway in order to explore the effect of rIFN-γ.

Résumé

Pour augmenter l'efficacité thérapeutique du facteur recombinant de nécrose tumorale alpha (rTNFα) et pour réduire les effets secondaires, on a élaboré un protocole utilisant une perfusion isolée des extrémités associée à une hyperthermie chez les patients atteints de métastases d'un mélanome. En raison d'un effet synergique antitumoral, de rTNFα et de l'interféron gamma recombinant (rIFN) d'une part et de rTNFα et des agents alkylisants d'autres part (effet rapporté dans la littérature), on a utilisé une triple combinaison de rTNFα à hautes doses, rIFN et melphalan. Vingt neuf patients d'âge moyen de 60 ans (extrêmes 22–82 ans) ont été inclus dans cette étude après avoir donné leur consentement éclairé. Ils ont reçu un total de 31 perfusions de la tripe association. Il y avait 24 femmes et 5 hommes ayant des métastases multiples extensives des membres inférieurs (stade III a ou II ab). rTNFα a été administré à une dose unique de 4 mg injectée en bolus par voie artérielle, dans des conditions d'hyperthermie modérée (40 à

40.5°C) pendant 90 minutes. rTNFα a été donné en sous-cutanée aux jours −2 et −1 mélangé à la perfusion de rTNFα à la dose de 0.2 mg. Le melphalan a été administré à la concentration de 40 mg/ml. Pour éviter un syndrome de choc rencontré chez deux patients traités hors protocole pour sarcome et carcinome, tous les patients de ce protocole ont reçu de la dopamine en perfusion continue à la dose de 3 μg/kg/mn depuis le début de la perfusion et pendant 48 heures, et n'ont eu qu'une hypotension modérée avec des frissons transitoires. La toxicité attribuée au rTNFα était minime. On a eu 16 cas de toxicité hématologique comprenant une neutropénie (11 cas, 1 grade 4, 1 grade 3) et neutropénie avec thrombopénie (12 cas, 1 grade 4, 4 grade 2). Dix huit patients avaient déjà été traités par Melphalane en perfusion isolée (13/39) ou en association avec du cisplatinium (2/29) ou par l'association rTNFα-melphalan (1/29) ou le rTNFα seul (2/29). Le suivi moyen était de 41 semaines. Sur les 29 patients évalués, il y a eu 26 rémissions complètes (RC) (90%), 3 rémissions partielles (RP) (10%) et ancun décès. Les survies actuarielles sans maladie et globale à 12 mois sont respectivement de 63 et de 73%. Dans tous les cas, les nodules se sont assouplis en moins de 3 jours après le début de la perfusion. Le délai de réponse au traitement variait entre 6 et 22 jours. L'analyse intermédiaire de l'étude de phase II suggère que de fortes doses de rTNFα peuvent être administrées avec une faible toxicité en perfusion associées à la dopamine et à une hyperhydratation. La réponse tumorale était évidente chez tous les patients avec une CR de 90%. De plus, l'association de rTNFα, IFN et melphalan semble donner une grande efficacité avec une toxicité minime, même en cas d'échec antérieur avec le melphalan utilisé seul.

Resumen

Se diseñó un protocolo que utiliza la perfusión aislada de las extremidades con hipertermia a fin de incrementar la eficacia terapéutica del factor necrotizante tumoral alfa recombinante (rFNTα) y reducir sus efectos secundarios sistémicos, en el tratamiento de metástasis en tránsito del melanoma. Se escogió una combinación triple de alta dosis de rFNTα + interferón-gamma recombinante (rIFN-γ) + melfalán en virtud del efecto sinergístico antitumoral del rTNFα con el IFN-γ y del rTNFα con los agentes alquilantes informados en la literatura. Veintinueve pacientes con edad promedio de 60 años (rango 22–82) ingresaron al estudio bajo consentimiento informado y recibieron un total de 31 perfusiones aisladas con la triple combinación. Hubo 24 mujeres y 5 hombres con metástasis en tránsito de melanoma de la extremidad inferior (estado IIa o IIIab). El rTNFα en la dosis única de 4 mg fue inyectado en bolo en la línea arterial, bajo condiciones levemente hipertermicas (40 a 50°C) por 90 minutos. El rIFN-γ fue administrado en los días −2 y −1 en el líquido perfusión, con rTNFα en la dosis de 0.2 mg. El melfalán fue administrado en el líquido de perfusión en una dosis para proveer una concentración de 40 μg/ml.

En todos los casos de perfusión aislada en el protocolo de triple combinación, y con el objeto de prevenir un cuadro del tripo de "shock-syndrome" que había sido observado en 2 pacientes tratados por sarcoma y carcinoma por fuera de este protocolo, se administró dopamina en infusión continua a una rata de 3 μg/kg/min desde el comienzo de la pefusión aislada y, por 48 horas; los pacientes sólo exhibieron hipotensión leve y escalofríos y fiebre transitorios. La toxicidad regional atribuible as rTNFα fue mínima. Se han presentado 16 casos con toxicidad hematológica consistente en nuetropenia (11 casos, uno grado 4 y uno grado 3) y neutropenia con trombocitopenia (12 casos, uno grado 4 y cuatro grado 2). Dieciocho de 29 pacientes habían sido previamente tratados con melfalán en perfusión aislada (13/29) o con cisplatino (2/29), rTNFα-melfalán (1/29) or rTNFα solamente (2/29). El promedio del sequimiento fue 41 semanas. Los 29 pacientes son valorables: ha habido 26 remisiones completas (90%), 3 remisiones parciales (10%) y ninguna falla. Las tasas de sobrevida actuarial libre de enfermedad y de sobrevida total han sido 63% y 73%, respectivamente, a 12 meses. En la totalidad de los casos apareció evidente el ablandamiento de los nódulos en los primeros 21 días después de la perfusión aislada y el intervalo hasta la respuesta difinitiva varió entre el día 6 y el día 22.

El análisis interim de un estudio de fase II sugiere que la alta dosis de rTNFα puede ser administrada con aceptable toxicidad por perfusión aislada con dopamina e hiperhidratación. Las respuestas tumorales pueden ser evidenciadas en la totalidad de los pacientes, con 90% de remisión completa. Además, la combinación de rTNFα, rIFN-γ y melfalán parece ser de elevada eficacia con toxicidad mínima, aún después de una falla terapéutica con melfalán sólo.

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