Synergistic Antitumor Activity of Histamine Plus Melphalan in Isolated Limb Perfusion: Preclinical Studies

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Background: We have previously shown how tumor response of isolated limb perfusion (ILP) with melphalan was improved when tumor necrosis factor alpha (TNF-α) was added. Taking into account that other vasoactive drugs could also improve tumor response to ILP, we evaluated histamine (Hi) as an alternative to TNF-α. Methods: We used a rat ILP model to assess the combined effects of Hi and melphalan (n = 6) on tumor regression, melphalan uptake (n = 6), and tissue histology (n = 2) compared with Hi or melphalan alone. We also evaluated the growth of BN-175 tumor cells as well as apoptosis, necrosis, cell morphology, and paracellular permeability of human umbilical vein endothelial cells (HUVECs) after Hi treatment alone and in combination with melphalan. Results: The antitumor effect of the combination of Hi and melphalan in vivo was synergistic, and Hi-dependent reduction in tumor volume was blocked by H1 and H2 receptor inhibitors. Tumor regression was observed in 66% of the animals treated with Hi and melphalan, compared with 17% after treatment with Hi or melphalan alone. Tumor melphalan uptake increased and vascular integrity in the surrounding tissue was reduced after ILP treatment with Hi and melphalan compared with melphalan alone. In vitro results paralleled in vivo results. BN-175 tumor cells were more sensitive to the cytotoxicity of combined treatment than HUVECs, and Hi treatment increased the permeability of HUVECs. Conclusions: HI in combination with melphalan in ILP improved response to that of melphalan alone through direct and indirect mechanisms. These results warrant further evaluation in the clinical ILP setting and, importantly, in organ perfusion. [J Natl Cancer Inst 2004;96:000–000] [J Natl Cancer Inst 2004;96:1603–10]

Isolated limb perfusion (ILP) is a treatment method in which high concentrations of drugs are administered to a limb containing an unresectable tumor that is temporarily isolated from the rest of the body’s circulatory system by the use of an extracorporeal perfusion circuit and a tourniquet placed at the root of the limb. ILP with tumor necrosis factor alpha (TNF-α) and melphalan is associated with synergistic antitumor effects against melanoma (1), large soft-tissue sarcomas (2,3), and various other tumors in the clinical setting (4–6). We have previously shown that the basis for the synergy is both a substantial enhancement of tumor-selective melphalan uptake (7) and the complete destruction of the tumor vasculature (2). The enhanced tissue uptake of different cytotoxic agents, when combined with TNF-α, shown in various limb and liver tumor models in our laboratory (7–12), prompted us to investigate a number of vasoactive substances for similar effects.

Histamine (Hi) is an obvious candidate to enhance tissue uptake of cytotoxic agents during ILP. It is an inflammatory mediator that is formed and stored mainly in the granules of mast cells and basophils, but it has also been identified in epidermal cells, gastric mucosa, neurons of the central nervous system, and in cells in regenerating or rapidly growing tissues. Its effect on fine vessels is to cause edema by increasing the flow of lymph and lymph proteins into the extracellular space and also by promoting the formation of gaps between endothelial cells, thus increasing transcapillary vesicular transport (13). The same mechanism that causes edema in fine vessels could potentially be used to increase drug concentrations in tumor tissues.

In this study, we performed ILP in a rat model by using combinations of Hi and melphalan to determine if Hi would increase the effects of melphalan. To determine the in vivo...
mecanisms involved, we measured melphalan uptake and performed histologic analysis after treatment. In addition, cultured sarcoma (14) and normal endothelial cells were treated in vitro with Hi, melphalan, or a combination of the two, and cytotoxicity, necrosis, apoptosis, and paracellular permeability were assayed.

**MATERIALS AND METHODS**

**ILP**

Male inbred Brown Norway rats weighing 250–300 g were obtained from Harlan-CPB (Austerlitz, The Netherlands) and were fed a standard laboratory diet ad libitum (Hope Farms, Woerden, The Netherlands). The studies were done in accordance with protocols approved by the Animal Care Committee of the Erasmus University Rotterdam (Rotterdam, The Netherlands).

Small fragments (diameter = 3 mm) of the spontaneous, nonimmunogeneic, syngeneic BN-175 sarcoma (14) were inserted subcutaneously in the right hind legs of the rats, as previously described (8). Tumor growth was measured daily with a caliper, and tumor volume was calculated using the formula $0.4(A^2 \times B)$ (where $B$ represents the largest tumor diameter and $A$ is the diameter perpendicular to it). When tumor diameter exceeded 25 mm or at the end of the experiment, rats were anesthetized and killed by cervical dislocation.

The treatment consisted of the experimental ILP previously described (8, 11). In brief, 7–10 days after tumor fragments were inserted (when they reached a diameter of 12–15 mm) rats were anesthetized by intraperitoneal ketamine and intramuscular hyoscine butylbromide. An incision parallel to the inguinal ligament was made, and the inguinal vessels were cannulated and connected by way of a low-flow roller pump (Watson Marlow, Falmouth, U.K.) to an oxygenated reservoir where drugs were added, in bolus, to the perfusate (total volume = 5 mL). A groin tourniquet was used to occlude collateral vessels, allowing a proper isolation of the limb. The temperature of the limb was maintained at 38 °C using a warm-water blanket.

The perfusate consisted of hemaccel alone (sham) (Boehringer Pharma, Amsterdam, The Netherlands), hemaccel plus 40 μg of melphalan (Alkeran Wellcome, Beckenham, UK), hemaccel plus 40 μg of melphalan and 1000 μg of Hi (kindly provided by Maxim Pharmaceuticals, San Diego, CA), or hemaccel plus 1000 μg of Hi.

To evaluate the role of the different Hi receptors in the Hi-based ILP, the Hi receptor blockers promethazine (H1-R) (Centrafarm, Etten-Leur, The Netherlands) and famotidine (H2-R) (Sigma, Zwijndrecht, The Netherlands) were added to the perfusate (200 and 50 μg/mL, respectively) and allowed to circulate into the limb for 5 minutes before melphalan and Hi were added.

Tumor dimensions were measured every day and used to monitor tumor volume. Volume on day 9 was compared with that on day 0, and response was classified as follows: progressive disease, increase of more than +25%; no change, volume between −25% and +25%; partial remission, decrease between −25% and −99%; or complete response, no palpable tumor.

Limb function was clinically observed as the ability to walk and stand on the perfused limb after ILP. On a scale from grade 0 to 2, grade 0 is severely impaired function in which the rat drags its hind limb, grade 1 is slightly impaired function (cannot use it in a normal way but can stand on it), and grade 2 is an intact function (normal walking and standing pattern) (8).

**In Vivo Melphalan Uptake**

To evaluate melphalan distribution, we killed 11 rats (six treated with Hi plus melphalan and five treated with melphalan alone) immediately after ILP was performed. Tumors and muscle from the limb were removed, snap-frozen in liquid nitrogen, and stored at −80 °C. Tissues were homogenized in 2 mL of acetonitrile with a PRO 200 homogenizer (Pro Scientific, Oxford, CT) and centrifuged at 2500 g and 4 °C. Melphalan concentration (reported as nanograms of melphalan per gram of tissue) was measured by gas chromatography–mass spectrometry on at least three different pieces of similar final weight per sample, as described previously (7, 15). Given the tumor and muscle values for melphalan uptake, the tumor-to-muscle ratio was calculated, considering the amount of melphalan measured in muscle as 100% and calculating the tumor value in comparison with it.

**Histologic Evaluation**

Two animals from each treatment group were killed by cervical dislocation directly after ILP; tumors and a piece of muscle from the limb were excised and cut in half. One half was fixed in 4% formaldehyde solution, embedded in paraffin, and stained with hematoxylin and eosin. Images of stained samples were taken on a Leica DM-RXA microscope (Leica Microsystems, Rijswijk, The Netherlands) with a Sony 3CCD DXC camera (Sony Netherlands, Badhoevedorp, The Netherlands).

**Cell Culture**

BN-175 cells (spontaneous rapidly growing and metastasizing soft-tissue sarcoma) (14) were grown in RPMI 1640 medium (Life Technologies, Leiden, The Netherlands) supplemented with 10% fetal calf serum (FCS) and 0.1% penicillin–streptomycin (Life Technologies). For growth assays, BN-175 cells were plated in 96-well flat-bottomed microtiter plates (Costar, Cambridge, MA) at 10^5 cells per well (in 100 μL) 24 hours before treatment and allowed to grow to confluence. Next, the cells were incubated at 37 °C in 5% CO₂ for 72 hours in the presence of medium alone or medium plus various concentrations of melphalan and Hi. Hi concentrations ranged from 0 to 200 μg/mL. Melphalan concentration ranged from 0 to 8 μg/mL.

HUVECs were prepared by collagenase treatment of freshly obtained human umbilical veins and cultured in human endothelial serum-free medium–RPMI medium (Cambrex Bioscience, Verviers, Belgium) supplemented with 10% heat inactivated human serum (Invitrogen Life Technologies, Breda, The Netherlands), 20% FCS, human epidermal growth factor, human basic fibroblast growth factor, and 0.1% penicillin–streptomycin (Life Technologies). For growth assays, HUVECs were plated 24 hours before treatment at 6 × 10^5 cells per well and cultured for 48 hours with various concentrations of Hi (0 to 200 μg/mL) and melphalan (0 to 200 μg/mL).

**Cell Growth**

Growth of BN-175 cells and HUVECs was measured using the Sulforhodamine-B (SRB) assay (16). In brief, cells were
washed with phosphate-buffered saline, incubated with 10% trichloroacetic acid for 1 hour at 4 °C, and washed again with phosphate-buffered saline. Cells were stained with SRB (0.5% SRB in 1% acetic acid) for 15 to 30 minutes, washed with 1% acetic acid, and air-dried. Protein-bound SRB was dissolved in Tris base (10 mM, pH 9.4). Absorbance at 540 nm was measured for each well, and tumor cell growth was calculated according to the following formula: percentage of tumor cell growth = (absorbance of test well/absorbance of control well) × 100%. The Hi concentration leading to 50% reduction in absorbance compared with control (i.e., 50% inhibitory concentration [IC50]) was determined and the 95% confidence intervals (CIs) were determined and reported.

**HUVEC Morphology and Necrosis–Apoptosis Assays**

HUVECs were plated 24 hours before treatment at 6 × 10^4 cells per well in flat-bottomed 12-well plates (Costar) in a volume of 900 μL per well and grown to confluence. Cells were then incubated at 37 °C in 5% CO2 with various concentrations of Hi for various times. At each time point, medium was discarded and replaced with 500 μL of HUVEC medium plus 0.05% YO-PRO for detection of apoptotic cells (Molecular Probes) or with propidium iodide to detect necrotic cells (Sigma). Cells were incubated for 30 minutes in the dark at 37 °C, and pictures were taken with a Zeiss AxioVert 100M inverted microscope (Carl Zeiss, Sliedrecht, The Netherlands).

Cells were cultured and treated using the time points above with the Vybrant Apoptosis assay kit #3 (Molecular Probes) for both adherent and detached cells. In brief, cells were treated with various concentrations of Hi alone (0 to 200 μg/mL), melphalan alone (0 or 8 μg/ml), or with combinations of the drugs for 15 or 30 minutes. Culture medium containing floating cells was removed from the wells and transferred to 5-mL tubes. Adherent cells were washed with RPMI medium, trypsinized with 300 μL of trypsin–EDTA (Biowhitaker), neutralized with 100 μL of HUVEC medium containing 20% FCS, and added to the 5-mL tubes. Tubes were centrifuged for 5 minutes at 250g, and the supernatant was discarded. Cells were then incubated in 200 μL of annexin binding buffer and propidium iodide, with or without annexin V (both reagents from the Vybrant Apoptosis assay kit) at room temperature for 15 minutes in the dark and evaluated by flow cytometry with a FACSscan (Becton Dickinson, Alphen aan den Rijn, The Netherlands) flow cytometer. Data were processed with Winmidi software version 2.7 (J. Trotter; Salk Institute, San Diego, CA). Experiments were done three times in duplicate, and the mean and 95% CIs of the percentage of living, apoptotic, and necrotic cells were reported.

**Endothelial Cell Monolayer Permeability Assay**

HUVECs were plated 48 hours before treatment at 6 × 10^4 cells per well in a monolayer on a fibronectin-coated polycarbonate membrane (diameter = 6.5 mm; pore size = 0.4 μm) in a transwell device (Costar). HUVEC medium (1 mL) was added to the lower compartment. Approximately 6 hours after the cells reached confluence, medium in the upper chamber was replaced with 50 μL of fluorescein isothiocyanate–bovine serum albumin (FITC–BSA) (1 mg/mL; Sigma) plus 250 μL of HUVEC medium containing various concentrations of Hi. At the same time, medium in the lower chamber was replaced with 700 μL of HUVEC medium. Fifty-microliter samples were taken from the lower chamber at various times, and FITC fluorescence was measured with a fluorescence spectrophotometer (Victor® FSR; Perkin Elmer, Bucks, U.K.) at 490 nm excitation and 530 nm emission. Values were compared with a standard curve based on known concentrations of FITC–BSA.

Next, to evaluate whether melphalan would have any effect on endothelial cell permeability, directly or in conjunction with Hi, the HUVEC monolayer was exposed to 250 μL of HUVEC medium alone (control), melphalan at 8 μg/mL, or Hi at 100 μg/mL with or without melphalan (8 μg/mL). Permeability was assayed as described above. Experiments were done three times in duplicate. The data were reported as the mean and 95% CIs of all values.

**Statistical Analysis**

Tumor growth curves were plotted as means and 95% CIs of the data from all animals. We used repeated-measure analyses of variance on the three most representative days, taken from the growth curve patterns 4, 8, and 10 using SAS Software release 8.2 for Windows 2000 (SAS institute, Cary, NC) using PROC MIXED. Main effects of treatment and day (three levels: days 4, 8, and 10) were included in the models, as was the interaction between treatment and day. For days in which response was statistically significant, interaction terms were further investigated by testing for differences following treatment on that day.

The data from HUVEC monolayer permeability assays was also analyzed as described above. The effects of treatment and time (5 levels: 0, 15, 30, 45, and 60 minutes) were evaluated.

Viability of HUVECs after Hi incubation data was presented and analyzed using the Kruskal–Wallis test with SPSS version 10.0 for Windows 2000.

Melphalan accumulation was shown both as mean values (with 95% CIs) of three measurements performed using different tumor areas and as a ratio between tumor and muscle values, expressed in percentages of tumor versus muscle melphalan uptake. Data were analyzed using the Mann–Whitney U test with SPSS version 10.0 for Windows 2000.

Synergism between Hi and melphalan was evaluated by determining whether tumor response after Hi alone or melphalan alone added together was different from the tumor response after Hi plus melphalan. First, the tumor response index was calculated by dividing the initial tumor volume by the tumor volume on a given day after treatment; then, the tumor response index of a rat from the Hi-treated group was randomly added to the tumor response index of a rat from the melphalan-treated group and compared with the tumor response index from the Hi-plus-melphalan group. Next, the data were analyzed with the Mann–Whitney U test (exact significance [2 × (one-tailed significance)] using SPSS version 10.0 for Windows 2000.

All statistical tests were two-sided. For all statistical tests, a P value less than .05 was considered statistically significant.
RESULTS

Tumor Response After Hi-based ILP

We previously showed that TNF-α improves the response to ILP by increasing the amount of melphanal delivered to tumor tissues (7). In this study, we used a similar model to test whether another vasoactive molecule, Hi, could also enhance melphanal uptake. A range of Hi concentrations were tested (20 to 200 μg/mL), and the concentration that led to optimal tumor regression was determined to be 200 μg/mL. Tumors grew exponentially in the Brown Norway rats after control ILP. However, the response to Hi plus melphanal ILP was striking, with a regression (more than a 25% decrease in tumor volume) in four (66%) of the six treated animals, including two (33%) with no palpable tumors approximately 10 days after treatment (P<.001). Perfusion with Hi or melphanal alone reduced or stabilized tumor growth—three stable (50%) and one regression (17%) (Fig. 1, A and Table 1). The combination of Hi plus melphanal showed a synergistic effect because the response index of the combination group was statistically significantly greater than that when the response index from the Hi and melphanal alone groups was randomly added (P = .043, Mann–Whitney U test [exact significance 2 × (one-tailed significance)]).

Perfusion with Hi, either alone or combined with melphanal, did not cause systemic toxicity. Only a transient, mild edema after Hi ILP, both with and without melphanal, was observed, leading to a temporary grade 1 toxicity in two rats for each group. After 2 days, the edema disappeared and limb function returned to normal.

Involvement of Hi Receptors in Hi-based ILP

To determine which Hi receptor (H1-R or H2-R) is involved in the effects observed above, specific Hi inhibitors were used during the treatment. Both pyrilamine, an H1-R blocker, and famotidine, an H2-R blocker, could block the effect of Hi in the ILP setting, which means that either H1 or H2 receptors are involved (Fig. 1, B).

Indirect Effect of Hi on Tumor Melphanal Uptake

We next evaluated whether Hi treatment could indirectly affect tumor-associated vasculature by increasing vascular permeability, which could cause more melphanal to accumulate in tumors than in normal tissue, as we previously showed using TNF-α combined with melphanal in ILP (7). To compare melphanal uptake in tumors and adjacent muscle, we excised tumors and muscle immediately after ILP with melphanal alone or melphanal combined with Hi and measured melphanal concentration. Hi addition not only led to a twofold increase in the amount of melphanal in tumor tissue (P = .024) but also reduced melphanal concentration in the muscle. As a result, adding Hi increased the ratio of melphanal in the tumor to that in the adjacent muscle by four (P = .02) (Fig. 2).

Table 1. Tumor response after histamine-based ILP

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>NC (%)</th>
<th>PD (%)</th>
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<tbody>
<tr>
<td>Sham (n = 5)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>100</td>
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<tr>
<td>Melphanal (n = 6)</td>
<td>—</td>
<td>—</td>
<td>17</td>
<td>17</td>
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<tr>
<td>HI (n = 6)</td>
<td>—</td>
<td>—</td>
<td>50</td>
<td>50</td>
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<tr>
<td>HI + melphanal (n = 6)</td>
<td>33</td>
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*=ILP = isolated limb perfusion. Volume on day 9 was compared with that on day 0, and response was classified as follows: PD = progressive disease, increase of more than 25%; NC = no change, volume between −25% and 25%; PR = partial remission, decrease between −25% and −99%; CR = complete response, no palpable tumor. Values are expressed in percentage of animals per response group. — = none.
Histology

To evaluate both the direct and indirect effects of Hi-based ILP on the tumor and the tumor-associated vasculature, we histologically examined tissue sections taken right after ILP was performed. After ILP with 200 μg/mL Hi alone, scattered vascular damage was observed (Fig. 3). After ILP with 200 μg/mL Hi and 8 μg/L melphalan, vascular damage became more pronounced. Perfusion with Hi alone resulted in vasodilatation of the tumor vasculature, extravasation of red blood cells into the tumor, and damage to the endothelial cell lining of tumor vessels. After ILP with Hi and melphalan, most of the tumor vessels were severely damaged and massive hemorrhage was observed. Tumor vessels showed loss of integrity and extensive gap formation, indicating edema. Red and white blood cells observed in the tissue suggested extravasation. We hypothesize that the edema observed in tumor tissue may indicate an augmented influx of melphalan from the blood stream into the tumor. In the muscle, however, no apparent changes in terms of hemorrhage, vasodilatation, or infiltrates after treatment, as above, were observed (data not shown).

These vascular effects were not observed when rats received sham ILP or melphalan via ILP (Fig. 3). After sham ILP, vessels were intact and tumor tissue was unaffected. When tumors were perfused with melphalan alone, some necrosis of the tumor tissue could be observed, but no vascular damage was seen. Together, these results indicate that Hi has tumor vascular–selective activity against the endothelial lining. This vascular effect was even more pronounced when Hi was combined with melphalan.

Cytotoxicity of Hi

The direct cytotoxic effects of Hi on BN-175 tumor cells and HUVEC endothelial cells were evaluated by means of in vitro cytotoxicity assays. Cell growth was inhibited in a concentration-dependent manner for both cell lines evaluated. BN-175 tumor cells were more sensitive to Hi, with an IC50 of 30 μg/mL. HUVEC appeared less sensitive to Hi with an IC50 of approximately 100 μg/mL (Fig. 4). The cytotoxic effect of Hi combined with melphalan in vitro was not synergistic, it was only additive.

Direct Effect on HUVEC: Morphology and Apoptosis Assay

In vitro, Hi was only slightly cytotoxic to HUVEC cells after long-term treatment (Fig. 4, B). Moreover, addition of Hi to melphalan did not enhance the sensitivity of HUVEC toward melphalan (Fig. 4, B). However, after ILP, a strong effect of Hi on the endothelial lining of tumor vessels was observed (Fig. 3). Therefore, we examined the morphology of HUVECs after short incubations (no longer than 60 minutes) with Hi plus melphalan. We observed a dose- and time-dependent effect of Hi on HUVEC, starting with the appearance of gaps between the cells. As time progressed, some cells became rounded and others became extended. In the higher concentration range or after prolonged incubation, cell fragments were seen in the medium (Fig. 5). Cells exposed to medium alone did not show these morphologic changes.

The observed differences in HUVEC morphology after Hi treatment prompted us to investigate whether these changes...
were irreversible, that is, whether they could lead to apoptosis or necrosis. With YO-PRO and propidium iodide to detect apoptosis and necrosis of adherent cells, respectively, we found no differences in the number of apoptotic or necrotic cells after exposure of HUVECs to Hi compared with exposure to medium alone (data not shown). When all cells, adherent as well as detached, were examined using the Vybrant apoptosis assay, no increase in the number of apoptotic cells or the number of necrotic cells was observed when Hi was added compared with medium alone (Fig. 6, A). Moreover, when Hi was combined with melphalan, no additional effect on permeability was observed after 15 and 30 minutes of incubation (curves start to parallel the control), respectively. Exposure of HUVECs to 200 μg/mL Hi resulted in an ongoing response of HUVECs as shown by the continuing permeability increase compared with control. Even at 60 minutes, the response of HUVECs to Hi did not parallel the control curve. Incubation with melphalan had no effect on the permeability of HUVEC monolayer, neither alone nor in combination with Hi (Fig. 7, B). The ongoing permeability increase might be essential to the observations in vivo.

**DISCUSSION**

This study shows for the first time, to our knowledge, the activity of Hi plus melphalan in ILP for the treatment of soft-tissue sarcomas. The strong effect of Hi-based ILP with melphalan was explained by three mechanisms: 1) direct cytotoxicity to the tumor cells, 2) direct cytotoxicity to the tumor-associated vasculature, and 3) an indirect effect through Hi-mediated, increased melphalan concentration in the tumor.
The direct inhibitory effect of Hi on tumor cells is in accord-
ance with previous reports on Hi receptor expression on dif-
ferent cell lines and human neoplasias, suggesting that it might
regulate tumor cell growth (18, 19). This growth-inhibitory
effect on the tumor cells, combined with the observed direct
effect on the endothelial cells, seen by us both in vitro and in vivo,
might be an explanation for the antitumor effect of Hi alone
(50% of the tumors stopped growing), compared with control
perfusion. More studies on the pharmacokinetics of higher doses
and evaluation in the clinical setting are, however, essential for
the clinical translation of Hi.

Our findings support a tumor endothelial cell–specific target-
ing effect of Hi resulting in dramatic hemorrhage and destruc-
tion of the endothelial cell lining of tumor vessels (confirmed
with CD-31 staining [data not shown]) in vivo. We hypothesize
that the pronounced direct effect of Hi on the endothelial cell
lining is fundamental for the better response than that achieved
by melphalan alone in the ILP model discussed here.

H₁ and H₂ Hi receptors were involved in Hi-induced tumor
regression in our model. Each receptor inhibitor alone blocked
the Hi effect in vivo. The two receptors are located in different
cell types and have independent mechanisms of action: H₁ has a
higher affinity, a rapid but short-lived effect, and is located in the
endothelial cells; H₂ has a lower affinity, a slower but more sus-
stained effect, and is located in the vascular smooth muscle
cells.

Toxicity would be unlikely to be a limiting factor for the use
of Hi in ILP in humans because no systemic toxicity was
observed, and the regional toxicity, affecting 33% of the rats
receiving Hi either alone or combined with melphalan, was very
mild and completely reversible after 2 days of recovery. Ac-
cordingly, ILP with TNF and melphalan in the clinical setting, as
Hi plus melphalan did in the animal model, also results in

Fig. 7. Effect of Hi on human umbilical vascular endothelial cell (HUVEC)
monolayer permeability. HUVECs were cultured on the filter of a transwell unit
for 48 hours before the addition of fluorescein isothiocyanate and bovine serum
albumin (FITC–BSA)–containing medium (control) or A) Hi in different concen-
trations. B) plus or minus melphalan to the upper compartment (see “Material
and Methods”). The amount of FITC–BSA in the lower compartment was
measured every 15 minutes for an hour. Values are from three experiments, each
done in duplicate. Error bars show 95% confidence intervals of the mean. * P
values using repeated-measure analysis of variance test P = .001 for 200 μg/mL
Hi at 15, 30, 45, and 60 minutes compared with control.
erythema and edema, which sometimes slightly impairs motility (grades II and III of Wieberdink, respectively) in most of the patients (6,22).

In conclusion, Hi combined with melphalan had a striking effect in the ILP for the treatment of soft-tissue sarcomas in rats. The mechanism of action involved both direct and indirect effects—cytotoxicity on the tumor and endothelial cells and tumor-associated vasculature with a twofold increase in the tumoral uptake of melphalan combined with a reduction in the uptake in the adjacent muscle. Therefore, Hi plus melphalan in ILP seems to be a promising alternative to TNF-α, to be evaluated in the clinical setting.

REFERENCES


NOTES

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