Potentiation of cyclophosphamide chemotherapy using the antiangiogenic drug thalidomide: Importance of optimal scheduling to exploit the normalization window of the tumor vasculature

J. segers[1], V. Difazio[2], R. Ansiaux[1], P. Martinive[1], O. Feron[1], P. Wallemacq[2], B. Gallez[1]
[1]UCL, Woluwe, Bruxelles, Belgium, [2]Saint-Luc Hospital, Woluwe, Bruxelles, Belgium

Introduction:

At the early phase of anti-angiogenic treatments, experimental evidence indicates a transient "normalisation" of the tumor vasculature, with a transient increase in tumor perfusion (1). This could provide enhancement of drugs delivery into the tumor. The aim of this work was to study how administration schedule affects potentiation of the chemotherapy drug cyclophosphamide by the anti-angiogenic agent thalidomide. To determine when transient normalization occurs in response to thalidomide, we used EPR oximetry. We previously showed that such measurements provide a surrogate marker for determining the reoxygenation associated with the increase in perfusion during the normalization produced by thalidomide (2). The tumor was treated with cyclophosphamide during this initial normalization period, then tumor regrowth delays were measured. Finally, we determined the penetration of the metabolite of cyclophosphamide (hydroxycyclophosphamide, or OH-CP) into tumors by liquid chromatography coupled with tandem mass spectrometry (HPLS-MS/MS).

Material and Methods:

Syngeneic TLT (Transplantable mouse Liver Tumor) was injected intramuscularly in the thigh of 5 week-old male NMRI mice. Oxygen pressure was measured by EPR oxymetry using charcoal with a 1.2 GHz spectrometer when the tumor diameter reached 8.0 ± 0.5 mm. Racemic thalidomide was dissolved in DMSO and given via i.p. injection (200 mg/kg). Cyclophosphamide was dissolved in saline and administered via i.p. injection (50 mg/kg, suboptimal dose). Using different combinations and schedules of treatments (Table 1), tumor size was monitored every day for all mice and the time for each tumor to reach 15 mm was calculated (regrowth delays). OH-CP was extracted from tumor and blood samples and finally reconstituted with 100 µl water and 100 µl acetonitrile for HPLC/MS-MS analysis.

Results

pO2 increased significantly after two to three days of daily treatment by thalidomide before eventually declining on day 4 (figure 1). We used this result as the rational basis for defining different schedules of treatments (see Table 1). Fig.2 shows the difference of regrowth delay between control (group 1) and each of the 6 treatment groups. The effect was not significant for groups 2, 3, 4, 5, or 7. The potentiation was maximal for group 6 (when cyclophosphamide was administered after two days of treatment with thalidomide). Thus the amount of cyclophosphamide metabolite (OH-CP) that enters the tumor cells was determined for group 6 and compared to corresponding control group (group 3). The amount of OH-CP was about two-fold higher in tumors that had received two days of thalidomide treatment than the control group (Fig.3A). To discriminate between an effect on the tumor vasculature and a possible effect of increase in production of OH-CP by the metabolism, we also performed a quantification of OH-CP in circulating blood. Figure 3B shows that the amount of this metabolite was not significantly different between the blood of treated and control mice.

Discussion

These results show that thalidomide potentiated the effect of cyclophosphamide. As predicted by EPR oximetry, the efficacy of the combination thalidomide/cyclophosphamide was dependent on the schedule of administration. This potentiation of chemotherapy was caused by an increase of the quantity of drug penetrating into the tumor.

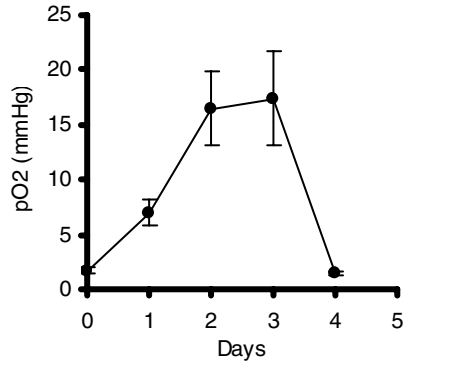

Figure 1 : Evolution of pO_2 in tumors treated with daily injection of thalidomide.

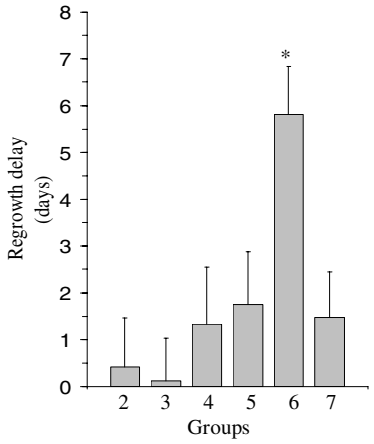

Figure 2 : Difference between regrowth delay of treated mice (groups 2-7) and control mice treated with vehicle only (DMSO, group 1).

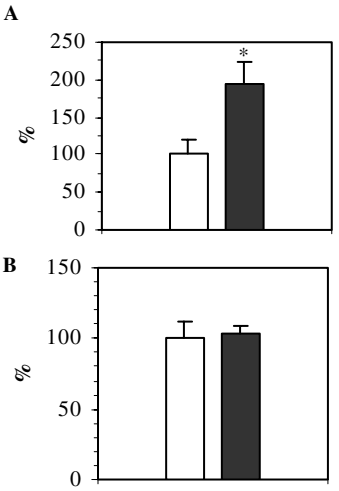

Figure 3. Dosage of OH-CP by HPLC/MS-MS in tumors (3a) and blood (3b) of normalized (dark) and control tumors (open).

	Day 1	Day 2	Day 3	Day 4	Day 5
Group 1	DMSO	DMSO			
Group 2	thalidomide	thalidomide			
Group 3	DMSO	DMSO	cyclophosphamide		
Group 4	Thalidomide + cyclophosphamide	thalidomide			
Group 5	cyclophosphamide	thalidomide	thalidomide		
Group 6	Thalidomide	thalidomide	cyclophosphamide		
Group 7	thalidomide	thalidomide	thalidomide	thalidomide	cyclophosphamide

Table 1. Schedule of treatments

References
(1) R.K. Jain, Nat Med 7:987-989, 2001.
(2) R. Ansiaux *et al*, Clin Cancer Res 11:743-750, 2005.