Valproate–doxorubicin: promising therapy for progressing mesothelioma. A phase II study

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ABSTRACT: No treatment is recommended for patients with malignant mesothelioma (MM) failing after first-line cisplatin-based chemotherapy. *In vitro* data suggested that valproic acid, a histone deacetylase inhibitor (HDACi), had a proapoptotic effect and synergised with doxorubicin to induce apoptosis in MM cells. Our primary end-point was to determine response rate of combined valproic acid and doxorubicin in patients with unresectable MM failing after platinum-based chemotherapy.

Treatment consisted of doxorubicin (60 mg·m⁻²) plus valproic acid. An interim analysis for response rate was planned after the first 16 registered patients. All the cases were centrally reviewed.

From July 2006 to March 2009, 45 eligible patients with pleural MM were registered. The majority of the patients were male (73%), had a performance status (PS) ≥ 80 (76%) and an epithelioid subtype (80%). There were seven partial responses (response rate 16%; 95% CI 3–25%), all in patients with PS 80–100. The best disease control rate was 36% (95% CI 22–51%). Two toxic deaths were observed (febrile neutropenia and cerebral thrombotic event), both in patients with poor PS (60–70).

Valproic acid, an HDACi, plus doxorubicin appeared an effective chemotherapy regimen in good PS (80–100) patients with refractory or recurrent MM, for which no standard therapy was available.

KEYWORDS: Chemotherapy, doxorubicin, histone deacetylase inhibitors, mesothelioma, valproic acid

Malignant mesothelioma (MM) is a rare malignancy with increasing incidence, due to the large use of asbestos and a poor prognosis. First-line chemotherapy combining cisplatin and pemetrexed is currently the standard care [1] for inoperable patients, having demonstrated improved response rate and survival in comparison with cisplatin alone in a phase III trial [2]. A second randomised study confirmed the activity of the antifolate raltitrexed in MM [3]. Currently, there is no standard second-line therapy for MM [1]. In a phase III trial comparing pemetrexed to palliative care alone, JASSEM *et al.* [4] demonstrated improved progression-free survival and time to progression without impact on overall survival. Few other studies assessed the effectiveness of chemotherapy as a second-line therapy. Response rates between 10% and 20% were noted with doxorubicin [5, 6], pemetrexed

[7], pemetrexed plus carboplatine [7], vinorelbine [8] or a combination of cisplatin, mitomycin and irinotecan [9]. As seen from these results, new treatments, including drugs with original activity profile are needed. The 2008 European Respiratory Society (ERS)/European Society of Thoracic Surgeons (ESTS) Task Force on MM management recommended that patients demonstrating prolonged symptomatic and objective response with first-line chemotherapy may be treated again with the same regimen in the event of recurrence. In other cases, inclusion of the patients in clinical trials is encouraged [1].

The purpose of the present phase II study was to evaluate the potentialities of a novel approach based on the concept of gene activation therapy. Our working hypothesis relied on the regulation of cell homeostasis, which is the result of a

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clinical balance between cell proliferation and death. A resistance to apoptosis is among the key mechanisms described in the pathogenesis of MM [10]. Our hypothesis was that the lack of apoptosis is the result of cell quiescence, correlating with an absence of gene expression of some apoptotic molecules. We proposed to relieve this silencing block by using a histone deacetylase inhibitor (HDACi). HDACi have a potential role in the regulation of gene expression, induction of cell death, apoptosis, cell cycle arrest of cancer cells, and inhibition of angiogenesis, motility and invasion of tumour cells by altering the acetylation status of chromatin and other non-histone proteins [11, 12]. HDACi exert profound modifications of the cell biology and some of these molecules have been proposed as potential anticancer agents [13].

HDACi include a variety of compounds belonging to several structural classes: hydroxamic acids (trichostatin A, suberoylanilide hydroxamic acid or vorinostat); cyclic peptides (trapoxin and depsipeptide), benzamides (MS-27-275) and short-chain fatty acids (butyric acid and valproic acid). Presently, the molecular basis of response to HDACi is not fully understood. Valproic acid, an old anti-convulsant drug widely used in patients with epilepsy, exhibited HDACi properties at therapeutic blood concentrations [14] without documented negative interactions with doxorubicin [15]. Valproic acid has demonstrated interesting inhibiting activity on different cell lines and tumour xenografts in mice. Growth inhibition and apoptosis were reported in haematological and solid tumours [16–22]. We showed that valproic acid increases the apoptosis induced by a combination of cisplatin and pemetrexed in MM cell lines and in tumour cells from patient's biopsies [23]. More recently, VANDERMEERS *et al.* also found that valproic acid synergised with doxorubicin to induce apoptosis in MM cells (F. Vandermeers, Gembloux Agro-Bio Tech and Interdisciplinary Cluster for Applied Genoproteomics (GIGA), University of Liège, Liège, Belgium; personal communication).

Valproic acid was tested in a few phase I-II studies in acute leukaemia and myelodysplastic syndrome, in combination with chemotherapeutic agents [12]. Several studies were performed in solid tumours in a limited number of patients, either alone or in combination with chemotherapy [24–27]. None of these studies included MM patients. Valproic acid was administered according to different schedules, most frequently orally at doses used for the treatment of epilepsy, either continuously during the whole duration of chemotherapy or during a specified time of the chemotherapy cycle. In two phase I studies performed in solid tumours, valproic acid was given intravenously at a very high dosage, up to 120 or 160 mg·kg⁻¹ [24, 25].

The aim of the present study was to test the combination of valproic acid and doxorubicin in patients with refractory or recurrent MM after standard first-line chemotherapy.

MATERIAL AND METHODS

This phase II study is an academic trial (EUDRACT 2006-001443-63) with the protocol available on the European Lung Cancer Working Party website (ELCWP); www.elcwp.org and Clinical Trials.gov (www.clinicaltrials.gov).

Selection criteria

To be eligible, patients had to present with histologically confirmed unresectable or inoperable MM failing after at least one prior chemotherapy regimen including platinum derivatives (cisplatin or carboplatin), whatever the response to the first-line treatment, and at least one evaluable or measurable lesion. Other eligibility criteria included Karnofsky performance status (PS) ≥60, and good renal (serum creatinine level ≤1.5 mg·dL⁻¹), hepatic (serum bilirubin level ≤1.5 mg·dL⁻¹ and aspartate aminotransferase/alanine aminotransferase less than twice the normal range) and haematological (neutrophil count ≥2,000 per μL and platelet count ≥100,000 per μL) functions. Coagulation tests (activated partial thromboplastin time, partial thromboplastin time and prothrombin time) and fibrinogen had to be in the normal ranges. Patients had to be accessible for participating in the detailed follow-up of the protocol and to have provided written informed consent.

Ineligibility criteria were the following: recent (<3 months) myocardial infarction, congestive heart failure (ejection fraction of the left ventricle <50%) or cardiac arrhythmia requiring medical treatment; uncontrolled infectious disease or other serious medical or psychiatric illness precluding adherence to the study protocol; prior history of malignancy except non-melanoma skin cancer or *in situ* carcinoma of the cervix and "cured" malignant tumour (>5-yr disease-free interval); active epilepsy needing a specific treatment; concomitant treatment with monoamine-oxidase inhibitor, carbamazepine, mefloquine, phenobarbital, primidone, phenytoin, lamotrigine, zidovudine; history of prior HIV infection; pregnancy or refusal to use active contraception; known allergy to valproic acid and/or doxorubicin; previous treatment with anthracycline derivatives.

Investigations

Initial work-up included clinical evaluation completed by weight, height, surface area and record of Karnofsky PS, complete blood sampling, electrocardiogram, chest radiograph and computed tomography scan, isotopic or echographic left ventricular fraction assessment. Blood counts were performed weekly. Blood chemistries including valproic acid measurement, chest radiograph and clinical examination were repeated before each new course. An evaluation after each three courses of chemotherapy was performed with the same tests as during the initial work-up and repeated every three cycles. After treatment completion, patients were followed every 2 months with clinical evaluation, chest radiograph and biological tests.

Treatment

After central registration at the ELCWP data centre, eligible patients were treated with oral valproic acid in order to obtain serum concentrations in the range of the recommended values for the treatment of epilepsy (50–100 μg·mL⁻¹), at the dose of 20–30 mg·kg⁻¹·day⁻¹ during meals. When stable therapeutic concentrations were obtained (generally after 1–2 weeks), patients were treated with doxorubicin at 60 mg·m⁻² as a short intravenous infusion every 3 weeks and valproic acid continued during the whole treatment.

Courses were repeated every 3–4 weeks, as soon as haematological (neutrophils >1,500 per mm³ and platelets >100,000 per mm³) function had recovered. If myelosuppression

persisted on day 36, the patient went off treatment. In absence of progression, treatment was continued until the best response was achieved, or until there was an unacceptable toxicity or cumulative dose of doxorubicin $>500 \text{ mg}\cdot\text{m}^{-2}$.

During the whole treatment, the following adaptations of doses were planned. If neutrophils nadir was $<500 \text{ per mm}^3$ and/or platelets $<25,000 \text{ per mm}^3$, the dose of doxorubicin was reduced to 75% for the next course. In the case of World Health Organization (WHO) grade 3 or 4 cardiac dysrhythmias, treatment was stopped; in the case of WHO grade 2, treatment continuation was discussed on a case by case basis. Treatment was stopped if a reduction of the left ventricular fraction of $>10\%$ from the baseline was observed or a reduction below the absolute value of 50%. In the case of WHO grade ≥ 3 stomatitis, the dose of doxorubicin was reduced to 75% for the next course. In the case of WHO grade ≥ 2 skin toxicity attributable to valproic acid, it was stopped and doxorubicin continued at the same dosage. For any other grade ≥ 3 WHO toxicity, if doxorubicin was responsible, the whole treatment was stopped, except for alopecia, nausea and vomiting or anaemia; if valproic acid was responsible, it was stopped and doxorubicin continued.

If plasma valproic acid was outside of the therapeutic range, oral doses were adapted in order to obtain serum concentrations between $50\text{--}100 \mu\text{g}\cdot\text{mL}^{-1}$. In cases of overdose, valproic acid was stopped and reintroduced only if all adverse events had resolved.

Criteria of evaluation

Response was evaluated after every three courses according to the WHO criteria. Response status, as well as the initial tumour, node, metastasis stage, was assessed during regular meetings of the group by at least three independent observers. Patients with early progression or death prior to evaluation due to malignant disease or toxicity, and treatment cessation due to toxicity were considered as treatment failures. WHO criteria were used to assess toxicity.

Duration of response was calculated from the first date of response documentation until the date of first observation of progressive disease or death without documented progression in patients with an objective response. Progression-free survival was defined as the period between the day of registration and the date of first progression or death. Survival was dated from the day of registration.

Statistical considerations

Sample size evaluation has been performed considering, as primary end-point, the antitumoural response and making use of the two-stage optimal design of SIMON [28]. In this second-line setting, we considered that a response rate reaching 15% was of clinical value and should be detected, if true, with a statistical power of 90% ($\beta=10\%$). Conversely, we considered that a theoretical response rate $\leq 2\%$ should lead to the rejection of the tested combination of doxorubicin and valproic acid with a probability of 95% ($\alpha=5\%$). On the basis of these assumptions, 16 evaluable patients needed to be registered in the trial in the first step. Early closure should occur if there was no response among these first patients. Otherwise, inclusion of patients had to be pursued until a total sample size of 41

patients. After this second step, the tested treatment had to be judged as warranting further research if at least three patients responded to treatment.

Univariate analysis for looking at factors predictive of response to the tested regimen were performed using Fisher exact tests, Chi-squared tests for categorical covariates or using logistic regression for a continuous covariate. Univariate analysis for survival was performed using Kaplan–Meier estimates and comparison of survival distributions for possible prognostic factors using log-rank tests. Multivariate analysis was planned to use forward methods for selection of covariates and required to be performed the identification of at least two covariates associated with a p-value of <0.20 .

RESULTS

Between July 2006 and March 2009, 46 consecutive patients were registered. One patient was deemed ineligible because of wrong histology. Characteristics of the 45 eligible patients are depicted in table 1. The majority of the patients were male (73%), had a good PS of ≥ 80 on the Karnofsky scale (76%) and presented with epithelial histological subtype (80%). The initial localisation of malignant mesothelioma was pleural in all cases. Previous first-line chemotherapy included the following regimens: cisplatin ($n=37$) or carboplatin ($n=4$) and

TABLE 1 Characteristics of the eligible patients with malignant mesothelioma

Eligible patients n	45
Sex	
Male	33 (73)
Female	12 (27)
Age yrs	69 (51–80)
Performance status	
Median	80
60	3 (7)
70	8 (18)
80	13 (29)
90	17 (38)
100	4 (9)
Weight loss	
$<5\%$	21 (47)
$\geq 5\%$	15 (33)
Unknown	9 (20)
Histology	
Epithelioid	36 (80)
Sarcomatoid	1 (2)
Biphasic (mixed)	7 (16)
Unspecified	1 (2)
Previous first-line chemotherapy	
Cisplatin + pemetrexed	37 (82)
Carboplatin + pemetrexed	4 (9)
Cisplatin + gemcitabine	4 (9)
Response to first-line chemotherapy	
Yes	10 (22)
No	29 (64)
Missing data	6 (13)

Data are presented as n (%) or median (range), unless otherwise stated.

pemetrexed, cisplatin and gemcitabine (n=4). Second- and third-line regimens included cisplatin (n=2) or carboplatin (n=1) and pemetrexed, pemetrexed alone (n=1), docetaxel plus gemcitabine (n=1), or vorinostat (n=4). At the time of analysis (September 2009) 35 patients were dead. Median follow-up for living patients was 12.4 months.

Two patients were unassessable for response because of treatment refusal before the first course of chemotherapy and loss to follow-up before radiological evaluation. Overall, 150 chemotherapy cycles were administered during the study, ranging from one to six per patient. The number of patients receiving one, two, three and six cycles was 10, 7, 14 and 14, respectively. Three partial responses were observed during the first step of the study, allowing further inclusions. Response rate at three cycles was 13% (intent-to-treat analysis). Best response rate was seven (16%) out of 45 (95% CI 3–25%). All the seven responding patients presented with good PS of 80 (n=1), 90 (n=5) or 100 (n=1). Five had epitheloid histological subtype of MM and three were male. Best disease control rate (partial response plus stable disease) was 36% (95% CI 22–51%) (table 2). Actuarial median response duration was 11.8 months with three patients still alive without documentation of progression at 8, 11 and 26 months after first documentation of response. Univariate analyses did not find any statistically significant factor predicting response to chemotherapy, although all objective responses were documented in good PS patients (table 3).

Median progression-free survival was 2.5 months (95% CI 2.0–3.0 months) (fig. 1). At 1 yr, 25% of patients were alive and 14% patients were alive without progression. Median survival was 6.7 months (95% CI 4.9–8.5 months) (fig. 1). In univariate analysis, only PS was a statistically significant prognostic factor for survival (table 4). Survival was also assessed according to response status using a landmark at 3.88 months (maximal time elapsed for having assessed as responder a patient after three courses of chemotherapy). Median survival time for responding patients was 16.7 months after the landmark. Among the 24 nonresponders alive at the landmark, median survival after the landmark was 4.5 months. The difference was statistically significant (p=0.03).

As expected, the main toxicities were leukopenia and neutropenia. Two toxic deaths, both in poor PS patients, were

TABLE 2 Response rates at three and six cycles in patients with malignant pleural mesothelioma treated with doxorubicin and valproic acid		
	At three cycles	At six cycles
Partial response	6 (13)	7 (16)
Stable disease	10 (22)	4 (9)
Progression	20 (44)	25 (56)
Early death by cancer	1 (2)	1 (2)
Toxic death	2 (4)	2 (4)
Stop for high toxicity	4 (9)	4 (9)
Unassessable	2 (4)	2 (4)

Data are presented as n (%).

TABLE 3	Univariate analyses of factors predicting response in patients treated with doxorubicin and valproic acid		
	Patients n	Response n	p-value
Performance status			
60–70	11	0	0.16
80–100	34	7	
Sex			
Male	33	3	0.07
Female	12	4	
Age yrs (continuous variable)			0.19
Histology			
Epitheloid	36	5	0.69
Other	9	2	
Response to first-line chemotherapy			
Yes	10	2	0.29
No	29	5	
Stage			
IV	13	3	0.39
I-III	32	4	

documented: one febrile neutropenia and one cerebral thrombotic event. One patient developed grade IV cardiac failure. Highest toxicity per patient during the whole course of treatment is reported in table 5.

DISCUSSION

Consistent with previous experimental data ([23] and VANDERMEERS *et al.* (F. Vandermeers, GIGA, University of Liège; personal communication), the combination of valproic acid, a HDACi, and doxorubicin appears as an effective chemotherapy regimen in good PS (80–100) patients with refractory or recurrent MM after a first-line cisplatin-based

FIGURE 1. Survival and progression-free survival curves of patients treated with doxorubicin and valproic acid. —: overall survival (OS); - - -: progression-free survival (PFS).

TABLE 4 Univariate analyses of prognostic factors for survival in patients treated with doxorubicin and valproic acid

	Patients n	Survival months	p-value
Performance status			
60–70	11	1.5	<0.001
80–100	34	8.8	
Sex			
Male	33	6.9	0.63
Female	12	3.5	
Age yrs (continuous variable)			0.27
Histology			
Epitheloid	36	6.9	0.44
Other	9	5.5	
Response to first-line chemotherapy			
Yes	10	5.9	0.39
No	29	6.9	
Stage			
IV	13	6.2	0.97
I–III	32	6.7	

chemotherapy, in accordance with the study statistical hypothesis. Despite a recruitment including some MM patients with poor PS, ranging from 60–100, we observed an interesting response rate of 16% and a best disease control rate of 36% when treating patients with valproic acid plus doxorubicin, resulting in a longer survival of responding patients compared to nonresponding subjects from a landmark at 3.88 months.

In fact, no drug has been validated for second-line chemotherapy for MM patients, leading the ERS/ESTS experts to recommend that these patients should rather be proposed to enter into clinical trials [1]. Among the different investigated agents, HDACi demonstrated *in vitro* and in clinical trials some potential anticancer activity in haematological and solid tumours [12]. In mesothelioma, the value of HDACi vorinostat and panobinostat were assessed in animal models [29, 30]. Vorinostat monotherapy has been tested so far in patients with MM failing after standard first-line chemotherapy. In a phase I trial [30], two patients out of 13 demonstrated a partial response. The results of a phase II trial (oral vorinostat *versus* placebo) are not published and a placebo-controlled randomised phase III study of second-line oral vorinostat is ongoing. Another HDACi monotherapy with belinostat (PXD101) showed no antitumour activity in MM, with two patients among 13 presenting with a stable disease [31]. Thus, our study is the first to report responses obtained with that therapeutic approach in MM.

VANDERMEERS *et al.* (F. Vandermeers, GIGA, University of Liège; personal communication) observed *in vitro* in MM cells that valproic acid can synergise with doxorubicin to induce apoptosis. Other authors showed similar results in anaplastic thyroid cancer cells [22]. It was suggested that the antitumour activity of doxorubicin, relying on its binding to DNA and the inhibition of topoisomerase II, is enhanced through histone

TABLE 5 Highest toxicity during the whole treatment among 45 patients receiving at least one cycle of chemotherapy

Toxicity	0	I	II	III	IV
Leukopenia	8 (18)	5 (11)	15 (33)	11 (24)	3 (7)
Neutropenia	4 (9)	4 (9)	8 (18)	11 (24)	15 (33)
Thrombopenia	30 (67)	3 (7)	7 (16)	2 (4)	
Nausea	19 (42)	18 (40)	2 (4)	1 (2)	
Diarrhoea	30 (67)	8 (18)	2 (4)		
Stomatitis	34 (76)	2 (4)	4 (9)		
Skin	36 (80)	2 (4)	1 (2)	1 (2)	
Infection	32 (71)	4 (9)	4 (9)		
Bleeding	39 (87)	1 (2)			
Neurological (encephalopathy)	37 (82)	2 (4)	1 (2)		
Neurological (peripheral)	34 (76)	5 (11)	1 (2)		
Constipation	36 (80)	2 (4)	1 (2)		
Respiratory	28 (62)		11 (24)	1 (2)	
Cardiac	36 (80)	1 (2)	2 (4)	1 (2)	1 (2)
Alopecia	15 (33)	6 (13)	6 (13)	14 (31)	
Loss of hearing	36 (80)	4 (9)	1 (2)		
Renal	41 (91)	2 (4)			
Febrile neutropenia	43 (96)			1 (2)	1 (2)
Vascular	42 (93)		1 non fatal (2), 1 fatal (1)		
Other	35 (78)	4 (9)	4 (9)	2 (4)	

Data are presented as n (%).

acetylation and increased apoptosis as shown by the increased caspase 3 activation and the enhancement of doxorubicin-induced G2 cell cycle arrest [32]. We observed an interesting response rate with the combination of valproic acid and doxorubicin that has not been previously reported with doxorubicin alone in two previous small studies; one patient among 11 and none among six showed partial response with second-line doxorubicin alone [5, 6]. Importantly, all the seven responding patients in our study presented with good PS (PS 80–100) (Eastern Cooperative Oncology Group PS 0–1). Consistently, PS was the only statistically significant prognostic factor for survival. Patients with poor PS at inclusion (PS 60–70) did not respond to treatment and showed severe toxicities (febrile neutropenia and thrombotic event). If we failed to detect PS as a factor with a statistically significant association with response to the tested regimen, it had a prognostic value for overall survival.

Neurocognitive impairment is a common side-effect reported in phase I–II studies with valproic acid. It consisted of confusion, somnolence, dizziness, hallucination or encephalopathy. It could be observed at therapeutic serum concentrations but was increased with higher doses of valproic acid [24, 25]. In contrast to previous studies, we did not observe grade 3–4 neurological toxicity. Other toxicities are difficult to compare with the previous studies, chemotherapy and population being noticeably different. A recent phase I/II trial

assessed valproic acid given at high intravenous dosages during 3 days in combination with epirubicin or a combination of 5-fluorouracil, epirubicin and cyclophosphamide in 44 patients with solid tumours [25]. Several partial responses were obtained. Outside of valproic acid associated neurovestibular disturbance, the most frequent side-effect was epirubicin-induced myelosuppression, as we found in the present study. Thus, in our study as in the literature, the HDACi valproic acid had limited side-effects and exhibited antitumour activity when combined with chemotherapy, which was responsible for more severe toxicities.

In conclusion, this study suggests that doxorubicin and valproic acid appear to be an effective chemotherapy regimen in good PS (80–100) patients with refractory or recurrent MM after first-line cisplatin-based chemotherapy. However, these results need confirmation in a randomised trial, which is the only way to validate the effectiveness of this chemotherapy regimen. It is noteworthy that this quite efficient regimen combines old but very cheap drugs, as MM incidence is expected to increase in the next decades in the developing countries still using asbestos without strict regulation.

Future directions include the search for clinical and biological markers to predict the response to HDACi and the prognosis of patients, the development of new HDACi and/or isoforms of previous HDACi with less toxicity than valproic acid or vorinostat but more antitumour effect, and the use of HDACi (valproic acid) in combination with first-line chemotherapy (cisplatin plus pemetrexed) in MM patients.

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CLINICAL TRIAL

This study is registered at ClinicalTrials.gov identifier number NCT00634205.

STATEMENT OF INTEREST

None declared.

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