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In Vitro Cytotoxicity of Peptichemio Compared to Melphalan and *Meta*-DL-Sarcolysin

LUCIANO MORASCA and EUGENIO ERBA

Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea, 62–20157 Milano, Italy

Abstract—*The cytotoxic activity of peptichemio was studied on primary cultures of Lewis lung carcinoma. Since peptichemio is a preparation of several peptides containing* meta-L-*sarcolysin, two control experiments were run with melphalan as representative of the clinically active phenylalanine mustard derivative, and* meta-DL-*sarcolysin, racemic form of the phenylalanine mustard derivative, included in the composition of peptichemio. Peptichemio was more active than the other two compounds and its cytotoxic activity cannot be explained merely in terms of the phenylalanine mustard the preparation contains.*

INTRODUCTION

AMONG the different steric structures of phenylalanine mustard, only melphalan, the L-isomer with dichloroethylamino group in *para* position, is an effective drug on human cancer. The *meta* derivative, *m*-DL-sarcolysin (NSC 27391), was more active but also more toxic both on experimental tumours and in patients [1–3]. When *m*-L-sarcolysin was included in peptidic structures, as peptichemio, several studies seemed to indicate a good activity in cancer patients [4]. This activity was recently confirmed by the clinical screening cooperative group of the European Organization on Research on Treatment of Cancer (EORTC) [5].

At this stage we became interested in comparing the *in vitro* cytotoxic activity of peptichemio with its closet congeners: melphalan as the most effective phenylalanine mustard derivative, and *m*-DL-sarcolysin as the closest structure to the *m*-L-sarcolysin in peptichemio peptides, since the latter was not available in a pure preparation.

MATERIALS AND METHODS

The 3LL carcinoma was excised from C57Bl mice 18 days after transplantation. After mechanical disgregation the cell suspension was filtered, counted by dye exclusion methods, and $8 \cdot 10^5$ cells were seeded in 1.8 ml of growth medium in Rose's chambers [6]. Growth medium was MEM containing 4× extra vitamins and 4× amino acid solution and 20% fetal calf serum.

The first morphological baseline evaluation was made 48 hr after seeding the cultures by choosing representative microscopic fields (score areas) in each culture that were marked on the outside of the chamber by a marker objective. These areas were then checked every 24 hr during the recovery periods, and alterations were scored as already described [6].

Scores of 1 and 2 were assigned for cytotoxic alterations, 4 and 5 for lethal effect, and 3 for intermediate conditions.

0 = normal epithelium; 1 = alterations of cellular shape; 2 = reduction of monolayer; 3 = fragmentation of monolayer with necrotic elements; 4 = few cells surviving (< 10 cells for score area); 5 = no surviving cells.

Score values in replicate experiments did not vary by more than ±0.5 units. Scoring was done by the same operator for the whole block of experiments. Each point is the median score of 10 experiments. As these data are non-parametric, the Lord test [7] was employed for statistical evaluation of results. Confidence limits of median values are reported for $P = 0.05$.

For treatments, the compounds were dissolved in growth medium. Peptichemio was available as a solution containing 10 mg of the peptide preparation equivalent to 4 mg of *m*-L-sarcolysin in 0.5 ml of a solvent containing

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propylene glycol p.p.4, dimethylsulfoxide p.p. 3.5, ethanol p.p. 2.5. Control treatments were made with the highest concentration of solvent present in the drug solutions. The solvent had no measurable toxicity at the concentration of 100 μl/ml. The concentrations of peptichemio reported in the tables are *m*-L-sarcolysin equivalents.

RESULTS

Cytotoxicity built up in peptichemio treated cells during the recovery time (Fig. 1). Treatment with the lowest concentration of 0.4 μg/ml equivalent of sarcolysin caused complete destruction of the cell population after 120 hr. When melphalan was tested (Fig. 2), as much of 40 μg/ml were needed to cause grade 3 damage and 100 μg/ml to reach grade 5 by the end of the recovery time. *m*-DL-sarcolysin (Fig. 3) gave a still lower activity score, reaching grade 5 cytotoxicity only at 400 μg/ml. Experiments with 24 hr exposure did not add any further information to the patterns of activity observed.

DISCUSSION

When the level of peptichemio cytotoxicity on this tumor is compared with previous data obtained on cell lines, this tumor, *in vitro*, appears to be more sensitive than EUE cell [8] and L 929 cells [11]. On the latter the drug has been shown to inhibit DNA synthesis [9], potentitated the hyperthermic effect on single strand breaks of the DNA molecule,

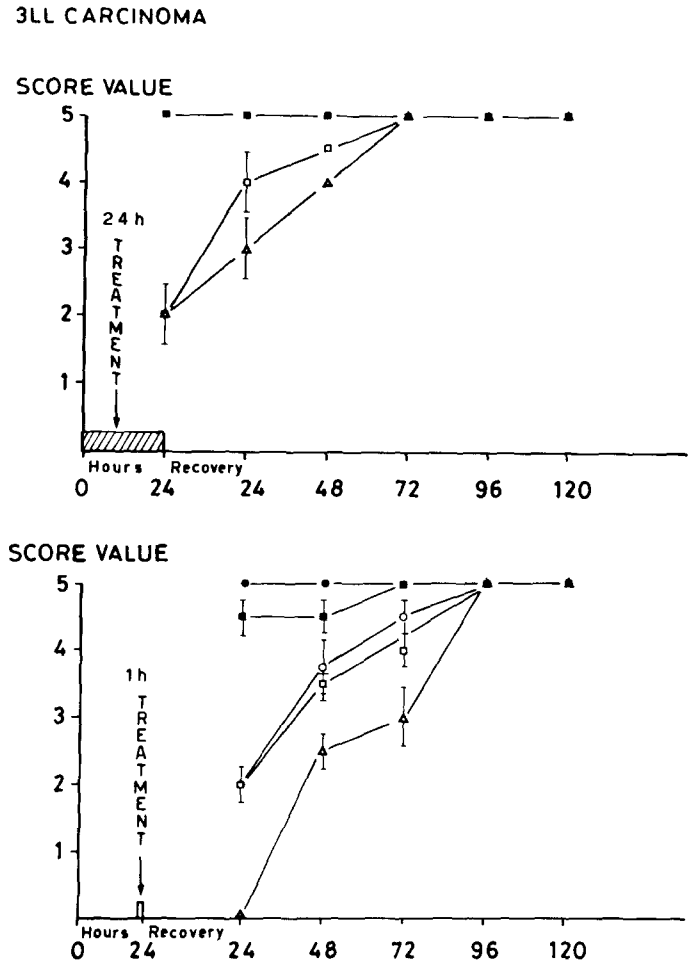

Fig. 1. Equivalents of m-L-*sarcolysin contained in peptichemio,* (●—●) 400, (▲) 200, (■) 100, (○) 40, (□) 4, (△) 0.4 μg/ml), *producing the scored effects described by median and* 0.05 *confidence limits. Two experiments are reported, illustrating* 24-hr *and* 1-hr *treatment of the cells. Scored toxicities are followed for* 120 hr.

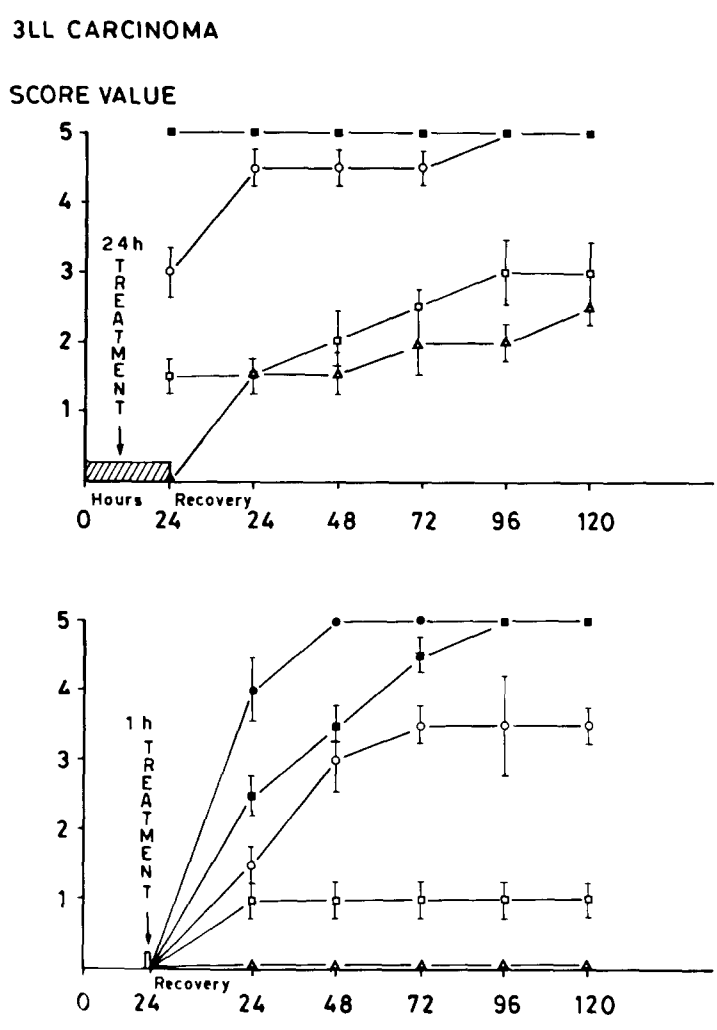

Fig. 2. Same as Fig. 1; the concentrations are of p-L-*sarcolysin* (*melphalan*).

and on the surviving fraction [10], decreasing, however, the cell number by about 1 log only after 6 days treatment at 30 μg/ml [11].

Peptichemio, melphalan and *m*-DL-sarcolysin maintained this order of cytotoxicity with sarcolysin equivalent respectively of 0.4 μg/ml, 100 μg/ml and 400 μg/ml required to reach grade 5 cytotoxicity. Such a large difference between the cytotoxic activities of the three compounds suggests that the peptidic organization given to phenylalanine mustard may have some effect. Any attempt to explain the activity of peptichemio in terms of the *m*-L-sarcolysin in its structure is in fact contradicted by this high activity.

From comparison of the racemic form *m*-DL-sarcolysin with the L-form in the peptichemio formulation, the activity of *m*-L-sarcolysin might be expected to be around 50% of that of *m*-L-sarcolysin [12]. This however is not enough to close the gap between the two levels of cytotoxicity on the cell types studied, to date, but it does bring into good agreement the cytotoxic activity of *m*-DL-sarcolysin and melphalan. The different activity of peptichemio is borne out by data on human lymphoma cells, indicating different patterns of interference with the mitotic cycle for melphalan and peptichemio [13].

In conclusion it seems that peptichemio has greater cytotoxic activity *in vitro* than its congeners melphalan and *m*-DL-sarcolysin, and that the 3LL carcinoma is particularly susceptible to this compound.

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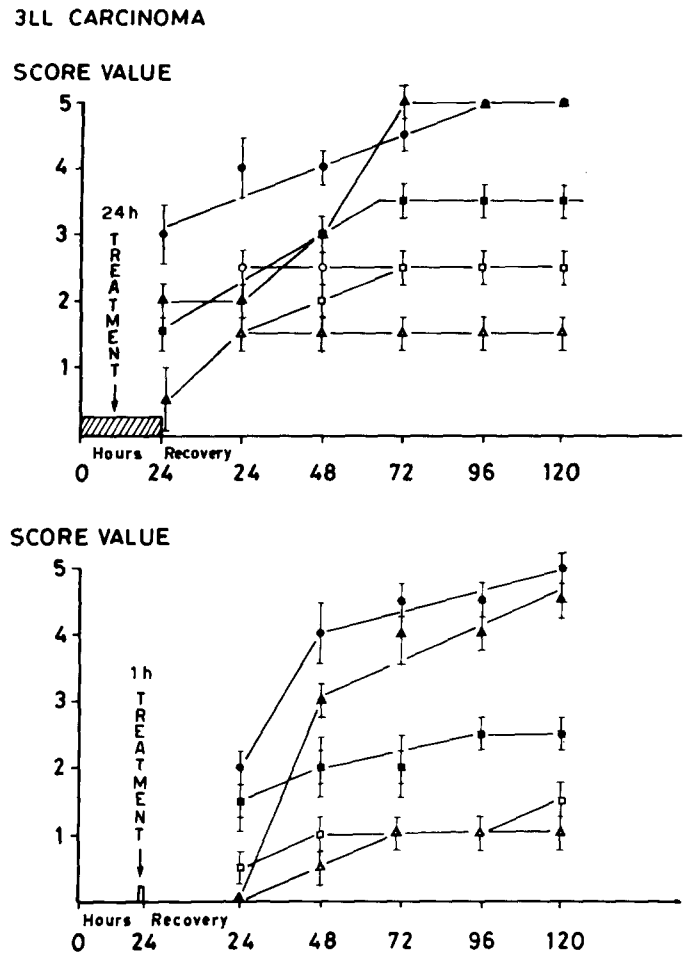

Fig. 3. *Same as Fig.* 1; *the concentrations are of* m-DL-*sarcolysin.*

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