Europ. J. Cancer Vol. 15, pp. 603–607.

0014–2964/79/0401–0603 $02.00/0

# Selective Antimetastatic Effects of *N*-Diazoacetylglycine Derivatives in Mice*

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**Abstract**—*Three* N-*diazoacetylglycine derivatives and two* N-*diazoacetylglycylglycine derivatives were examined for their differential effects on subcutaneous tumor growth and lung metastasis formation in mice bearing Lewis lung carcinoma. None of the tested compounds caused marked inhibition of primary tumor growth, even at maximum tolerated dosages. However, the two* N-*diazoacetylglycine and* N-*diazoacetylglycylglycine amide derivatives respectively caused a dramatic and a moderate inhibition of lung metastasis formation. The lack of correlation between inhibition of subcutaneous tumor growth and formation of lung secondaries indicates that the two amide derivatives possess selective antimetastatic properties.*

## INTRODUCTION

*N*-DIAZOACETYLGLYCINE derivatives are a class of substances possessing antineoplastic effects. Different degrees of activity have been observed, depending on the structure of the compound examined and the experimental model used [1]. In addition to the reported effects, *N*-diazoacetylglycine hydrazide (DGI) also shows significant activity against L1210 leukemia (unpublished data). Recently three of these compounds DGA, DGE and DGI (see Table 1) have been preliminarily examined for their effects in mice bearing Lewis lung carcinoma. In that investigation DGA proved highly active as a selective antimetastatic agent [2].

The purpose of this investigation is therefore that of examining in more detail the effects of these diazoacetylglycine derivatives and also those of two *N*-diazoacetylglycylglycine derivatives in mice bearing Lewis lung carcinoma. The chemical structure of the compounds examined and the abbreviations used are reported in Table 1. The differential effects of the tested compounds on primary tumor growth and formation of lung metastases using different dosages is hereafter reported.

## MATERIALS AND METHODS

### *Synthesis*

The synthesis of the compounds used in this investigation was performed following previously reported procedures (Table 1).

### *Animal treatment*

The compounds were administered i.p. as freshly prepared aqueous solutions in 0.1 N $NaHCO_3$ in volumes of 0.1 ml/10 g of body weight: the control animals received only the solvent. The treatment was performed daily for 14 days following tumor implantation.

### *Tumor transplantation and evaluation*

The Lewis lung carcinoma was transplanted in BDF1 mice implanting aseptically tumor fragments s.c. in the axillary region [3]. Primary tumor weight was determined by caliper measurements, assuming tumor density to be equal to 1, as the volume of the rotation elipsoid having the long and the short axes equal to $a$ and $b$ respectively:

$$\text{Tumor weight} = \pi/6 \times a^2 \times b. \qquad (1)$$

The number of lung metastases was determined at sacrifice using a dissection magnifying lens. The mass of metastasis was estimated

Accepted 28 September 1978.
*Supported by Consiglio Nazionale delle Ricerche grants CT 77.01110.03 and CT 77.01370.04.
‡Recipient of a Research Grant by the Italo-American Medical Education Foundation: permanent address the same as ¶.

*Table 1. Chemical structure and abbreviation used for the tested compounds*

| R | $N_2$—CH—CO—NH—$CH_2$—CO—R | Abbreviation | Reference |
|---|---|---|---|
| —$NH_2$ | *N*-diazoacetylglycinamide | DGA | (18) |
| —$NHNH_2$ | *N*-diazoacetylglycine hydrazide | DGI | (19) |
| —O—$CH_2$—$CH_3$ | *N*-diazoacetylglycine ethyl ester | DGE | (20) |
| —NH—$CH_2$—$CONH_2$ | *N*-diazoacetylglycylglycinamide | DGGA | (18) |
| —NH—$CH_2$—CO—O—$CH_2$—$CH_3$ | *N*-diazoacetylglycylglycine ethyl ester | DGGE | (21) |

as the sum of their individual weight, determined using equation (1).

Forty controls were used, and each treated group consisted of 10 mice. Primary tumor weight and the examination of lungs for metastases were made on days 15 and 21 from tumor implantation respectively. Primary tumor weight, the number and the mass of metastases are expressed as their average value per mouse.

## RESULTS AND DISCUSSION

The effects of the tested compounds on primary tumor growth are illustrated in Fig. 1. The highest dosages used for the diazoacetylglycine derivatives are equitoxic and equal to 1/3 of the $LD_{50}$, obtained in normal mice receiving daily administrations for 4 days using the method of Litchfield and Wilcoxon [4]. The limited amounts of the *N*-diazoacetylglycylglycine derivatives available did not allow a toxicity evaluation and examination of the effects of dosages greater than 600 mg/kg. No toxic death was observed in the treated tumor bearing animals. Primary tumor growth was not markedly reduced by any of the substances examined. DGE and DGGA caused no significant effect at any of the doses examined, and DGGE caused only a marginal inhibition. DGA and DGI reduced primary tumor weight at the end of treatment, to about 75% of the controls at the highest dose used.

The inhibitory effects on pulmonary metastasis formation are, on the contrary, markedly pronounced for DGA (Table 2). At the maximum tolerated dose, the number and mass of lung metastases were considerably reduced

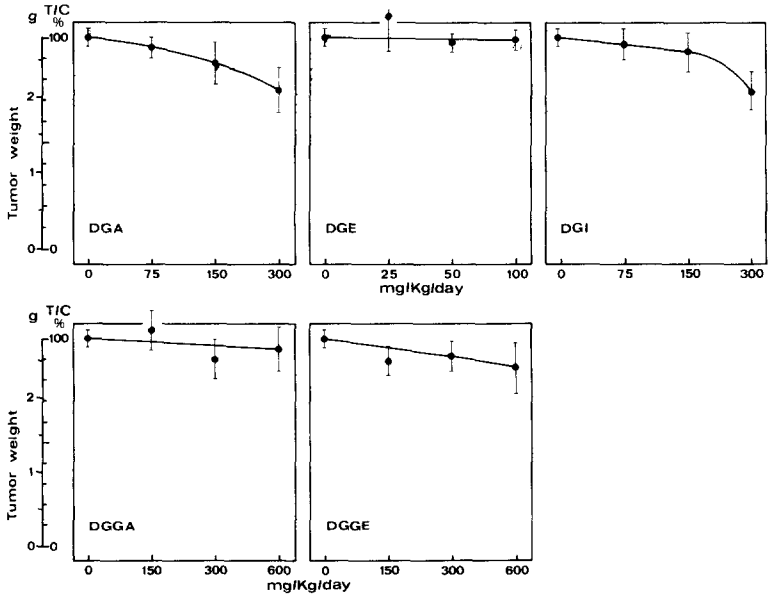


*Fig. 1. Effects of the tested compounds on primary tumor growth. The treatment was performed daily for* 14 days, *starting on* day 1 *after tumor transplantation: the tumor weight was determined on* day 15. *Each value is the mean* ± S.E. *obtained in groups of* 10 *animals* (40 *controls*).

*Table 2. Effects of the tested compounds on lung metastases*

| Compound | Daily dose mg/kg/day | Average number of metastases per mouse ± S.E. Small† | Large | Total | Estimated mass of metastases | Animals free of large metastases |
|---|---|---|---|---|---|---|
| — | — | 100.0 ± 6.1 | 100.0 ± 8.8 | 100.0 ± 5.7 | 100 | 0/40 |
| | | (58.6 ± 3.6)* | (6.7 ± 0.59)* | (65.3 ± 3.7)* | (121.1 mg)* | |
| DGA | 75 | 43.2 ± 3.4‡ | 74.6 ± 17.9 | 46.4 ± 4.7‡ | 68.5 | 1/10 |
| | 150 | 52.9 ± 9.4‡ | 29.9 ± 14.9‡ | 50.5 ± 8.9‡ | 34.3 | 2/10 |
| | 300 | 39.2 ± 10.1‡ | 0 | 35.2 ± 9.0‡ | 5.9 | 10/10 |
| DGE | 25 | 95.2 ± 13.7 | 104.5 ± 12.5 | 96.2 ± 7.7 | 99.7 | 0/10 |
| | 50 | 72.0 ± 15.9 | 79.1 ± 7.2 | 72.7 ± 13.8 | 76.5 | 0/10 |
| | 100 | 71.7 ± 11.8 | 79.1 ± 14.2 | 72.4 ± 10.9‡ | 76.5 | 0/10 |
| DGI | 75 | 99.8 ± 6.7 | 123.9 ± 10.6 | 102.3 ± 6.5 | 120.3 | 0/10 |
| | 150 | 80.4 ± 10.1 | 97.0 ± 11.9 | 82.1 ± 9.9 | 100.2 | 0/10 |
| | 300 | 73.4 ± 15.9 | 111.9 ± 4.3 | 77.3 ± 14.6 | 108.8 | 0/10 |
| DGGA | 150 | 89.2 ± 10.2 | 29.9 ± 8.7‡ | 83.2 ± 9.5 | 49.9 | 0/10 |
| | 300 | 75.6 ± 4.8 | 44.8 ± 19.3‡ | 72.4 ± 4.3‡ | 38.6 | 0/10 |
| | 600 | 50.3 ± 6.0 | 17.9 ± 3.9‡ | 47.0 ± 5.4‡ | 22.5 | 1/10 |
| DGGE | 150 | 114.3 ± 18.1 | 101.5 ± 11.2 | 113.0 ± 17.2 | 99.8 | 0/10 |
| | 300 | 93.3 ± 8.0 | 153.7 ± 25.4 | 99.5 ± 6.6 | 82.5 | 0/10 |
| | 600 | 50.0 ± 11.8‡ | 64.2 ± 30.3 | 51.5 ± 7.5‡ | 62.3 | 0/10 |

*Actual finding.
†Diameter smaller than 2 mm.
The animals, whose primary tumor weight is reported in Fig. 1, were sacrificed on day 21 after tumor transplantation, and the macroscopically detectable lung colonies counted. Each value is expressed as the percentage ratio of the average for the treated group to that of the controls. The statistical analysis performed is the Student–Neumann–Keule test [17].
‡Significantly different from the controls ($P<0.05$).

and no large metastases were seen in any of the treated animals. The effects were dose dependent, being still evident at the two lower dosages. The effects of the other two glycine derivatives, DGI and DGE, and those of DGGE were markedly less pronounced and of little significance, even at the highest dosages used. DGGA, though less active than DGA, showed some activity consisting of a significant reduction of the mass of lung colonies at all the dose levels used. The reduction in the total number of lung colonies was more evident at the highest dosage, and the number of large metastases was particularly lowered, when compared with the total.

From the data reported so far, it appears that all of the compounds examined cause only marginal reduction in primary tumor growth. The two amide derivatives, DGA and DGGA, cause a dramatic or a moderate inhibition respectively of the formation of lung metastases. It should be noted that higher dosages of the glycylglycine derivatives might be tolerated, and that the effects obtainable with maximum tolerated dosages, particularly for DGGA, could be more pronounced. These results indicate for the compounds examined the absence of correlation between inhibition of the growth of the primary tumor and depression of metastasis formation. This finding is in contrast with the effects observed with purely cytotoxic agents, such as cyclophosphamide, which cause a parallel inhibition of subcutaneous and pulmonary tumor growth [5]. This indicates for DGA and DGGA selective antimetastatic properties, similar to those found for ICRF159 [6, 7]. As far as the mechanism of the antimetastatic effect is concerned, it is worth noting that α-diazocarbonyl derivatives of amino acids are a class of irreversible inhibitors directed at the active site of thiol [8, 9] and acid proteases [10, 11]. DGA and DGI have also been reported to inhibit neutral proteases [12]. At the same time, some protease inhibitors have been found capable of reducing the formation of metastases in mice [13, 14]. This finding is in accord with the suggested role of proteolytic enzymes in the early stages of the process of metastasis formation, namely microvascular invasion [15] and tumor cell detachment from the primary tumor [16]. These considerations seem to suggest that the mechanism of the selective antimetastatic effects of DGA might consist of the inhibition of tumor proteases. They also indicate a therapeutic potential for DGA as an antimetastatic adjuvant to the surgical treatment of solid tumors. Work is in progress on detecting any inhibition of tumor proteases and on the possible role of DGA as an antimetastatic adjuvant in animals.

**Acknowledgements**—One of the authors (TG) wishes to thank Dr. A. Goldin, Dr. D. A. Cooney and Dr. P. Schein for support, encouragement and discussion throughout this work.

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