Effects of Some N-Diazoacetyl-Glycine Derivatives on the Growth of *E. coli*

Diazoacetyl derivatives of amino acids are a group of substances possessing interesting pharmacological and biological properties. In particular azaserine and 6-diazo-5-oxo-L-norleucine (DON) have been extensively studied because of their antibacterial and antitumour activities[1]. This fact prompted BALDINI et al.[2,3] to synthesize a series of diazoacetyl derivatives of glycine, glycyl-glycine, alanine and phenylalanine[2-5]. Some of the derivatives of glycine, particularly DGA and DGI (see chemical structure) were shown to possess a marked immunodepressive activity[6,7] and to have inhibitory properties on the growth of some transplantable tumours in mice and rats[2,3].

The aim of this communication is to report on the results of a series of experiments performed to study the effects of the diazoacetyl derivatives of glycine, including some new molecules (see structure), on the growth of *E. coli*.

Materials and methods. *E. coli* strains K12, W3110 and the DNA repairing system lacking pol A$^-$[8] (obtained through the courtesy of Dr. CAIRNS) were grown in minimal medium (MM)[9], supplemented for the W3110 and pol A$^-$ strains with 0.38 m*M* thymine. A loopful of 18 h culture was used to inoculate 4 ml of medium containing the stated amount of the drug to be tested. The incubations were carried out overnight at 37 °C: the bacterial growth was evaluated by measurement of the optical density of the cultures by a Leitz photometer model M, using an A filter.

The partition coefficients $p = C_{oct}/C_{H_2O}$ of the drugs between *n*-octanol and 10 m*M* phosphate buffer pH 7.4 were determined essentially as described by FUJITA et al.[10]. Typically, a sample giving an extinction at 250 nm of about 0.5 in the buffer was partitioned with an equal volume of *n*-octanol previously saturated with the buffer. The extinction of the 2 phases was determined by UV-spectrophotometry at 250 nm. Adherence to Beer's law was checked for each substance in both solvents.

Results and discussion. Data reported in Table I show that the highest inhibitory activity on the growth of *E. coli* K12 strain was exhibited by DGE, with a DI_{50} = 50 μg/ml. Among the other compounds tested, only DGEA, DGPA and DGIPA had a DI_{50} lower than 1000 μg/ml. The liposolubility of these substances, expressed as the log *p*, showed a good correlation with their activity. In particular, the DI_{50} for DGE remained unchanged, also when the incubations were carried out at 22 °C or under anaerobic conditions.

Data reported in Table II show that the effects of DGE are not reduced when the bacteria are grown in the presence of this drug and adenine, at a concentration sufficient to relieve significantly the effects of azaserine. This finding recalls the result obtained with another diazoacetyl-glycine derivative, DGA, in tumour cells, where the inhibition of DNA synthesis caused by the drug could not be reduced by adenine[11].

The same degree of inhibition of bacterial growth has been obtained for DGE and the other drugs when tested on *E. coli* W3110 and pol A$^-$ strains. Since the latter strain is lacking the DNA repairing system[8], its use provided a useful tool to investigate the occurrence of interactions between these diazo compounds and DNA.

Chemical structure of N-diazoacetyl derivatives of glycine

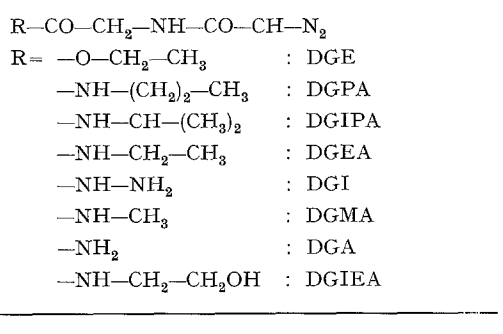

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Table I. Effects of N-diazoacetyl-glycine derivatives on the growth of *E. coli* (strain K12)

	Drug concentration (μg/ml)					
	10	30	100	300	1000	log *p*
DGE	30.4	42.5	59.9	84.3	89.9	−0.0820
DGPA	2.7	0	11.0	13.7	74.7	−0.238
DGIPA	0	− 8.8	−18.2	21.0	77.7	−0.411
DGEA	6.7	5.5	13.2	13.7	66.1	−0.620
DGI	5.5	—	2.9	—	31.7	−0.762
DGMA	4.1	8.2	− 3.1	− 3.1	34.7	−0.873
DGA	8.2	5.5	1.2	2.7	39.0	−1.260
DGIEA	6.2	16.1	18.9	20.0	26.9	−1.349

The values are % inhibition of the growth of bacteria in MM in the presence of the drug, in respect to the controls. Each value is the mean of 3 determinations.

Table II. Effects of adenine on the inhibition of the growth of *E. coli* strain K12 caused by azaserine and DGE

	Azaserine concentration (μg/ml)					
Adenine	0	0.0001	0.001	0.01	0.1	1
–	14.6	8.9	28.1	82.3	100	–
+	0	0	6.2	14.6	91.5	100

	DGE concentration (μg/ml)				
Adenine	0	1	10	100	1000
–	14.6	8.9	14.6	35.4	91.5
+	0	3.1	14.6	49.6	91.5

The values are the % inhibition of the growth of bacteria in MM, supplemented when indicated with 0.38 m*M* adenine, in the presence of the drugs, in respect to the controls. Each value is the mean of 3 determinations.

Interactions of this type have been reported to occur when purified calf thymus DNA is incubated in vitro with DGA[11]. The lack of a differential sensitivity between W3110 and pol A⁻ strains suggested that these drugs did not react extensively with the bacterial DNA.

An interesting comparison can be made between the effects of these drugs on bacterial and mammalian cells. DGA and DGI, which do not affect the growth of *E. coli*, are very active immunodepressant and antitumour agents[2,6]. In contrast, DGE, which in this group of substances exhibits the greatest capacity of inhibiting bacterial growth, has no effect on tumour or immunocompetent cells. At first sight it might be suggested that the selective activity of these diazoacetyl-derivatives is related to their different liposolubility, and accordingly, to the diversity of surface structures of bacterial and mammalian cells. However, the analysis of the chemical structures and biological activities for some alkylating agents did not show any strict linear correlation between the partition coefficients and the antitumour properties for the drugs considered[12]. Further, the screening of various sulphonamide derivatives has shown that no relationship exists between partition coefficient and minimal concentration required for inhibition of bacterial growth in vitro[13].

In any case, the preliminary analysis of the properties of the drugs discussed in this paper seems to encourage the synthesis and the evaluation of the biological properties of new molecules characterized by the presence of the α-diazocarbonyl moiety. In this context, particularly attractive seem to be derivatives of metabolites involved in biosynthetic pathways peculiar to bacteria, since possibly drugs having the antibiotic effectiveness of azaserine, with a much lesser toxicity to the host, might result[14].

Riassunto. Gli effetti di una serie di N-diazoacetil derivati della glicina, alcuni dei quali possiedono notevole attività immunosoppressiva ed antineoplastica, sono stati studiati sulla crescita di *E. coli*. La diazoacetilglicina etilestere, praticamente priva di effetti farmacologici, si è dimostrata la più efficace nell'inibire la crescita batterica, con una DI_{50} di 50 μg/ml.

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Plasmodium juxtanucleare: An Electron Microscopic Study of the Exoerythrocytic Stages[1,2]

Plasmodium juxtanucleare, a malarial parasite of the domestic fowl, was first described in Brazil by Versiani and Gomes[3]. Since the first studies it has been observed that this parasite provokes only a slight infection in the blood of chicks, which causes a weak immunity and very seldom gives exoery throcytic infection[4].

The exoerythrocytic stages, as an intermediate between the sporozoites from the mosquito and the erythrocytic phase, constitute an essential link in the cyclical development of a malarial parasite. Few ultrastructural studies have been done on these stages[5–9].

In the present paper preliminary results will be given of a study of the ultrastructure of *Plasmodium juxtanucleare* as it appeared in the spleen of a chick with exoerythrocytic infection.

Materials and methods. The strain of *Plasmodium juxtanucleare* used in this work was isolated in our laboratory from the blood of a naturally infected fowl bought in the commercial market of Rio de Janeiro (Brazil). For about 5 years, the parasite has been maintained in chicks by successive blood passages. During this period, no exoerythrocytic infections were seen when