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Effects of *N*-diazoacetyl-glycine amide on the pool of 5-phosphoribosyl 1-pyrophosphate in Ehrlich ascites tumour cells

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N-Diazoacetyl-glycine amide (DGA) posesses various biological properties [1–3], including activity against the growth of some rodent tumours [4]. The mechanism of its antitumour effects has been shown to be inhibition of DNA synthesis [5, 6], concomitant with an inhibition of purine nucleotide biosynthesis [5]. A detailed study of the effects of DGA on purine nucleotide metabolism revealed that many stages were inhibited by the drug, although to different degrees, the most affected one being the 'salvage' conversion of hypoxanthine to XMP [7]. The latter enzymatic process is dependent on the concentration of 5-phosphoribosyl 1-pyrophosphate (PP-ribose-P), which in its turn is involved in other earlier stages of *de novo* purine biosynthesis. We thought it, worthwhile therefore, to study the effects of DGA on the cellular pool of PP-ribose-P, in order to ascertain whether the reported effects of DGA on XMP formation are due to a depression of its cellular pool or to an inhibition of hypoxanthine phosphoribosyl transferase.

The extraction procedure from tumour cells and the enzymatic assay for PP-ribose-P were performed as described by Henderson and Khoo [8]. The assay uses a cell extract as the source of PP-ribose-P for the enzymatic conversion of labelled adenine to AMP: the PP-ribose-P concentration is rate limiting for this process. At the end of the assay, the deproteinized incubation mixture was concentrated by freezing and drying and redissolving in 200 μl of water. 50 μl aliquots, with 1 μg of carrier AMP added, were then spotted on a cellulose thin-layer plate (Merck), and subjected to ascending chromatography with 5% (w/v) Na_2HPO_4 in water as the solvent. The radioactivity of adenine and AMP resolved on the plates was subsequently determined by scraping off 2 × 0·5-cm areas from the plate and counting them in a toluene-based scintillator. Blanks containing no cell extract, and standards containing known amounts of PP-ribose-P (Calbiochem, B grade), were run.

The effects of *in vivo* treatment of Ehrlich ascites-bearing mice with DGA on the tumour cell pool of PP-ribose-P are reported in Table 1. As reported by Henderson and Khoo [8], glucose administration 5 min before sacrifice results in more than a 3-fold increase in the PP-ribose-P pool. The treatment with DGA also causes a sharp increase in the PP-ribose-P content of the tumour cells, up to about three times that of the controls. When DGA and glucose are administered respectively 2 hr and 5 min before sacrifice, the PP-ribose-P level in the tumour cells is about six times higher than that of untreated controls.

These findings show that the reported inhibition caused by DGA on the 'salvage' conversion of hypoxanthine to XMP in tumour cells [7], is not attributable to a reduction of the cellular pool of PP-ribose-P caused by the drug. They also indicate that the observed increase in the cellular content of PP-ribose-P caused by DGA, and its enhancement caused by glucose administration, might be related to the inhibition of at least one enzyme, hypoxanthine phosphoribosyl transferase, for which PP-ribose-P is a substrate. Further investigation, which is in progress, is needed to examine the possible occurrence of inhibition caused by DGA of hypoxanthine phosphoribosyl transferase or of other enzymes for which PP-ribose-P is the substrate, the mechanism of DGA inhibitory effects, and the relevance of these findings to the mechanism of antitumour activity of this compound.

Table 1. Effects of DGA on the cellular pool of PP-ribose-P in Ehrlich ascites tumour cells

Treatment DGA	Treatment Glucose	PP-ribose-P cellular pool (μmoles/g of cells)	T/C
–	–	0·362 ± 0·048	–
–	+	1·22 ± 0·077	3·37
+	–	1·15 ± 0·042	3·17
+	+	2·23 ± 0·69	6·16

Male Swiss albino mice, weighing 20–25 g, were inoculated i.p. 5 days before the experiment with 5 × 10^6 Ehrlich ascites tumour cells. When indicated, the animals received DGA 1·5 g/kg i.p. 2 hr before sacrifice, or glucose 10 mg per mouse 5 min before sacrifice. The tumour cells were then collected and washed by centrifugation at 500 *g* for 5 min and resuspended in Dulbecco phosphate buffered saline [9]. The final cell concentration was adjusted to 2% (v/v) after a microhematocrit determination. Each value is the mean ± S.E.M. of individual determinations performed on groups of three mice.

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