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Inhibition of galactosyltransferase by 5-fluorouracil

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It has been reported from our laboratory that the specific activity of UDP-galactose glycoprotein: galactosyltransferase (EC 2.4.1.22) in homogenates prepared from ovarian epithelial tumours is 2–15-fold higher than that from normal ovaries. The levels of this enzyme in the sera of ovarian cancer patients correlated well with the clinical status of the patient [1–4]. Immediately after tumour reductive surgery, serum levels of the enzyme drop sharply [1, 3]. This was interpreted as showing that at least part of the serum enzyme is of tumour origin. Serial determination of serum enzyme level may be useful for evaluating the effectiveness of therapeutic programmes. 5-Fluorouracil is frequently used in combination chemotherapy for treatment of pre- and post-operative ovarian cancer patients. Since various uridine derivatives act as competitive inhibitors with respect to UDP-galactose substrate for the galactosyltransferase [5], we thought it worthwhile to find out whether 5-fluorouracil has any effect on galactosyltransferase.

5-Fluorouracil was obtained from two different sources, Roche Laboratories, N.J. and Sigma Chemical Co., St. Louis, MO., and was diluted to the desired concentration with distilled water immediately before use. Sources of other materials and procedure for the preparation of homogenates, collection of serum and assay of galactosyltransferase have been described in earlier publications [1–4]. In this particular study the enzyme from tissue homogenate and serum was partially purified by precipitating by 50% saturation of ammonium sulphate (pH 7.0). This treatment almost quantitatively precipitates the enzyme. The precipitate was dissolved in 10mM Tris–HCl, pH 7.4, and dialysed against the same buffer to remove any endogenous uridine derivatives.

Drug dose-dependent inhibition of galactosyltransferase from tumour tissue was observed with 5-fluorouracil (Fig. 1). However, high concentrations of 5-fluorouracil is necessary to observe any inhibition of galactosyltransferase. The inhibition is unlikely to be due to any contaminant present in the drug, since 5-fluorouracil from two different sources produced identical inhibition curves. Enzyme from serum also showed drug dose-dependent inhibition of galactosyltransferase activity (Fig. 2), but again only at a high concentration of 5-fluorouracil. The concentration required to produce a 50% reduction in enzyme activity was around 1mg/ml of the drug.

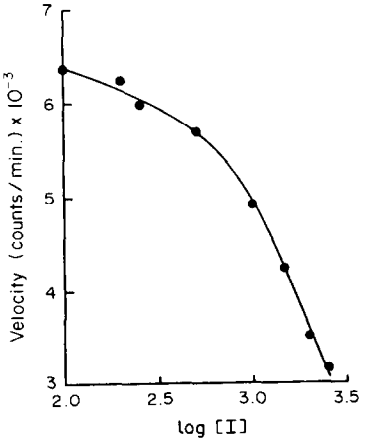

Fig. 1. Inhibition of galactosyltransferase from ovarian tumour tissues by 5-fluorouracil. Concentration of the drug was given in μg/ml.

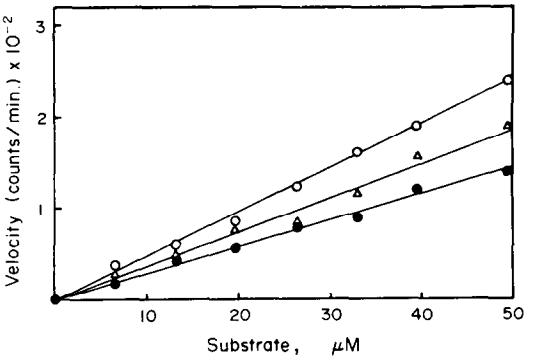

Fig. 2. Inhibition of galactosyltransferase from serum of ovarian cancer patient by 5-fluorouracil at varying concentration of the substrate. Concentration of 5-fluorouracil; ○—zero, △—7.7 mM, ●—15.4 mM.

Kinetics of 5-fluorouracil inhibition of serum galactosyltransferase activity was studied using different concentrations of the donor substrate, UDP-galactose. A double reciprocal plot of these data according to Lineweaver and Burk [6] suggested that the inhibition by 5-fluorouracil was noncompetitive; the K_m and K_i values were 3.33×10^{-6} M and 30.76×10^{-3} M respectively (Fig. 3). Khatra *et al.* [5] reported that uridine, UMP and UDP-glucose competitively inhibit galactosyltransferase from human milk with K_i values of 13.8, 0.82 and 0.079 mM respectively.

At the therapeutic dose, the maximum concentration of 5-fluorouracil in serum immediately after i.v. infusion is 0.1 mg/ml. This concentration is too low to have any significant effect on galactosyltransferase assay; however, we are not sure whether the drug could inhibit shedding of galactosyltransferase, an ectoenzyme, from plasma membrane, which was suggested to be the mechanism of appearance of this enzyme in serum [7].

5-Fluorouracil produced drug dose-dependent inhibition in UDP-galactose glycoprotein: galactosyltransferase from human serum and ovarian tumour tissues. Enzyme kinetics studied suggested a non-competitive inhibition with K_m and K_i values of 3.33×10^{-6} M and 30.76×10^{-3} M respectively.

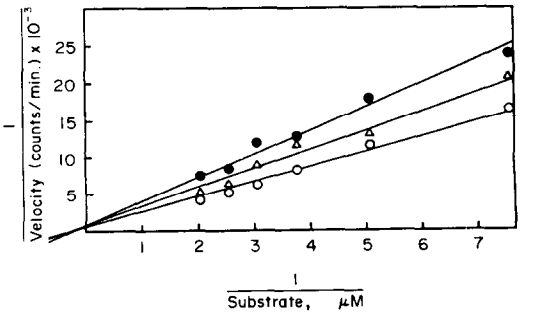

Fig. 3. Double reciprocal plot of the data given in Fig. 2.

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The effect of indomethacin and its ester on lysosomal enzyme release from polymorphonuclear leukocytes and intracellular levels of cAMP and cGMP after phagocytosis of urate crystals

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Lysosomal enzymes are implicated in the pathogenesis of articular tissue degradation in several rheumatic diseases. The main sources of these enzymes are polymorphonuclear leukocytes and mononuclear phagocytes which release lysosomal enzymes after exposure to various phagocytic stimuli. Release of lysosomal enzymes from invading polymorphonuclears and other cells could be one of the targets for the therapeutic action of antirheumatic drugs. Many studies performed so far have yielded controversial results. This confusion originated from a great number of variable conditions used in experimental systems. Various authors used cell-free systems, cultured polymorphonuclear leukocytes and macrophages, different types of phagocytic stimuli (zymosan, aggregated IgG, urate crystals etc.). Relatively good agreement has been achieved when cultured polymorphonuclear leukocytes and macrophages have been used as test system—most clinically active non-steroidal antirheumatic drugs inhibited the release of lysosomal enzymes in concentrations higher than those achieved in clinical therapy. This is well documented in the case of indomethacin which in some experimental systems has been active in inhibiting lysosomal enzyme release in concentrations of 10^{-3} M to 10^{-4} M [1]. Lower concentrations were not significantly effective [2, 3], whereas higher concentrations (above 10^{-4} M) resulted in one experiment even in cytotoxicity and stimulation of zymosan-induced enzyme release [4].

The objective of our study was to examine the effect of indomethacin and ester of indomethacin with tropic acid—Tropesin (2-phenyl-3-(1-*p*-chlorbenzoyl-2-methyl-5-methoxy-3-indolyl) (acetoxypropionic acid in racemic form) both on the release of selected lysosomal enzyme from PMN leukocytes and on the levels of cyclic nucleotides involved in regulatory mechanism of the lysosmal enzymes release into the extracellular space. Microcrystals of sodium urate were used as stimulators of phagocytosis. Indomethacin and Tropesin were prepared in Research Institute for Pharmacy and Biochemistry, Prague. Monosodium urate (MSU) microcrystals (0.5–30 μm) were a gift from Dr. Továrek, University Hospital, Brno, Czechoslovakia. Phenolphthalein-β-D-glucuronide was from Koch-Light and cyclic AMP and cyclic GMP RIA kits from Amersham.

Separation of leukocytes [5]. Leukocytes were obtained from venous blood of healthy young men. Blood (450 ml) was drawn into plastic flasks with 0.9 ml of heparin (5000 U per ml) and 90 ml of a 6% dextran solution. Sedimentation was allowed to proceed in the same flasks for 40 min at room temperature. The cell-rich supernatant was sedimented at 100 *g* for 8 min at room temperature. The erythrocytes were removed by hypotonic lysis (90 ml 0.85% NaCl for 30 sec, 270 ml distilled water added for 20 sec, 90 ml 2.6% NaCl added), and the leukocytes were washed two more times in 0.15 M NaCl and resuspended in the buffered medium to a concentration of 5×10^7 leukocytes per ml medium (1% glucose in phosphate buffered saline, pH 7.4, containing 500 U of heparin per 100 ml). Neutrophils were 60–75 per cent of total leukocytes.

Measurement of enzyme release. Portions of cell suspension (0.7 ml) were dispensed into 10×75 mm plastic test tubes. The cells were incubated at 37° with gentle shaking with indomethacin or with Tropesin in various concentrations. Autologous serum was added to a concentration of 10%. After 1 hr incubation the cells were exposed for 1 hr to particles of microcrystalline monosodium urate. The final concentration was 0.5 mg urate per ml medium. At the end of experiments, tubes were centrifuged at 755 *g* at 4°. The cell-free supernatant fractions were used for enzyme determination. Portions (0.5 ml) of 0.05 M Tris–HCl buffer (pH 7.5) containing 4 mM EDTA were added to sediments (EDTA acts as a phosphodiesterase inhibitor to prevent degradation of cyclic nucleotides by plasma enzymes). Samples were heated to 100° and centrifuged at low speed.