N-Acetyltransferase activity in cultured cells

ANGELA CHRISTIE, ANNE P. BURGESS and EDITH SIM*
Wellcome Toxicology Unit, Department of Pharmacology, South Parks Road, Oxford OX1 3QT, U.K.

Procainamide is widely used as an anti-arrhythmic agent. However, long-term administration is limited by the occurrence of a toxic condition resembling systemic lupus erythematosus (SLE) (Reidenberg, 1981). N-Acetylation of procainamide occurs via liver N-acetyltransferase and constitutes the major route of metabolism in man, Rhesus monkey and rat. Such N-acetylation is under genetic control and humans demonstrate either a rapid or slow acetylator phenotype. Fast and slow acetylators differ in their susceptibility to procainamide-induced SLE; symptoms occur in both phenotypes but take longer to appear in fast acetylators. Although work has been done on N-acetyltransferase from hamster, rabbit and mouse, little is known about the human enzyme (for review, see Weber & Hein, 1985). We have investigated human N-acetyltransferase activity in a hepatoma cell line, HepG2, using p-aminobenzoic acid as a substrate. The monolayer cell line of human origin (Knowles et al., 1980) retains many specialized functions usually lost upon culturing, including the synthesis and secretion of major plasma proteins and retention of cytochromes P-450 (Dawson et al., 1985). We have also looked at the effect of procainamide on the growth of these cells.

HepG2 cells were maintained in Dulbecco's minimum essential medium (DMEM) containing 10% fetal calf serum, penicillin (100 units/ml) and streptomycin (100 μg/ml). Cells were incubated at 37°C in an atmosphere of 5% CO₂. Cells were detached, to allow passage, by incubation (37°C, 5 min) in the presence of 1 ml of trypsin (250 mg in 5 mM-EDTA/150 mM-NaCl). All tissue culture products were from Gibco (Paisley, Scotland, U.K.). Growth of cells was determined from a series of identical 25 cm² flasks each initially containing approx. 10⁶ cells. Procainamide was included as indicated. At appropriate times, the cell number in six flasks was determined by the detachment of cells with 0.5 ml of trypsin as before, then 20 ml of complete DMEM was added and the mixture centrifuged (400g, 2 min). The cell pellet was washed three times in 20 ml of unsupplemented DMEM. The cell pellet was finally resuspended in 0.5 ml of trypsin (250 mg in 50 mM-potassium phosphate/l mM-dithiothreitol, pH 7.4, 20°C for at least 1 h). The pellet was thawed at 37°C and lysis was assured by the addition of 20 μl of ice-cold water. N-Acetyltransferase activity was then determined by the method of Hein et al. (1982). Protein content was measured according to Lowry et al. (1951). All results are expressed as the means ± the standard error of the mean.

Growth of HepG2 cells in the presence of 0.5 mM-procainamide is no different from that of the controls (Fig. 1). After an initial lag period the cells entered a phase of exponential growth. Maximum cell number was reached at 36 h both in the presence and absence of procainamide. The pH of the culture medium was observed to decrease after 42 h and is the likely cause of the decline in cell number. Cell viability in all cultures remained between 70 and 80%.

Abbreviations used: SLE, systemic lupus erythematosus; DMEM, Dulbecco’s minimum essential medium.

*To whom correspondence should be addressed.

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