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98 DETERMINATION OF ANTIGLIADIN ANTIBODIES IN SALIVA FROM COELIAC PATIENTS BY ELISA-BASED ASSAY

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Coeliac disease is a permanent intolerance to dietary gliadin (alcohol soluble protein fraction of wheat gluten) resulting in small intestinal villous atrophy with consequent malabsorption and malnutrition (Trier 1991) and the presence of high levels of sera antigliadin antibodies of IGA class (Engstrom et al 1992). A Gluten-free diet is life-long treatment for such patients. Diagnostic procedure is a long lasting and highly invasive procedure including at least three duodenal biopsies (Meeuwise 1970).

The main aim of our work was development of a specific ELISA-based assay for detection and quantification of antigliadin antibodies in saliva from coeliac patients that can be used in diagnostic procedures.

Saliva from 12 coeliac patients on a gluten-free diet, as well as saliva from a control group of 12 healthy human subjects was collected without stimulation through filter paper in tubes with an enzyme inhibitor (Benzamidine-HCl 6 mmol L^{-1} in ε-amino capronic acid 30 mmol L^{-1} in phosphate buffer pH 7.4) and samples were examined using an ELISA assay with wheat gliadin (commercial and extracted by ourselves) as antigen and prolamins from maize and rice as negative control. Secondary antibody was rabbit antihuman IgA + goat antirabbit IgG (H + L) labelled with horseradish peroxidase (Bio-Rad). Specific substrate for peroxidase was fast OPD (Sigma). Developed colour was measured at 492 nm in a microplate reader Titertek Multiscan (Flow Laboratories). Results showed the presence of high levels of IgA antigliadin antibodies in all investigated saliva from coeliac patients and very good correlation with clinical status. The proposed technique can be used in diagnostic procedures and in monitoring of the clinical status and dietary treatment for patients on gluten-free diets.

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99 A RAPID HPLC METHOD FOR THE DETERMINATION OF LAMOTRIGINE IN SMALL SAMPLE VOLUMES OF PLASMA

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Lamotrigine is a new and effective antiepileptic drug of use in the management of partial and tonic-clonic seizures. A number of hplc assays are available for the measurement of lamotrigine in plasma (e.g. Meyler et al 1993; Sinz & Remmel 1991). Several of these methods involve large volumes of organic solvents, lengthy extraction procedures and poor recovery. The present work describes a simple, rapid, selective and reproducible reversed-phase hplc micro-assay of the determination of lamotrigine in plasma.

The drug was extracted from 100µl of plasma with chloroform: isopropanol (95:5%, v/v) in the presence of 100µl of phosphate buffer (10mM). The extract was evaporated and the residue was reconstituted with mobile phase and injected onto the hplc system. The drug and the internal standard (chloramphenicol) were eluted from a Symmetry C_{18} stainless steel column at ambient temperature with a mobile phase consisting of 0.01M potassium phosphate: acetonitrile:methanol (70:20:10%, v/v), adjusted to pH 6.7, at a flow rate of 1.3ml/min, and the eluate was monitored at 214nm. Retention times were 4.0 min (lamotrigine) and 6.65 min (chloramphenicol). Quantitation was achieved by measurement of the peak-area ratio of the drug to the internal standard and the lower limit of detection for lamotrigine in plasma was 20ng/ml. The intraday precision ranged from 3.34 to 6.12% CV and the interday precision ranged from 2.15 to 8.34% CV. The absolute and relative recoveries of lamotrigine ranged from 86.93 to 90.71% and from 95.18 to 107.13% respectively. None of the commonly used antiepileptic drugs or their main metabolites interfered with the assay.

The method was applied to the determination of lamotrigine in plasma collected at various times, after an oral dose (18.6mg/kg), from a group of 6 New Zealand White rabbits. C_{max} values of 5.11±0.69µg/ml were reached in 0.94±0.48h. The $t_{0.5}$ was 5.5±1.65 h and the $AUC_{0-\infty}$ was 23.9±6.05µg h/ml. This reliable micro-method would have application in pharmacokinetic studies of lamotrigine where only small sample sizes are available e.g. paediatric patients.

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