Determination of Chloramphenicol in Tissues—Problems With *In Vitro* Metabolism

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Residues of drugs and other chemicals used in meat production are covered by EEC Directives; compliance with such Directives requires sensitive analytical techniques for residues analysis. One such drug, chloramphenicol, is used in farm animals and is a potential problem for the residues analyst because it is metabolised rapidly. The cytochrome P_{450} mediated metabolism occurs both *in vivo* and *in vitro* in tissues removed from the animal. It is shown here that chemical inhibition of cytochrome P_{450} significantly increases the recovery of chloramphenicol from spiked liver samples. It is suggested that piperonyl butoxide, a potent cytochrome P_{450} inhibitor, be used to inhibit the enzyme system during the determination of chloramphenicol.

Keywords: *Chloramphenicol determination; cytochrome P_{450} inhibition; piperonyl butoxide; tissue residues*

Chloramphenicol is a potent bacteriostatic antibiotic and has found widespread application in veterinary practice. It is used both in treatment and prophylactically.[1] The latter use will be controlled by an EEC Directive in 1989.[2] Such control relies on the analysis of samples of tissues from abattoirs as part of a food surveillance scheme.

Chloramphenicol is metabolised rapidly[3]: phase I metabolism involves oxidation catalysed by the cytochrome P_{450} system followed by phase II glucuronic acid conjugation.[4] Cytochrome P_{450} is present at high activity in the liver and at lower activities in other tissues.[5] For this reason metabolism of chloramphenicol might occur *in vitro* in tissue samples containing the drug.

A good analytical system relies on quality control. In this instance tissue samples spiked with chloramphenicol are used. During the course of our studies it became apparent that chloramphenicol recoveries from spiked liver were poor. This paper describes the results of a study designed to investigate this phenomenon and offers an approach to its solution.

The determination of chloramphenicol has been reviewed extensively[6,7]; the method used here is a modification of a previously published technique.[8]

Experimental

Extraction of Chloramphenicol From Tissue Samples

A tissue sample (10 g) is homogenised in water (40 cm^3) and centrifuged at 3×10^3 rev min^{-1} for 40 min. An aliquot (18 cm^3) of the supernatant liquid is loaded on to an Extrelut column and allowed to stand for 15 min. The column is then eluted with dichloromethane (2×35 cm^3). The dichloromethane eluate is evaporated in a stream of nitrogen at 40 °C and the residue is dissolved in 0.4 cm^3 of water, ultrasonicated for 1 min using a Decon FS200 ultrasonic bath (Decon Ultrasonics, Hove, UK), then extracted with toluene (2×2.0 cm^3), centrifuged at 3×10^3 rev min^{-1} for 10 min and the toluene layer discarded.

All traces of toluene are removed from the aqueous phase using a stream of nitrogen and a water-bath at 40 °C. The aqueous phase is then diluted to 10 cm^3 with water and extracted with 2,2,4-trimethylpentane - trichloromethane (2 + 3; 7.5 cm^3), centrifuged at 3×10^3 rev min^{-1} for 5 min and the aqueous phase passed through a C_{18} SepPak cartridge. The SepPak column is washed with 3 cm^3 of water and the bound material (including chloramphenicol) is eluted with acetonitrile - water (1 + 1; 3 cm^3). The acetonitrile is removed from the eluate by evaporation in a stream of nitrogen at 40 °C and the remaining aqueous phase (approximately 1.7 cm^3) is extracted with 10 cm^3 of ethyl acetate. The upper organic phase is removed, evaporated in a stream of nitrogen at 40 °C and the residue dissolved (aided by sonication) in 0.4 cm^3 of water. This solution is then analysed by HPLC as described below.

HPLC Analysis

An aliquot (0.1 cm^3) of the final aqueous solution is injected on to a reversed-phase C_{18} HPLC column.

HPLC system

The HPLC system consisted of a Rheodyne injection port, a Spherisorb C_{18} column, 200 × 3 mm i.d., 5 μm (Chrompack, Millharbour, London), a Chrompack C_{18} guard column cartridge, a Spectroflow 773 detector (ABI Analytical, Kratos Division, Warrington, UK), a 30 cm column block heater (Jones Chromatography, Hengoed, Glamorgan, UK) and a PU 4015 dual reciprocating LC pump (Philips, Cambridge, UK).

The mobile phase consisted of sodium acetate buffer (0.01 M, pH 4.3) - acetonitrile (75 + 25). The following conditions were used: solvent flow-rate, 0.6 cm^3 min^{-1}; temperature, 25 °C; and detector wavelength, 278 nm.

Inhibition of Cytochrome P_{450} Activity

Bovine liver samples were homogenised as described above. Piperonyl butoxide (PB; 1 cm^3) was added to each 10 g of homogenate and the whole was allowed to stand at room temperature for 15 min.

Preparation of Spiked Samples

Chloramphenicol (20 ng g^{-1}) was added to either liver homogenate containing PB or to untreated homogenate. The homogenates were analysed immediately as described above.

Results and Discussion

The level of chloramphenicol in untreated liver homogenate was found to be 6.6 ± 0.67 ng g^{-1} (mean ±SD, n = 3), whereas in samples containing PB the level was 12.1 ± 0.77 ng g^{-1} (mean ±SD, n=3). These results represent 32.9 and 60.7%, respectively, of the added chloramphenicol. This clearly demonstrates that inhibition of cytochrome P_{450}

activity by PB enhances the level of chloramphenicol present in the tissue sample. This is probably due to the fact that the rapid (*ca.* 40 min during the sample preparation period) metabolism of chloramphenicol *in vitro* in spiked tissues is inhibited by PB.

In the experiments described above, PB was added to the liver homogenate prior to spiking with chloramphenicol and therefore non-PB inhibited homogenates had sufficient time to metabolise chloramphenicol during the 40-min centrifugation period. The addition of PB to tissue homogenates can therefore be used to prevent a decrease in the chloramphenicol levels *in vitro* in spiked tissues used in quality control schemes. It is recommended that samples taken for analysis should be cooled or, ideally, be frozen immediately after excision in order to minimise metabolism and that the samples are homogenised in water containing 2.5% *m/V* PB. This procedure should minimise the loss of chloramphenicol caused by *in vitro* metabolism.

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