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Biotransformation of the topical glucocorticoids budesonide and beclomethasone 17α,21-dipropionate in human liver and lung homogenate

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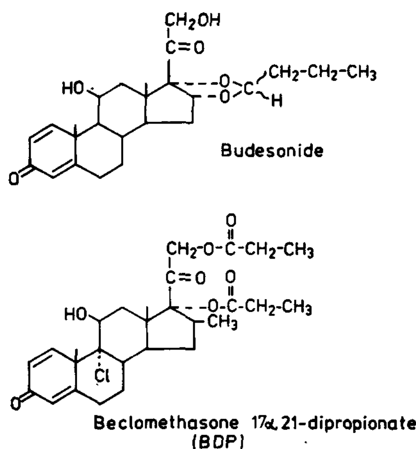
Tritiated budesonide and beclomethasone 17α,21-dipropionate (BDP) were incubated with the 9000*g* supernatant of human liver homogenate. BDP was immediately hydrolysed to beclomethasone 17α-propionate (BMP). BMP was then further biotransformed to polar metabolites. Budesonide was rapidly biotransformed (2–4 times more rapidly than BMP) to metabolites of low glucocorticoid potency. The compounds were also incubated with the 1000*g* supernatant of human lung homogenate. BDP was rapidly hydrolysed to BMP and then more slowly to beclomethasone. Budesonide was not biotransformed in the lung.

The glucocorticoids budesonide I and beclomethasone 17α,21-dipropionate (BDP, II) are both used in the topical treatment (inhalation) of respiratory disorders such as asthma and rhinitis (Hansen & Mygind 1974; Toogood et al 1977; Ellul-Micallef et al 1980; Balle 1982). As judged from vasoconstriction data budesonide is 2–3 times more potent than BDP (Johansson et al 1982), which indicates that it is clinically more potent than BDP. However, budesonide is significantly less potent that BDP in depressing plasma cortisol and changing the total or differential white blood cell count especially after oral administration but also after inhalation (Johansson et al 1982). This difference may depend on a more efficient first pass metabolism of budesonide giving metabolites with low biological activity. We describe the in-vitro biotransformation of budesonide and BDP in human liver and lung.

Methods

Tritiated [1,2-^{3}H]budesonide and [1,2-^{3}H]beclomethasone 17α,21-dipropionate ([1,2-^{3}H]BDP) were obtained from the Radiochemical Centre, Amersham, England. The specific activity was 76·6 mCi mg^{-1} [^{3}H]budesonide and 19·0 mCi mg^{-1} [^{3}H]BDP. Hplc analysis using a Nucleosil C_{18} (5 μM) column and ethanol–water as mobile phase (45:55 for [^{3}H]budesonide and 50:50 for [^{3}H]BDP) showed a radiochemical purity higher than 95%. Non-labelled budesonide, beclomethasone 17α-propionate (BMP), beclomethasone 21-propionate and beclomethasone were supplied by Dr A. Thalén, AB Draco, Sweden. Non-labelled BDP was obtained from LARK SpA, Milan, Italy. The water used was Millipore filtered and dichloromethane, chloroform, heptane and ethanol were of spectroscopic grade. D-Glucose-6-phosphate, NADP and glucose-6-phosphate dehydrogenase were obtained from Sigma, Sweden.



Four human liver samples (samples: 13, 16, 17 and 18) were supplied from the liver bank at Huddinge Hospital, Sweden (von Bahr et al 1980). The sex and age of the donors were F53, M52, M24 and F59, respectively. Each sample was homogenized in ice cold buffer (0·1 mol litre^{-1} K_2HPO_4, KH_2PO_4, pH 7·4, also containing 0·2 mol litre^{-1} of sucrose) with a Polytron PT20 homogenizer for 20–30 s. The homogenates were centrifuged at 9000*g*. The protein concentration was estimated according to Lowry et al (1951) and diluted to 1 mg ml^{-1}. Three human lung samples were obtained from lobectomy patients at the University Hospital of Lund, Sweden. The age of the donors was 51, 58 and 75 years. The lungs were pooled and homogenized as described above. The homogenate was centrifuged at 1000*g*. The protein concentration was measured and diluted to 4 mg ml^{-1}.

[^{3}H]Budesonide and [^{3}H]BDP were incubated at a concentration of 0·1 μmol litre^{-1} with the 9000*g* liver supernatants and with the 1000*g* lung supernatant at 37 °C in the presence of carbogen gas (95% O_2, 5% CO_2). Each incubation (volume 6 ml) also contained 6 mg of protein (liver) or 24 mg of protein (lung), 60 μmol of $MgCl_2$, 45 μmol of glucose-6-phosphate, 1·8 μmol of NADP, and 2·5 units of glucose-6-phosphate dehydrogenase. Blank incubations using denatured (boiled) supernatants were also performed.

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After an equilibrium period for 10 min at 37 °C, the incubations were started by the addition of 60 μl ethanol containing either [^{3}H]budesonide or [^{3}H]BDP. One human serum sample (diluted 1 : 1 with buffer) was also incubated with [^{3}H]budesonide and [^{3}H]BDP. Aliquots (0·5 ml) were taken from each incubation flask and transferred into borosilicate test tubes containing either non-labelled budesonide (60 μg) or BDP, BMP and beclomethasone (71, 63 and 56 μg, respectively) as internal standard in 0·5 ml ethanol. The samples were then frozen on dry ice (−60 °C). To each sample, 4·0 ml of dichloromethane was added. Extraction was by gentle shaking for 30 min, followed by centrifugation for 15 min at 2000 rev min^{-1}. The organic phase (3·0 ml) was collected and taken to dryness under a gentle stream of nitrogen. The residue was then dissolved in 200 μl of the mobile phase used in hplc. By extraction of denatured incubation samples from liver, a recovery of 89·5% (s.d.: 0·8%, n = 5, [^{3}H]budesonide) and 101·8% (s.d.: 5·6%, n = 5, [^{3}H]BDP) of the total radioactivity were obtained.

The analyses of [^{3}H]budesonide and [^{3}H]BDP–BMP were by hplc (Waters): stationary phase Nucleosil C_{18} (5 μM) (Column: 5 × 200 mm), mobile phase ethanol–water (45 : 55 and 50 : 50, respectively), flow rate 1·0 ml min^{-1}. After injection (100 μl) the effluent was collected into scintillation vials and the tritiated peaks were assayed for radioactivity. The uv-peaks of the internal standards were recorded by a uv-detector and the ratio between radioactivity and area under uv-peak was calculated. At 0 min of incubation, the ratio was considered to reflect a relative concentration of 1. [^{3}H]Budesonide, [^{3}H]BDP, [^{3}H]BMP and [^{3}H]beclomethazone were identified on the basis of their retention times compared with those of the internal standards.

Results and discussion

During addition of [^{3}H]BDP to the 9000*g* liver supernatants, the drug was immediately biotransformed to a metabolite having a similar hplc retention time as beclomethasone 17α-propionate or beclomethasone 21-propionate. The identity of the metabolite was checked by gel chromatography on a Lipidex 5000 (Packard) column with heptane–chloroform (1 : 1) as mobile phase. In this system the 17α- and 21-propionate esters of beclomethasone separated. All radioactivity co-eluted with beclomethasone 17α-propionate (BMP). Since the vasoconstriction potency of BMP is almost as high as that for BDP, the hydrolysis of BDP to BMP does not represent an inactivation step (Harris 1975). To be deactivated, BMP has to be hydrolysed to beclomethasone and/or biotransformed by oxidative or reductive pathways to polar metabolites. The relative biotransformation rate of [^{3}H]budesonide and [^{3}H]BMP in homogenates from the human livers followed a pattern shown in Fig. 1. The [^{3}H]budesonide was biotransformed 2–4 times more rapidly than

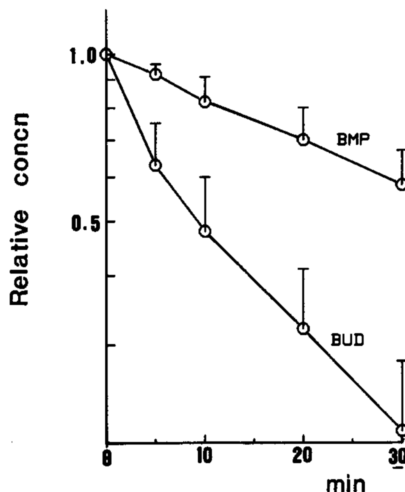

FIG. 1. Relative concentrations of unchanged budesonide (BUD) and beclomethasone 17α-propionate (BMP) during incubation of BUD and beclomethasone 17α,21-dipropionate (BDP) in four human liver 9000*g* supernatants. Initial steroid concentration: 0·1 μmol litre^{-1}. The data points are expressed as mean ± s.e.m. Part of this figure has been published in Eur. J. Resp. Dis. 1982, 63 (122): 86–95.

[^{3}H]BMP. While [^{3}H]budesonide was biotransformed to the relative inactive metabolites 6β-hydroxybudesonide and 16α-hydroxyprednisolone (Edsbäcker et al 1983; Dahlberg et al 1984). [^{3}H]BMP was biotransformed to unknown metabolites. None of these was found to co-elute with beclomethasone during hplc analyses. No biotransformation occurred when incubating [^{3}H]budesonide and [^{3}H]BDP with denatured incubation medium.

During incubation with the 1000*g* lung supernatant, [^{3}H]BDP was rapidly hydrolysed to [^{3}H]BMP (Fig. 2). After 5 min only18% of the radioactivity was attributed to [^{3}H]BDP and the rest to [^{3}H]BMP, which then was very slowly biotransformed to a compound with the same retention time as beclomethasone.

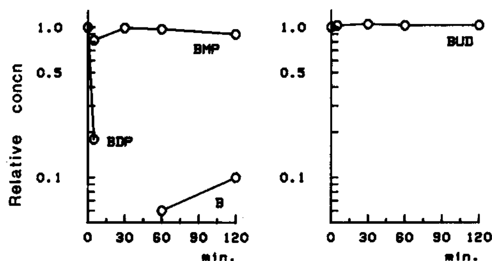

FIG. 2. Relative concentration of unchanged beclomethasone 17α,21-dipropionate (BDP), beclomethasone 17α-propionate (BMP) and beclomethasone (B) and of unchanged budesonide (BUD) during incubation of BDP and BUD with the human lung 1000*g* supernatant. Initial steroid concentration: 0·1 μmol litre^{-1}.

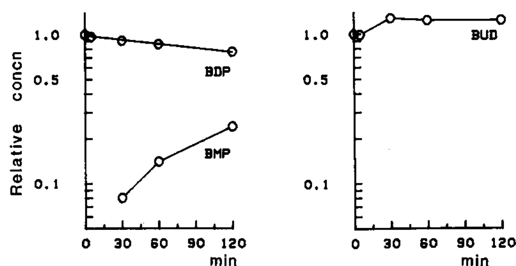

FIG. 3. Relative concentration of unchanged beclomethasone 17α,21-dipropionate (BDP) and beclomethasone 17α-propionate (BMP) and of unchanged budesonide (BUD) during incubation of BDP and BUD with human serum (diluted 1:1 with buffer). Initial steroid concentration: 0·1 μmol $litre^{-1}$.

The results are in agreement with those reported by Martin et al (1974) and Ronca-Tesloni (1983) *and* are due to a relatively high activity of esterase enzymes in the lung. They also verify that an ester group in the 21-position is more easily hydrolysed than that in the 17α-position (O'Neill & Carless 1980). Budesonide, on the other hand, was not biotransformed in the lung (Fig. 2). Since budesonide in the liver is biotransformed mainly by oxidative and to some extent also reductive pathways, the results indicate a very low activity of these enzymes in the lung.

The two drugs were also incubated with one human serum sample (diluted 1:1 with buffer). [^{3}H]BDP was slowly hydrolysed to [^{3}H]BMP (Fig. 3). At 2 h of incubation about 76% of the radioactivity was attributed to unchanged [^{3}H]BDP and the rest to [^{3}H]BMP. No [^{3}H]beclomethasone could be detected. [^{3}H]budesonide was not biotransformed in serum (Fig. 3).

The present data help to elucidate the pharmacodynamics of the two glucocorticoids. After topical deposition in the respiratory tract, both drugs are systemically absorbed, probably with most of their biological activity preserved. However, in topical (aerosol) therapy, the major fraction of the discharged dose is swallowed and then absorbed from the gastrointestinal tract (Newman et al 1980; Davies 1982). The absorbed drug can induce systemic side effects. To minimize these effects it is essential that an efficient first pass inactivation in the liver takes place. For steroid esters, inactivation by hydrolysis in the lumen and/or mucosa of the small intestine can also be of importance. In this study, [^{3}H]budesonide was found to be more rapidly inactivated by biotransformation than [^{3}H]BDP in human liver homogenate. The efficient liver biotransformation of budesonide is also reflected by a low oral availability (10·7 ± 4·3%) as was shown by Ryrfeldt et al (1982) in healthy volunteers. These results may partly explain the relatively lower systemic effects found with budesonide compared with BDP at higher clinical dosages.

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