Development, Evaluation and Data Acquired with a Tape-Stripping Technique for Measuring Dermal Exposure to Budesonide at a Pharmaceutical Manufacturing Site

INGRID E. LILJELIND1,2*, INGEGERD MICHEL3, MARIA DAMM3 and KÅRE A. ERIKSSON2

1Occupational Medicine, Department of Public Health and Clinical Medicine, Umeå University, S-901 87 Umeå, Sweden; 2Occupational and Environmental Medicine, University Hospital of Northern Sweden, S-901 85 Umeå, Sweden; 3AstraZeneca AB, Sweden Safety Health & Environment, SE-151 85 Södertälje, Sweden

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Objectives: Although corticosteroids have been used for over 50 years as anti-inflammatory and anti-proliferative agents, few studies have examined their exposure levels and health effects on workers employed in the corticosteroid manufacturing industry. The aims of the study reported here were to develop a tape-stripping technique for monitoring budesonide (a corticosteroid used in inhalators for treating respiratory diseases) and to apply the method in a pilot study to estimate the potential dermal exposure to budesonide among workers at a pharmaceutical formulation site.

Methods: The tape-stripping method was evaluated by applying 0.5 and 2.07 μg of budesonide dissolved in ethanol on tape strips. The same amounts were also applied on a cleaned glass plate and human skin of volunteers, which were then stripped by series of tapes immediately, and 30 min later, the amounts collected by the tapes were measured. Finally, the technique was used to study the exposure of budesonide among eight employees at a pharmaceutical industry site. Three exposure sites were tested: the tip of the forefinger, palm of the hand and ventral part of the lower arm. Five consecutive tape strips per sampling site were used in both the recovery studies and the field study.

Results: The mean overall recoveries from spiked tapes and the glass plate were 96 and 81%, respectively, while for human skin the corresponding figure was 38%, (for applications of 2.07 μg; no detectable amounts were recovered from human skin after 0.5 μg applications). The recovered amount was found on two consecutive tapes after 0 min, but only on the first tape strip after 30 min. The inter-individual variability was 4-fold. In the field, quantifiable amounts were found for four of eight employees and a concentration gradient was detected along the two or three consecutive tape strips. The tip of the forefinger and the palm of the hand were the most highly exposed sites to budesonide.

Conclusions: A tape-stripping method can be used to determine potential dermal exposure to budesonide. The results also indicate that budesonide is taken up by the skin of operators who are exposed to the substance at their workplace.

Keywords: budesonide; occupational exposure; potential dermal exposure; tape-stripping technique

INTRODUCTION

Corticosteroids have been used for over 50 years because of their anti-inflammatory and anti-proliferative effects, especially successfully in skin therapy. However, adverse health effects have also been reported in some cases; most frequently, local effects including atrophy, rosacea, perioral dermatitis, acne and purpura, but also some systemic effects including hyperglycemia, glaucoma and adrenocortico insufficiency (Hengge et al., 2006). Nevertheless, this knowledge is based
on experience from therapeutically topical treatment of clinical patients, 5% among whom became allergic (type IV allergy, contact allergy) to corticosteroids (Isaksson, 2004). Few studies have addressed exposure and/or health effects among workers employed in the corticosteroid manufacturing industry. Heron and Pickering (2003) concluded in a recent review that there is limited epidemiological evidence of severe adverse health effects such as mortality and morbidity due to occupational exposure to active pharmaceutical ingredients, but adverse effects can occur. Two studies, by Newton et al. (1982) and Pezzarossa et al. (1987), have shown symptoms of adrenal insufficiency in workers exposed to corticosteroids. In the first cited study, no exposure data were reported, but in the second, corticosteroid levels of 2.5–10.2 mg m⁻³ were found in dust samples collected in the work area. Another study (Moroni et al., 1988) found reversible local effects such as acne and erythema and systemic effects such as hypertension and Cushing’s syndrome among exposed workers. The exposure in this case was measured by monitoring urinary 17-OH corticosteroids and plasma cortisol.

The workers examined in our study were potentially exposed to the corticosteroid budesonide, formulated for use in inhalators for treating respiratory diseases. Exposure to budesonide has been found in the air, but mostly at very low levels compared with the exposure limit of 0.01 mg m⁻³, voluntarily agreed by the industry. Workplace measurements were performed in 2002 and 2004 and generally air levels were found to be between <0.00001 and 0.002 mg m⁻³, but in one extreme case, 0.013 mg m⁻³ was detected (I. Michel, unpublished data). Despite the low air levels, skin reactions such as acne have been reported. Therefore, samples from different workplace surfaces (1 dm²) were collected in 2002–2005 using a swab sampling technique. The results showed that environmental samples contained detectable amounts (12–37 μg) of budesonide, and visible dust could contain 135–235 μg of the substance. Generally, no budesonide was found in swab samples from areas of workers’ skin covered by their protective clothes. However, in one case, 2 μg was found on the hands and arms of a worker who had taken off his/her protective clothes (I. Michel, data not published).

As no occupational exposure limit, detected by swab sampling, has been set, it is not possible to compare the amounts found with levels recognised as ‘safe’. However, the company decided that 200 μg dm⁻² would be consistent with the quality assurance (GMP) procedures. To further examine the exposure to budesonide, which might explain the skin effects found among the workers, their dermal exposure was measured using a tape-stripping method.

The aims of this study were to develop a tape-stripping technique for budesonide, to apply the method in a pilot study among exposed workers in a pharmaceutical industry and to estimate their potential dermal exposure to the compound.

MATERIAL AND METHODS

Tape stripping

The measurements of budesonide were performed by a tape-stripping technique (Mattorano et al., 2004), using Fixomull® (BSN medical GmbH & Co, Germany), which has been found to have better sampling and analytical qualities, and to remove another class of organic contaminants (multifunctional acrylates) more efficiently than available alternatives (Surakka et al., 1999). Pre-cut, 10 cm² (2.5 × 4 cm²) pieces of the adhesive tape were applied to sites of exposed skin. After 1–2 min of adhesion, the tape was removed slowly with constant force applied at a 45° angle to the skin (or glass surface in the preliminary recovery study) using cleaned forceps. Each tape was placed in a clean glass scintillation vial which was closed with a screw-tight lid. Further tapes (2–5) were applied to the same site as the previous tape. When the sampling procedure was complete, the tape samples were placed in an exicator protected from light at room temperature, awaiting analysis. The tapes were removed from the sampling skin site by an individual wearing unused vinyl gloves (Vinyl Exam gloves, Evercare, Sweden) and the forceps were rinsed in acetone (Burdick & Jackson, USA) between handling each sample to avoid cross-contamination.

Recovery studies

Tapes and glass plate. To each of a set of five-tape strips, 0.5 μg of budesonide dissolved in 5 μl ethanol (ETAX AaS 99.5%, Solveco chemicals AB) was applied and 2.07 μg to each of another set of five. In addition, to examine the removable capacity of the tape, 0.5 μg of budesonide dissolved in ethanol (ETAX AaS 99.5%, Solveco chemicals AB) was evenly applied to eight 10-cm² areas on a cleaned glass plate and 2.07 μg to another eight. Four sites with each amount were tape stripped five times immediately thereafter and the other sets of four 30 min later. The tapes were handled as described above and used for both the recovery study and the stability test. Sample stability. The stability of the budesonide samples was evaluated over two and a half months to determine how long the samples could be stored as unextracted tape samples in an exicator or extracts of the tape samples stored at 4°C and analysed in a time series.

The spiked tapes and tapes samples from the glass plate experiments (both 0.5 and 2.07 μg) were
desorbed (see below) at various times during the two and a half months following preparation. Spiked tapes 1, 2 and 3 and glass plate samples 1 and 2 were all desorbed and analysed within one month, spiked tape 4 and glass plate samples 3 after one and a half months, while spiked tape 5 and glass plate samples 4 were analysed two and a half months after sampling. To evaluate the extract stability from tape samples, spiked tapes 3 and 4 were analysed one and a half months and one month after desorption.

**Human skin.** Six volunteers (three men, three women) were exposed at room temperature to 0.5 and 2.07 μg of budesonide dissolved in 5 μl ethanol (ETAX AaS 99.5%, Solveco chemicals AB) at separate sites on their ventral part of the lower arms, for 0 and 30 min, respectively. They were not allowed to leave the room or touch the exposed skin area. Each exposure site was tape stripped five times and the tapes were handled as described above.

**Analysis**

The tapes that had been placed in clean glass 20 ml scintillation vials were desorbed by ultrasonically agitating them (in a 8510 ultrasonic tank, Branson) for 20 min in 5 ml of Methanol (Rathburn Chemicals Ltd. Scotland), adding 5 ml of Millipore water, then ultrasonically agitating them again for 20 min. The extracts from the tapes were analysed as follows: 100 μl of each sample was automatically injected by an auto sampler (Agilent 1100) into a high-pressure liquid chromatograph (Agilent 1100) equipped with a variable wavelength detector set at 248 nm. The mobile phase was 70/30 (v/v) high-pressure liquid chromatograph Zorbax SB-C18 (Agilent Technologies, USA) column and then 150 ml of Methanol (Rathburn Chemicals Ltd, Scotland) was added. When the substance was completely dissolved, further methanol was added to bring concentration to 100 ng ml⁻¹ and then 150 ml of Methanol was added. A third task (T3; operator C, D, G and H) was preparing batches of budesonide solution (each containing 2.4 kg pure budesonide) for use in the first steps in the process described above and this work was performed in a glove box. The last task (T4; operator I, J, K and L) studied was weighing 6.3 kg pure budesonide and cleaning the room with both ethanol and vacuum cleaner. All the workers were instructed to use task-specific full personal protection suits (PPE) to avoid dermal and inhalation exposure. Observations on relevant phenomena, e.g. individual behaviour and compliance procedures regarding personal protection equipment were recorded by a work environment engineer who observed each worker performing their specific work tasks.

When the assigned work tasks were completed, samples from operators were collected by tape stripping, as described above, in a separate room. Workers A–H entered this room after removing their protective equipment, but not their protection suits. To comply with quality assurance (GMP) procedures, which could not be ignored, workers I, J, K and L also removed their protection overalls and passed through a clean air lock. None of the workers (except L) washed their hands before sampling. The tapes were applied at three different sites: the tips of the right and left forefingers, palm of the hand and wrist. At each site, five consecutive tapes were applied and the tapes were placed in scintillation vials, then stored in an exciator in the dark until analysis. The tapes were handled as described above.

**Exposure assessment**

Worker exposure was measured during the formulation of a solid drug containing budesonide. Budesonide, the active ingredient, is not manufactured at this facility but budesonide solution is sprayed on pellets, which then are coated with an acid-resistant layer and dried in a closed system including a fluid bed dryer, and then sieved.

The formulation process is taken place in a closed system, but exposure can occur at several points. Eight exposed workers employed at two different sections of the facility participated in the study and performed either one or two of four defined tasks. The first task (T1; operator A and F) included sieving pellets containing budesonide into a can and exchanging parts of spray gun used to apply budesonide to the pellets. Another closely related task (T2; operator B and E) involved cleaning of a glove box with ethanol. A third task (T3; operator C, D, G and H) was preparing batches of budesonide solution (each containing 2.4 kg pure budesonide) for use in the first steps in the process described above and this work was performed in a glove box. The last task (T4; operator I, J, K and L) studied was weighing 6.3 kg pure budesonide and cleaning the room with both ethanol and vacuum cleaner. All the workers were instructed to use task-specific full personal protection suits (PPE) to avoid dermal and inhalation exposure. Observations on relevant phenomena, e.g. individual behaviour and compliance procedures regarding personal protection equipment were recorded by a work environment engineer who observed each worker performing their specific work tasks.
On each sampling occasion, one field blank for each worker was prepared.

The Umeå University Ethics Committee approved the study (Un dnr. 04-175).

RESULTS

Analysis

Regression analysis showed that the signals obtained from the standard solutions were linear across the concentration tested, with R²-values of 0.9999–1.0000. The LOD and LOQ were estimated to be 100 ng/tape and 300 ng/tape, respectively, for standard solutions.

The estimated within-day variability, calculated as the coefficient of variance for 10 standard solution samples analysed on the same day, was 2.7%. The between-day variability, obtained by repeatedly analysing a sample on five alternate days, was estimated to be 1.94%.

Recovery studies

Tape and glass plate. The recovery was 95 ± 9.4% for 0.5 µg and 97 ± 5.8% for 2.07 µg budesonide (overall mean for the ten spiked samples, 96 ± 7.4%). All measured levels reported in this paper are corrected for the overall mean recovery from the spiked tape.

The overall mean recoveries of the four tests on a glass plate were 78 ± 13.6% and 84 ± 8.7% for the 0.5 µg and 2.07 µg applications, respectively, Table 1. Similar results were obtained for both exposure times; 81% for both 0 and 30 min. For 0.5 µg, samples exposed for both 0 and 30 min, only the amounts of budesonide extracted by the first tape strip are reported. In two cases, the amount analysed was close to the LOQ (Gp1:30, Gp2:30, i.e. glass plate 30 min exposure samples 1 and 2) (Table 1). After application of 2.07 µg, detectable amounts of budesonide were found on two consecutive tapes and in one case on three consecutive tapes (Gp4:30), in decreasing amount (data not shown). However, in each case, the amounts on the last tape with detectable levels from glass plates 2 and 4 were close to LOQ (Table 1) and were not therefore included in the recovery calculations. All the spiked tape samples and the tape samples from the glass plate experiment were included in the sample stability test described below. Therefore, glass plate 4 samples were analysed two and a half months after sampling, and the resulting chromatogram showed higher noise level than expected, which may have interfered with the quantification, especially for the third tape.

Sample stability. The results, as described above, show that the tapes could be stored for at least two months in an excicator before desorption and analysis. However, there were high noise levels in the chromatogram obtained from one set of the tapes (from glass plate 4) stored for two and a half months before analysis. The reason(s) for the high noise levels must be further evaluated. Stability tests were also performed on tape extracts that had been stored in a refrigerator (temperature 2–6°C). Extracts from spiked tape 3 were analysed one and a half months after desorption and extracts from spiked tape 4 were analysed one month after desorption. The mean recovery was calculated to be 98% for the low concentration and 93% for the high (data not shown). Further evaluation is needed to establish the time that desorbed samples that can be stored.

Human skin. The results from the high dose (2.07 µg) applications are shown in Table 2. No detectable amount was found on the tapes after application of the low dose (0.5 µg). The mean overall recovery from all of the individuals was 38%, corresponding to 0.78 µg of budesonide. The data presented in Table 2 show that there was an almost 4-fold range in recoveries among the tested permutations of individuals and exposure times. The mean recovery was 40 ± 14% for 0 min exposure time and 36 ± 8% for 30 min exposure time. This difference was not statistically significant (P > 0.05, Student’s t-test). Budesonide was found on two consecutive tapes after 0 min exposure time from three individuals, however quantifiable levels of budesonide were only found on the first tape for all individuals after 30 min exposure.

The concentrations of budesonide in all the blank samples were below the LOD.

Table 1. Recoveries from applications of 0.5 and 2.07 µg of budesonide on a glass plate (Gp) followed by repeated tape stripping after 0 and 30 min

<table>
<thead>
<tr>
<th>Gp:min</th>
<th>Applied amount, 0.5 µg</th>
<th></th>
<th>Applied amount, 2.07 µg</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured (µg)</td>
<td>Recovery %</td>
<td>Measured (µg)</td>
<td>Recovery %</td>
</tr>
<tr>
<td>Gp1:0</td>
<td>0.44</td>
<td>88</td>
<td>1.49</td>
<td>72</td>
</tr>
<tr>
<td>Gp1:30</td>
<td>0.30a</td>
<td>60</td>
<td>1.74</td>
<td>84</td>
</tr>
<tr>
<td>Gp2:0</td>
<td>0.34</td>
<td>69</td>
<td>1.84b</td>
<td>88</td>
</tr>
<tr>
<td>Gp2:30</td>
<td>0.30a</td>
<td>60</td>
<td>1.71</td>
<td>82</td>
</tr>
<tr>
<td>Gp3:0</td>
<td>0.40</td>
<td>80</td>
<td>1.69</td>
<td>84</td>
</tr>
<tr>
<td>Gp3:30</td>
<td>0.40</td>
<td>80</td>
<td>1.87</td>
<td>94</td>
</tr>
<tr>
<td>Gp4:0</td>
<td>0.48</td>
<td>96</td>
<td>1.48</td>
<td>74</td>
</tr>
<tr>
<td>Gp4:30</td>
<td>0.45</td>
<td>90</td>
<td>2.01c</td>
<td>97</td>
</tr>
<tr>
<td>Mean</td>
<td>0.39</td>
<td>78</td>
<td>1.73</td>
<td>84</td>
</tr>
</tbody>
</table>

The values shown are corrected for the recovery of the spiked tape samples.

aAt the LOQ.

bOne of the tape strips close to LOQ, if included 2.1 µg and 101%.

cOne of the tape strips at the LOQ, if included 2.31 µg and 115%.
Exposure assessment

The tip of the forefinger, as expected, and the palm of the hand were the most exposed sites of the operators performing work tasks T1, T2 and T3, while the wrist was less exposed in all the work tasks (Table 3). Following work task T4, no detectable amounts of budesonide were found on any tape. The highest dermal exposure levels were associated with work task T2 (sieving and cleaning glove box), especially for operator B, whose exposure also showed a skewed distribution, as higher levels of budesonide were found on the right side than on the left side of samples (Table 3). Quantifiable amounts were also obtained from most of the sampled sites of operator E (who performed the same task as operator B). Furthermore, decreasing amounts were seen on the two consecutive tapes for operator B for samples taken from the forefinger tip. The same was seen for operator D who was one of the operators performing work task number three (preparing budesonide solutions in a glove box). This might indicate a concentration gradient of budesonide.

The concentrations of budesonide in all the blank field samples were below the LOD.

DISCUSSION

The results from the recovery studies on spiked tapes, glass plates and the field study of human exposure show that it is possible to measure the potential dermal exposure to budesonide with the tape-stripping technique. In all, the mean level of 85% of the budesonide applied on the spiked tapes and glass plate were recovered. The amount recovered from the 0.5 \( \mu g \) applications was very close to the LOQ in two cases, indicating that 0.5 \( \mu g \) is close to the limit that could be validly measured. This may explain the lower recovery from the 0.5 \( \mu g \) applications. However, we used the same amount on human skin to evaluate what happens at very low concentrations, as we expected to find low amounts in the field study. After applying 2.07 \( \mu g \) of budesonide to the glass plate, the amount was quantifiable in all cases not only on the first tape, but also the second tape. This finding indicates that the method...
may not have efficiently removed budesonide from the glass surface. A possible explanation for this finding is that the budesonide solution was unevenly spread on the glass plate and saturated the tape at some points, which the second tape then ‘cleaned up’. However, it is difficult to think of any plausible chemical reaction that would cause budesonide to stick to the glass plate, and it is highly unlikely that significant amounts of the substance would evaporate at room temperature during the short exposure periods.

The recovery from human skin of samples collected immediately after application vary from 15 to 55% between the individuals, but there was less between-individual variability among the sample collected after 30 min, Table 2. Some of the variabilities may have resulted from imprecise tape-stripping of the application areas or imprecise application within the defined area. Nevertheless, more studies are warranted to examine inter-individual variability with respect to factors such as age and gender.

The recoveries were approximately halved in comparison with the recovery from the glass plate (Tables 1 and 2) for 2.07 μg, indicating that the human skin may have absorbed some of the budesonide. This hypothesis is supported by the absence of detectable levels on the tapes after applications of 0.5 μg of the compound and the recovery levels were higher (albeit non-statistically significantly, possibly because of the small size of the study group) after 0 min than after 30 min, following the applications. Another explanation can be that the tape strips did not succeed in removing the budesonide from the skin, but this has to be further investigated. However, after 30 min exposure, budesonide was only recovered on the first tape. An explanation for the findings after 30 min could be the reservoir property of the stratum corneum. Pelchrzim, et al. (2004) have shown that the first upper cell layer of stratum corneum may act as a corticosteroid reservoir. Two hours after application of two different corticosteroid formulations (cream and emollient), the skin was repeatedly tape stripped and in the penetration profile it was evident that most of corticosteroid (how much is not said) was trapped in the stratum corneum; and penetrate into the stratum corneum. Therefore, the relevance of the results from the recovery study to the field study is debatable. However, it is not straightforward to develop a recovery method for particle-bound substances even if the exposure at the workplace is predominantly via budesonide-containing dust. On the other hand, some of the exposure occurs during the work tasks including ethanol cleaning of the glove box, and gloves are not worn continuously during the tasks performed by operators A–H.

The exposure assessments were performed during normal production days and/or according to standard operating procedures. We found quantifiable and detectable amounts among the workers exposed to budesonide performing three out of four examined work tasks (Table 3). Four workers clearly had quantifiable amounts of the substance on their skin, especially on the tips of their forefingers, palms of their hands and in one case on the wrists. Interestingly, there were also indications of differences in this respect between the right and left sides of at least three operators (B, G and H), who were all right-handed.

It should be noted that all workers were instructed to use personal protective equipment (PPE), but this procedure was not followed strictly and/or it was unclear where and when use of such equipment was mandatory. In the cases where the highest exposures were found, several factors affecting exposure levels were observed. Operators B and E performed parts of their work tasks without wearing gloves and touched contaminated surfaces. Operator D lifted a bag containing pure budesonide into a glove box and the bag could have been contaminated. Operator A had to lie on the floor beneath the equipment to dismount the spray guns, while doing so dust containing budesonide could have occasionally slipped in through gaps of the protective clothes. Visual observations during the exposure assessment revealed that gaps may arise between the glove and sleeve of the protective suits used by operators A–H. Operators I to L used an appropriate full protection suit all the time. The operators were told to keep their hands in the air before the tape stripping, but their hands may also have been contaminated/exposed to some degree while moving to the adjacent room before completely changing their clothes.

The dermal exposure among the workers is difficult to value since no comparable data are available. This is the first study in which the dermal exposure of budesonide has been assessed. We have shown that exposure and possibly uptake occur, since budesonide was found on consecutive tape strips in two cases in the field study. From the recovery study, it was shown that ~40% of the applied amount was recovered, but the vehicles are not completely comparable with the field study.
In the present study, samples were taken from a small group of potentially exposed people. Therefore, further studies will be performed. In this study, only hands and arms were assessed, but effects of exposure to budesonide can appear at other sites of the body, for example on the face, where it is also possible to use the tape-stripping method. Based on the results from this study, three consecutive tape strips might be sufficient to collect the budesonide exposure. However, the tape-stripping method is not judged to be invasive since each tape will take away $\sim 1\text{–}2$ cell layers ($5$ strips $\sim 6\text{–}10$ cell layers) of stratum corneum and this does not cause any observable effects on the skin site. The cells are replaced usually in one week.

To compare the results between individuals, the budesonide levels could be standardized to the amount of keratin (Chao and Nylander-French, 2004) found on the tape strip. Several published studies have shown that tape stripping can be used for diverse substances, such as diisocyanates, jet fuel, metals and acrylates (Cullander et al., 2000; Nylander-French, 2000; Mattorano et al., 2004; Fent et al., 2006). Therefore, the method has high potential for studying exposure during other work tasks at the investigated workplace, and it could also be used to assess dermal exposure to other active pharmaceutical ingredients. Its convenience makes it an attractive option in surveillance assessments.

**CONCLUSIONS**

The results of this study show that the tape-stripping method can be used to determine potential dermal exposure to budesonide. They also indicate that budesonide may be taken up by the skin of operators exposed to the substance in their workplace, despite instructions to use personal protection suits.

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