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Urinary excretion profile of prednisolone and prednisone after rectal administration: Significance in antidoping analysis

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Abstract

The rectal administration of glucocorticoids, as well as any injectable, and oral ones, is currently prohibited by the World Anti-Doping Agency when occurs "in competition." A reporting level of 100 ng/ml for prednisolone and 300 ng/ml for prednisone was established to discriminate the allowed and the prohibited administration. Here, the urinary excretion profiles of prednisone and prednisolone were evaluated in five volunteers in therapy with glucocorticoid-based rectal formulations containing prednisone or prednisolone caproate. The urinary levels of the excreted target compounds were determined by liquid chromatography–tandem mass spectrometry (LC-MS/MS) following the procedure validated and currently in use in our laboratory to detect and quantitate glucocorticoids in urine. Predictably, the excretion trend of the analytes of interest were generally comparable with those obtained after oral administration, even if the excretion profile showed a broad interindividual variability, with the absorption rate and the systemic bioavailability after rectal administration being strongly influenced by the type of formulations (suppository or rectal cream, in our case) as well as the physiological conditions of the absorption area. Results showed that the target compounds were detectable for at least 30 h after drug administration. After suppository administration, prednisolone levels reached the maximum after 3 h from drug administration and then dropped below the reporting level after 15–21 h; prednisone reached the maximum after 3 h from drug administration, and then dropped below the reporting level after 12–15 h. After cream administration, both prednisone and prednisolone levels remained in a concentration below the reporting level throughout the entire monitored period.

KEYWORDS
doping analysis, LC-MS/MS, prednisolone, prednisone, rectal route

Loredana Iannella and Fabio Comunità contributed equally.

1 | INTRODUCTION

The oral route is the most convenient drug delivery route in clinical practice, allowing the administration of different dosage forms and systems in a well-tolerated and noninvasive way. The rectal administration of drugs is not commonly prescribed in clinical practice, but it is useful to obtain systemic therapeutic effects in certain patients. For instance, the rectal administration of drugs, even if generally less commonly used, represents an effective alternative when the swallowing capabilities are compromised, mainly in pediatric and unconscious patients.[1,2]

In comparison to the upper gastrointestinal tract, the rectum exhibits a smaller surface area (about 200–400 cm^2 in adults), constant and stable environment not containing villi or microvilli, and low enzymatic activity. Compounds showing low oral bioavailability or causing gastric mucosa irritation may be reliably administered through the rectum, particularly if with high logP (partition coefficient) value and low molecular weight (mainly <500 g/mol).[3] Three are the main categories of the commercially available rectal formulations: Solid (suppositories, rectal capsules, or tablets), liquid (enemas or microenemas), and semi-solid (gels, foams, and creams). Besides these, new drug delivery strategies, mostly based on lipid and protein nanotechnologies, have been recently investigated, displaying promising pharmacokinetic profiles for future therapeutic applications.[4–6] The extent of the rectal absorption depends not only on anatomic and physiological factors but also on the site of drug delivery: If located in the lower part of the rectum, the drug is transported directly in the systemic circulation, by-passing the hepatic first-pass metabolism, and readily reaching high hematic levels.

Several rectal options are currently available, in local or systemic forms of administration, to treat pain, nausea and vomiting, fever, anal fissures, hemorrhoids, migraine, and bowel diseases and in preoperative medication to induce anesthesia and sedation status.[7–11] Among them, only few formulations containing glucocorticoids are easily traceable on the pharmaceutical market. Nonetheless, due to their potential systemic effects, the rectal administration of glucocorticoids-based products is prohibited in sport by the World Anti-Doping Agency (WADA) and included in the section S9 of the List of Prohibited Substances and Methods.[12] For indeed, glucocorticoids exert a beneficial impact on muscle responsiveness and recovery during sport performances, by increasing the availability of metabolic substrates and reducing the feeling of fatigue and the pain of efforts, especially if systemically and short-term administered.[13–15] The WADA have therefore restricted their use in sport, also considering the several adverse reactions they can induce.[16,17] According to the current limitations, glucocorticoids are banned if administered by any injectable, oral and rectal routes during competition. A reporting level of 30 ng/ml was initially established by the WADA to discriminate the allowed (topical) and the prohibited (systemic) administration.[18] Data reported by previous investigations carried out after administration by different routes of prednisone, prednisolone,[19–27] betamethasone,[28] budesonide,[29,30] or triamcinolone acetonide[31,32] have showed the need to adequately modify the WADA guidelines. New reporting levels were, therefore, established by the WADA for several glucocorticoids, including prednisone (i.e., 300 ng/ml) and prednisolone (i.e., 100 ng/ml).[33]

The excretion profile of prednisone and prednisolone after local (eye drops, nasal spray, ointment) and oral (tablet, vial) intake was extensively investigated in antidoping field.[20–23] In this work, the excretion profile of two rectal formulations containing prednisolone or prednisone (Scheriproct® and Rectodelt®) has been evaluated in five volunteers after acute intake, by the liquid chromatography–tandem mass spectrometry (LC-MS/MS) specific method already developed and validated in our laboratory and described elsewhere.[23,34] We herein verified whether the urinary levels of prednisone and prednisolone are compatible with systemic or local absorption and assessed the suitability of the WADA criteria: Evidence for a predominantly local action of similar rectal products have been already demonstrated in the past.[35]

2 | EXPERIMENTAL

2.1 | Chemicals and reagents

Certified standards of prednisolone, prednisone, 20β-dihydro-prednisolone, and prednisolone-2,4,6,6,9,11,12,12-d8 (internal standard), used for the quality control, were purchased from TRC (North York, Canada). β-Glucuronidase from *Escherichia coli* was supplied by Roche Diagnostic (Mannheim, Germany). Solvents (tert-butylmethyl ether, and methanol) and reagents (potassium carbonate, sodium phosphate, sodium hydrogen phosphate, sodium hydrogen carbonate) of analytical or high-performance liquid chromatography (HPLC) grade were from Sigma-Aldrich. Water was obtained from a Milli-Q water purification system (Millipore S.p.A., Milano, Italy).

TABLE 1 Retention times and mass spectrometric parameters of prednisone, prednisolone, 20β-dihydro-prednisolone, and internal standards (underscored transitions are intended for the quantitative results)

Compound	Retention time (min)	Precursor ion Q1 (*m/z*)	Product ions Q3 (*m/z*)	Collision energy (eV)
Prednisone	9.8	359	121; 147; 171; 313; 323; 341	35; 35; 35; 30; 30; 25
Prednisolone	9.7	361	121; 147; 171; 307; 325; 343	35; 35; 35; 30; 30; 25
20β-dihydro-prednisolone	9.2	363	121; 147; 171; 309; 327; 345	35; 35; 35; 30; 30; 25
ISTD (prednisolone d8)	9.8	369	171	35

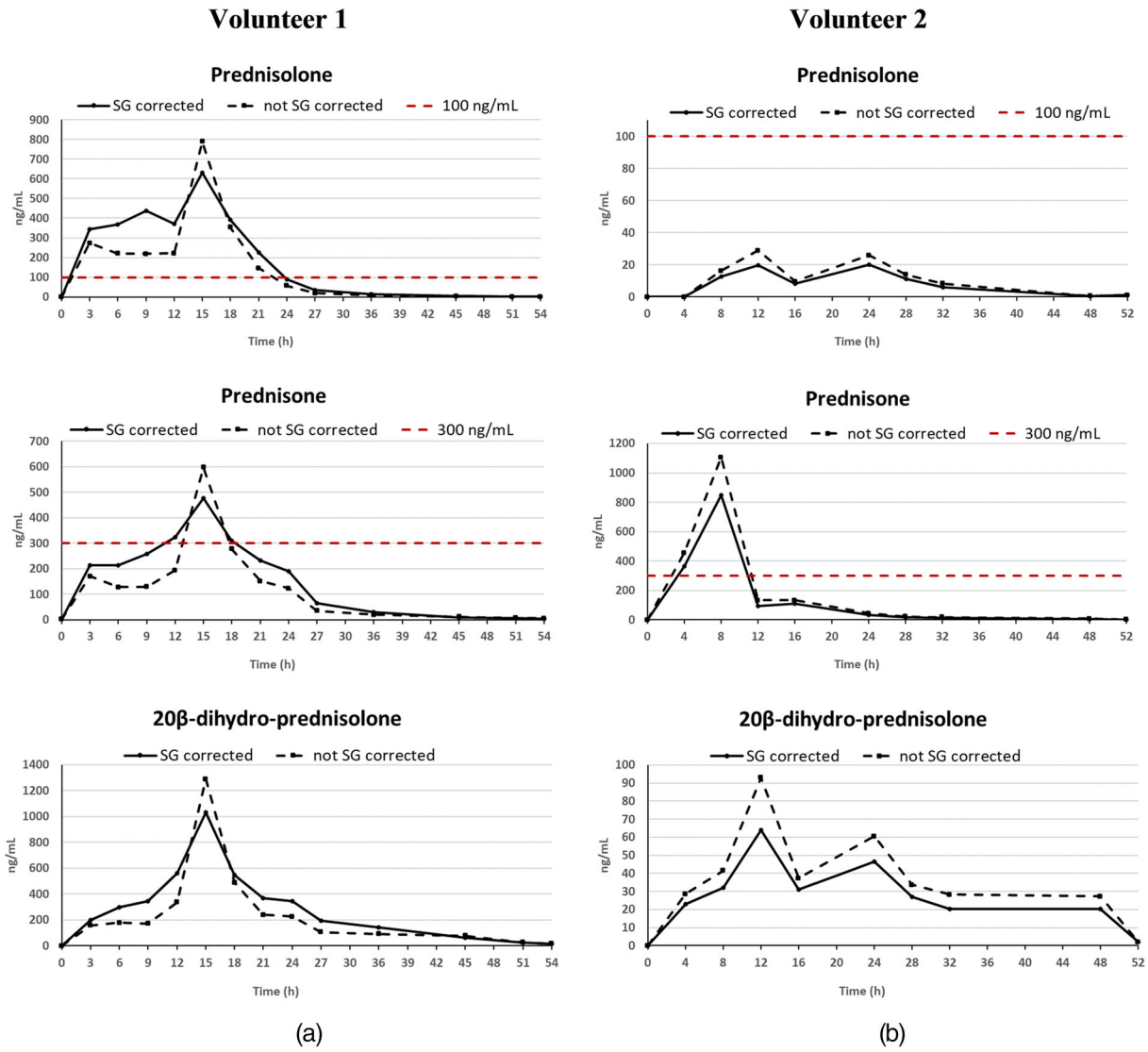


FIGURE 1 Excretion profile of prednisolone, prednisone, and 20β-dihydro-prednisolone after administration of one single suppository of Rectodelt® for Volunteer 1 (a), Volunteer 2 (b), Volunteer 3 (c), Volunteer 4 (d), and Volunteer 5 (e)

2.2 | LC-MS/MS analysis

LC-MS/MS assays were performed by a method already described,[23,34] with few modifications. Briefly, the instrumental setup was constituted by an Agilent 1200 Rapid Resolution liquid chromatographer with binary HPLC gradient pump system and automatic injector (Agilent technologies S.p.A, Cernusco sul Naviglio, MI, Italy) coupled to an API 4000 triple quadrupole mass spectrometer (ABSciex), equipped with electrospray source operating in positive ionization mode and with acquisition in multiple reaction monitoring (MRM) mode. Chromatographic separation via reversed-phase liquid chromatography was performed using an octadecyl reverse-phase column from Supelco (particle size of 5 μm, length of 15 cm, and internal diameter of 2.1 mm), setting the column temperature at 30°C, and selecting water (eluent A) and acetonitrile (eluent B) as mobile phases, with 0.1% of formic acid as mobile phase modifier. Flow rate was set at 250 μl/min. Gradient program started at 10% B and increased to 60% B in 7 min and then, after 6 min, to 100% B in 1 min. The column was flushed for 1 min at 100% B and finally re-equilibrated at 10% B for 4 min. Mass spectrometric conditions were the following: A curtain gas pressure of 15 psi, a source temperature of 550°C, an ion source gas 1 pressure of 25 psi, an ion source gas 2 pressure of 25 psi, a declustering potential of 80 V, an entrance potential of 10 V, and a needle voltage of 5500 V. Collision-induced dissociation (CID) experiments were performed with nitrogen as collision gas at 5.8 mPa, obtained from a dedicated nitrogen generator system Parker-Balston model 75-A74, gas purity 99.5% (CPS Analitica Milano, Italy). All aspects of instrument control, method setup parameters, sample injection, and sequence operation were controlled by Analyst software (version 1.6.2).

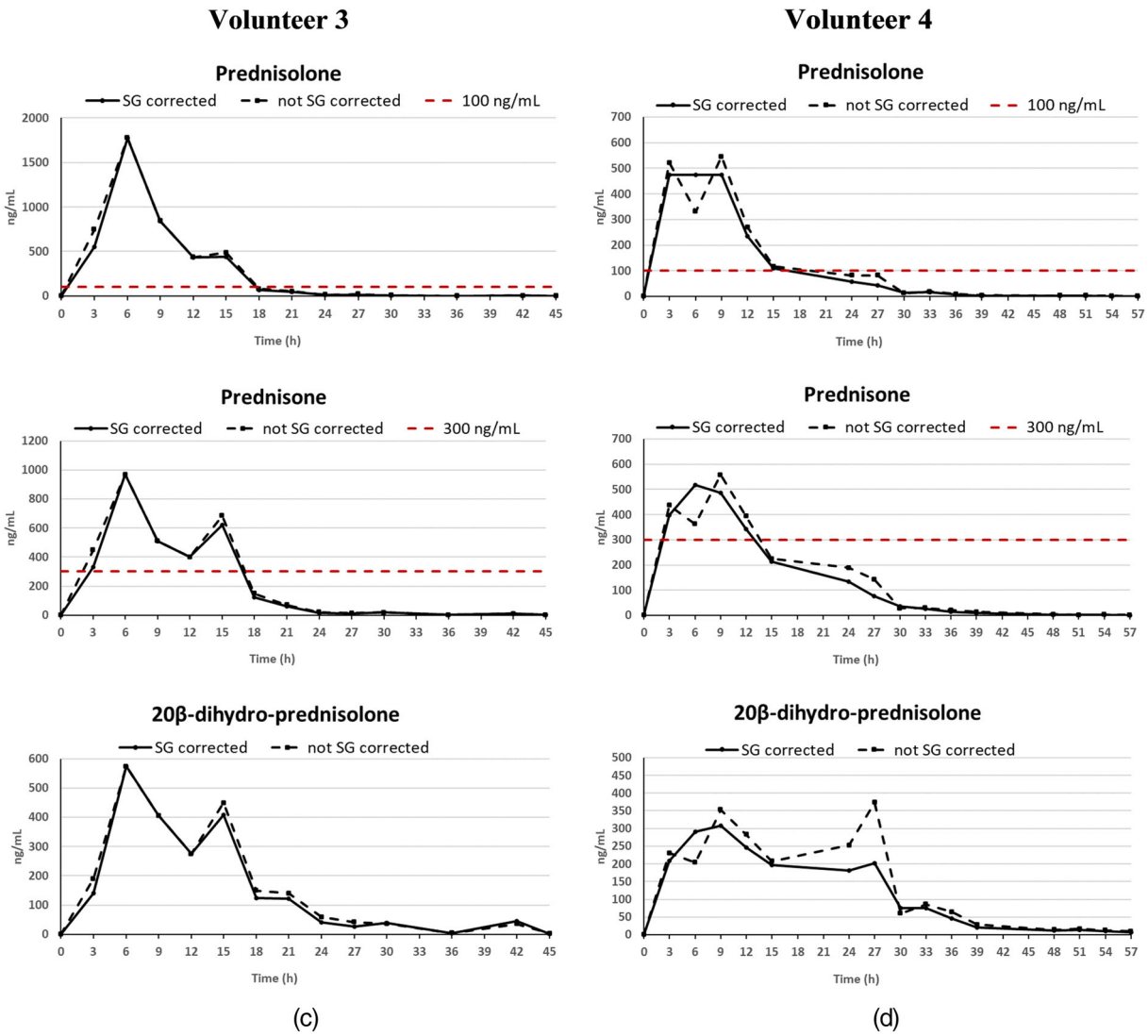


FIGURE 1 (Continued)

The specific target analytes were identified and quantitated by checking the retention time and the characteristic ion transitions. The selected diagnostic ion transitions for each compound are listed in Table 1. Urinary concentration of each target compound was calculated by the peak area ratios of the detected signals and the deuterated internal standard, using the most suitable calibration sample.

2.3 | Sample pretreatment

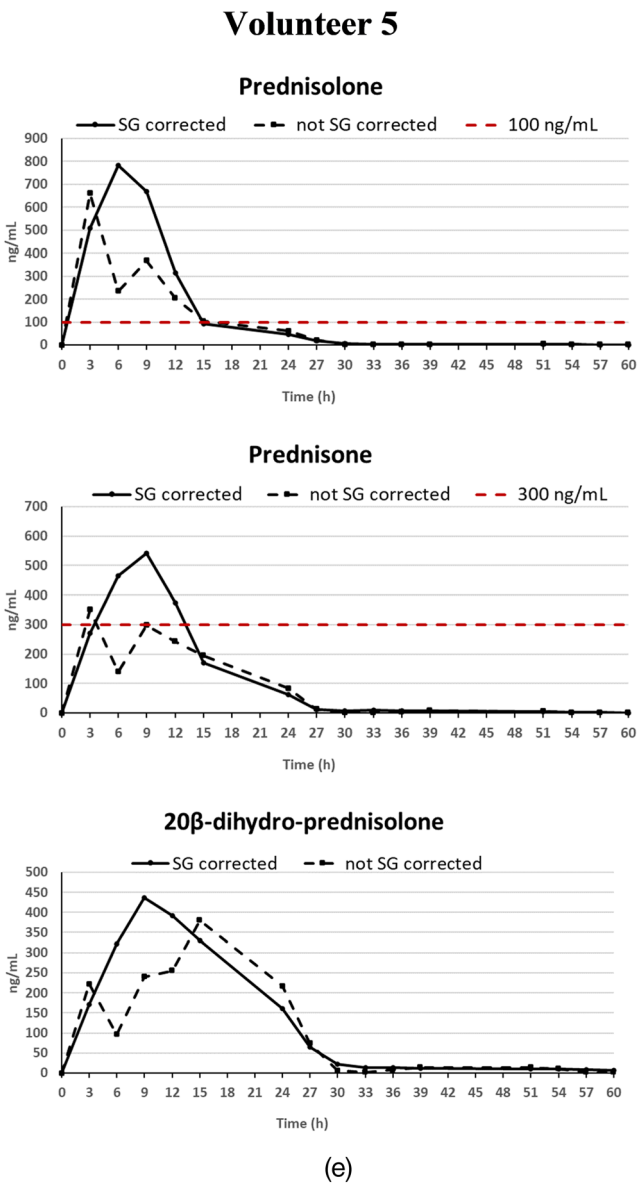
Three milliliters of urine were added with 50 µl of internal standard (final concentration 30 ng/ml) solution and hydrolyzed with β-glucuronidase from *E. coli* (1 h, 50°C, pH 7.4). One milliliter of carbonate buffer 1 M (pH 9) and 10 ml of tert-butyl methyl ether were then added to accomplish the liquid/liquid extraction (by mechanical shaking for 5 min). After centrifugation at 3000 rpm for 3 min, the organic layer was transferred in a conical tube and evaporated under a gentle stream of nitrogen at room temperature until dry and then reconstituted with 50 µl of mobile phase (initial composition). Aliquots of 10 µl were, finally, injected into the LC-MS/MS system to determine the total (free and glucurono-conjugated) concentrations of the three analytes under investigation through the method already validated in accordance with the ISO17025 and WADA guidelines.[36–38] All reported concentrations were then adjusted for a value of the specific gravity of 1.020, following the equation below:

$$\mathrm{Conc_{corr}} = \mathrm{Conc_{measured}} * (1.020 - 1)/(\mathrm{SG} + 0.002 - 1)$$

where SG is the measured specific gravity of the sample.

2.4 | Standard solution and calibration samples

Stock solutions of glucocorticoids and internal standards at 1 mg/ml were prepared by appropriate dissolution of reference powders into



(e)

FIGURE 1 (Continued)

methanol and stored at −20°C. An internal standard solution containing 1.8 μg/ml of prednisolone d_8 in methanol was also prepared. A proper amount of stock solutions was added to negative urine of healthy volunteers to obtain calibration samples at 10, 30, 60, 100, 500, and 1000 ng/ml. Quality control samples were prepared by spiking the urine samples of the five volunteers collected before drug administration with the target compounds at concentration of 100 and 300 ng/ml.

2.5 | Administration study design

The urinary concentrations of glucocorticoids and their major metabolite were determined in five subjects (two males [V1 and V2] and three females [V3, V4, and V5] aged between 29 and 50 years) in therapy with the prednisolone or prednisone formulations herein investigated (Scheriproct® and Rectodelt®). The observational study was approved by the Ethics Committee Lazio 1 (ref. 2464/CE Lazio 1 and 201/CE Lazio 1) and the volunteers signed an informed and anonymous written consent to allow the use of their urine samples for research purposes. They all collected a first urine sample before the rectal administration prednisone (Rectodelt® is produced by Mibe Pharma Italia S.r.l in a pharmaceutical form of suppositories) or prednisolone (Scheriproct® is an ointment manufactured by Bayer) and then one sample at regular intervals of 3 or 4 h for the next 3 days. Urinary samples were collected in sterile screw cap urine container and stored at −20°C until assayed. As illustrated in their respective package inserts, one Rectodelt® suppository application contains 100 mg of prednisone, while a single therapeutic dose of Scheriproct® is estimated to be

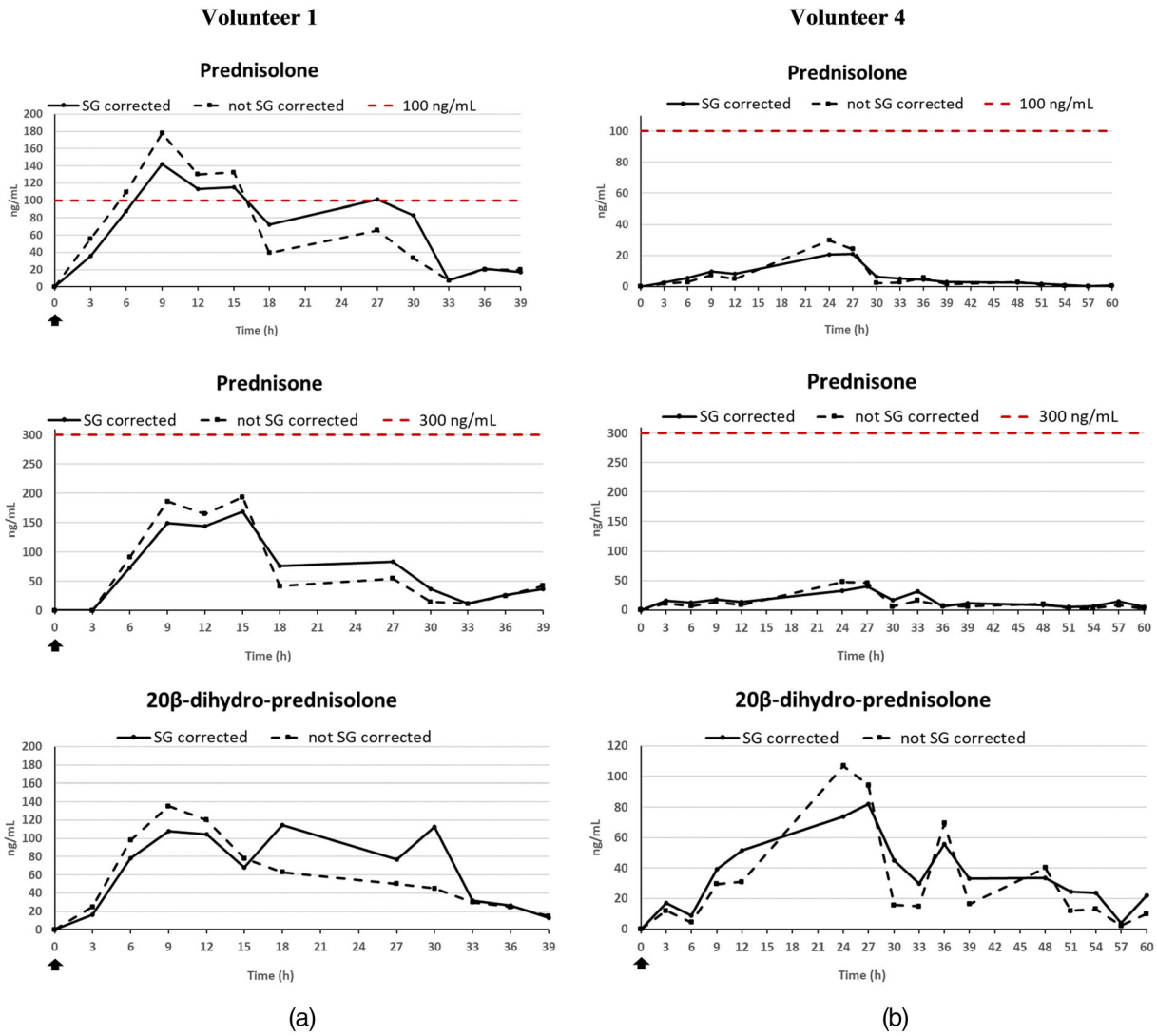
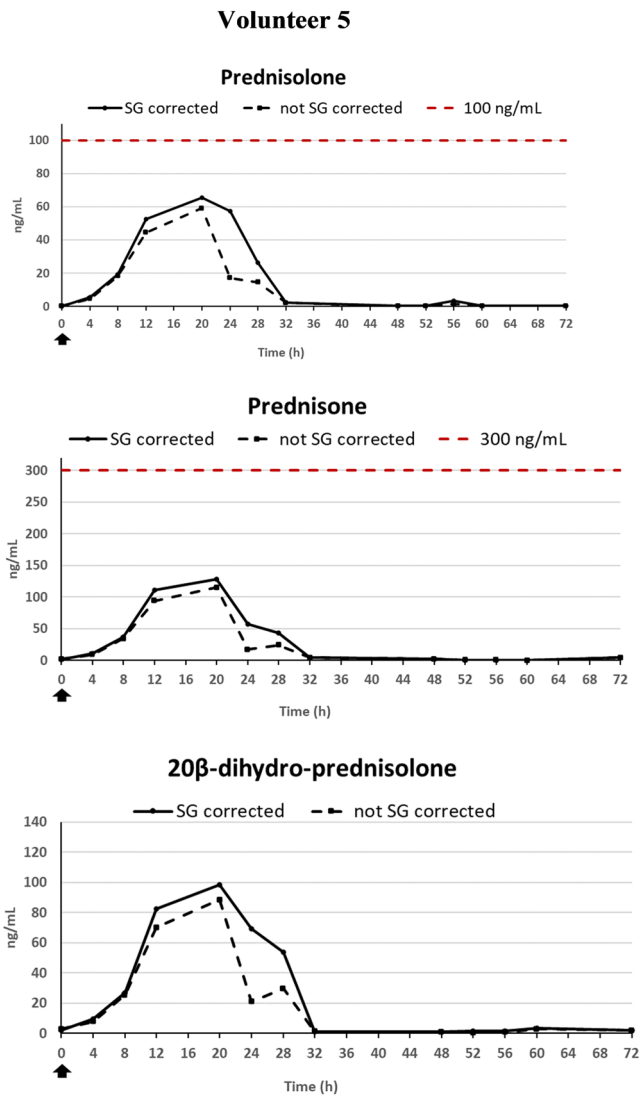


FIGURE 2 Excretion profile of prednisolone, prednisone, and 20β-dihydro-prednisolone after administration of a therapeutic dose of the cream formulation Scheriproct® for Volunteer 1 (a), Volunteer 4 (b), and Volunteer 5 (c)

in the range 5–15 mg, because the ointment contains 1.9 mg of prednisolone caproate (corresponding to 1.5 mg of prednisolone) per 1 g of product. The urinary excretion profile of prednisolone, prednisone, and 20β-dihydro-prednisolone (the main target metabolite) was evaluated for 3 days following the rectal administration of glucocorticoid with a washout period of 30 days between treatments. The excretion profile of prednisone, prednisolone, and their main diagnostic metabolite 20β-dihydro-prednisolone was followed in accordance to the validated analytical procedure currently adopted by the Anti-doping Laboratory of Rome to detect glucocorticoids in urine.[23]

3 | RESULTS AND DISCUSSION

Due to its systemic effects, the rectal use of glucocorticoids, similarly to any injectable and oral administration, is prohibited by the WADA "in competition" only. The excretion profile of prednisone and prednisolone after local (eye drops, nasal spray, ointment) and oral (tablet, vial) intake was extensively investigated in the antidoping field.[19–27] In this work, we explored the rectal administration of both prednisolone and prednisone, verifying whether it should be considered as a systemic route, regardless of the type of drug formulation used. As already stated, analyses were carried out by the analytical procedure currently adopted in our laboratory to detect (the LOD for prednisone and prednisolone is 1 ng/ml) and confirm (the LOI for prednisone and prednisolone is 5 ng/ml) the presence of glucocorticoids in urine for doping control tests. The values of the urinary concentration of each target compound were calculated by the peak areas of the detected signals relative to the internal standard, using the calibration samples described in the experimental section. The measured concentrations were then reported versus the collection time to evaluate the excretion profile. All data were normalized for the specific gravity.



(c)

FIGURE 2 (Continued)

3.1 | Excretion studies after administration of prednisone suppository

Five volunteers in therapy with Rectodelt® prednisone suppository collected their urine samples each 3 or 4 h for at least 48 h. The urinary excretion profile of prednisone, prednisolone (produced by the hepatic 11β-hydroxysteroid dehydrogenase from prednisone through a reversible reaction), and 20β-dihydro-prednisolone (one of the most abundant metabolite of both prednisone and prednisolone) were followed. For the volunteers V1, V3, V4, and V5, the urinary excretion trend of prednisone and prednisolone was similar to those previously obtained after the administration of prednisolone/prednisone oral formulations[23] (see Figure 1a–e). Indeed, the maximum urinary concentration of prednisolone (from 550 to 1800 ng/ml), prednisone (from 550 to 1000 ng/ml), and their metabolite 20β-dihydro-prednisolone (from 300 to 1300 ng/ml) were reached within 9 (V3, V4, V5) or 15 h (V1) after dosing and were detectable for more than 24 h (see again Figure 1a–e). Prednisolone reached levels higher than the reporting level (100 ng/ml) after 3 h from drug administration and remained in concentrations higher than the reporting level for 18 h in V1 (from 3 to 21 h from drug administration, Figure 1a) and for 12 h in V3, V4, and V5 (from 3 to 15 h from drug administration, Figure 1c–e). In V2, the enzymatic conversion of prednisone to prednisolone did not significantly occur, as well as the metabolic production of 20β-dihydro-prednisolone. In this case, prednisolone levels remained always lower than the reporting level (see Figure 1b). Regarding prednisone, it reached concentrations higher than the reporting level (300 ng/ml) after 15 h in V1 and after 3 h in V2, V3, V4, and V5 (see Figure 1a–e). Its levels remained higher than the reporting level for 2 h in V1 (see Figure 1a), for 4 h in V2 (from 4 to 8 h from drug administration, Figure 1b), for 12 h in V3 (from 3 to 15 h from drug administration, Figure 1c) and for 9 h (from 3 to12 h from drug administration,

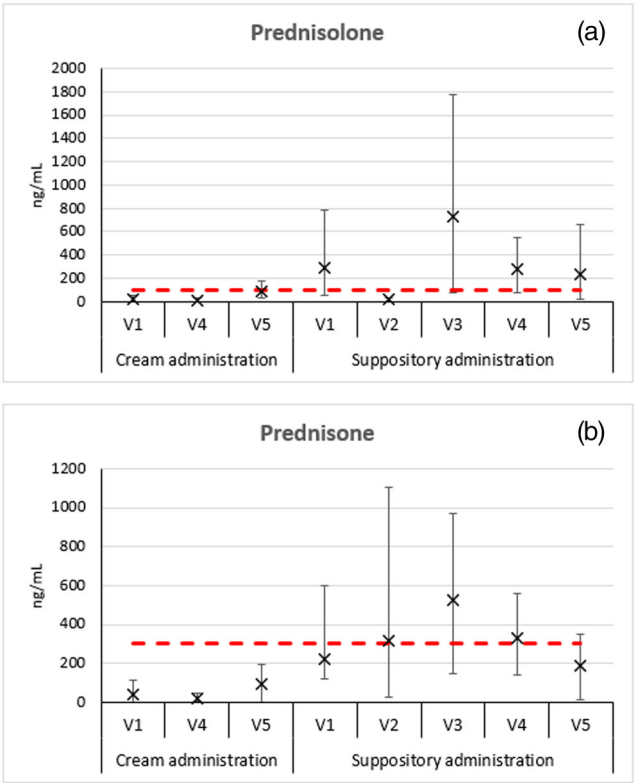


FIGURE 3 Prednisolone (a) and prednisone (b) data distribution comparison in the volunteers evaluated after either suppository or cream rectal administration

Figure 1d–e) in V4 and V5. The correction of the concentration for the specific gravity did not change the results with the exception of V5 where prednisone levels after correction never reached values higher than the reporting level (see Figure 1e).

3.2 | Excretion studies after administration of prednisolone rectal ointment

Additional observational studies were performed on urine specimens collected from V1, V4, and V5 undergoing pharmacological therapy with Scheriproct®. The excretion profile of prednisolone, prednisone, and 20β-dihydro-prednisolone after the administration of a single dose (estimated to be in the range 5–15 mg of prednisolone) through the proper applicator was reported in Figure 2a–c. Data collected showed that the excretion profile of the target compounds presents a broad interindividual variability. Indeed, in volunteer V1, the maximum concentration of the target compounds (180 ng/ml for prednisolone, 170 ng/ml for prednisone, and 110 ng/ml for 20β-dihydro-prednisolone) was measured after 9–15 h from drug intake and remained detectable for more than 24 h. The concentrations of prednisolone reached levels higher than the reporting level after 6 h and remained above this level for 9 h (from 6 to 15 h from drug administration). On the contrary, prednisone did not exceed the reporting level for the entire monitored period of the study (see Figure 2a). In V4, prednisone (40 ng/ml) and prednisolone (20 ng/ml) reached the maximum after 27 h from drug administration and was detectable for the entire monitored period; 20β-dihydro-prednisolone, similar to prednisone, reached the maximum (80 ng/ml) after 27 h from drug administration and was detectable for the entire monitored period (see Figure 2b). In V5, urinary concentration of compounds of interest peaked the maximum excretion level (66 ng/ml for prednisolone, 128 ng/ml for prednisone, and 98 ng/ml for 20β-dihydro-prednisolone) after 20 h from the drug application (see Figure 2c) and remained detectable for the entire monitored period. After correction for the specific gravity, the results reported above did not change for all the volunteers considered.

4 | CONCLUSIONS

The purpose of this work was to investigate the excretion profile of prednisone, prednisolone, and 20β-dihydro-prednisolone following rectal administration of pharmaceutical preparations containing prednisone or prednisolone. The urinary excretion profile herein presented could be helpful to verify the suitability of the criteria currently required by WADA in reporting findings for glucocorticoids, especially when administered via the less common and poorly studied rectal route. We found that all analytes were detectable 3 h after the drug intake and peaked within 9 h from the suppository insertion or 30 h after the cream application. Without evaluating the pharmacological properties of rectally administered treatments and the extent of local or systemic action, results suggest that a consistent fraction of the administered dose is absorbed in the bloodstream, exerting systemic effects (see Figure 3 for the comparison of the results obtained after rectal administration). The systemic absorption after suppository administration is proved by the high urinary concentrations of the analytes of interest, mainly comparable with those described in other studies focused on the oral route. The rectal delivery of drugs is less efficient in term of absorption compared with the oral route, and higher doses (up to 100 mg in Rectodelt®, while the oral commercially available formulations typically contain 1–25 mg of active substance) are needed to reach similar therapeutically effective levels. Nevertheless, prednisolone, prednisone, and 20β-dihydro-prednisolone urinary levels after ointment application were significantly lower and similar to those reported in previous articles after local administration. Even if there is not a definite correlation, we should not exclude that the higher urinary levels obtained after suppository administration are strictly related to higher systemic bioavailability. In future, we aim to increase the number of volunteers and formulations to increase knowledge on the systemic effect of the rectal administration route and to assess the suitability of the reporting levels fixed by the WADA for prednisone and prednisolone.

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DATA AVAILABILITY STATEMENT

All data not specifically included in the final version of the manuscript are available on request.

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