View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

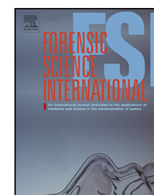
provided by Elsevier - Publisher Connector

Forensic Science International 244 (2014) 1–6

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint



Positive doping results caused by the single-dose local injection of triamcinolone acetonide

Chih-Wei Chang [a,1], Tai-Yuan Huang [b,1], Yi-Chun Tseng [c], Guo-Ping Chang-Chien [d], Su-Fan Lin [d], Mei-Chich Hsu [a,*]

[a] Department of Sports Medicine, Kaohsiung Medical University, 100, Shih-Chuan 1st Rd., Sanmin, Kaohsiung 807, Taiwan, ROC
[b] Department of Orphopedic Surgery, Yuan's General Hospital, 162, Cheng-Kung 1st Rd., Lingya, Kaohsiung 802, Taiwan, ROC
[c] Department of Sports Science, National Taiwan Sport University, 250, Wen-Hua 1st Rd., Kweishan, Taoyuan County 333, Taiwan, ROC
[d] Super Micro Mass Research & Technology Center, Cheng Shiu University, 840, Cheng-Cing Rd., Niaosong Township, Kaohsiung 833, Taiwan, ROC

ARTICLE INFO

Article history:
Received 14 March 2014
Received in revised form 18 July 2014
Accepted 23 July 2014
Available online 30 July 2014

Keywords:
Triamcinolone
Glucocorticoid
LC–MS/MS

ABSTRACT

Triamcinolone acetonide (TA) is classified as an S9 glucocorticoid in the 2014 Prohibited List published by the World Anti-Doping Agency, which caused it to be prohibited in-competition when administered orally, intravenously, intramuscularly or rectally. The Minimum Required Performance Level (MRPL) for the detection and identification of glucocorticoids is 30 ng/mL. Other common local injection routes, such as intraarticular, intratendinous, or intrabursal injection, are not prohibited. The purpose of this study was to analyze the TA and triamcinolone (T) concentrations in urine after a single injection of TA in patients to determine if it would produce a positive result. This study was performed on 40 patients with sports injuries or joint pains. TA was administered locally (doses varied from 12 to 80 mg). Samples were extracted using a solid-phase extraction column, followed by hydrolysis and liquid extraction using diethyl ether. The elution solvents were collected and dried. The dried residue was reconstituted and assayed by performing liquid chromatography–tandem mass spectrometry (LC–MS/MS) in positive ionization mode using electrospray ionization and multiple-reaction monitoring as the acquisition mode. The results demonstrated that the concentrations of both TA and T in urine exceeded the MRPL (30 ng/mL) after a single local injection. We obtained positive results for TA in 25 patients, and a positive result for T in one patient. Furthermore, the metabolic situation of TA, a long-acting glucocorticoid, was not an exact linear model. The highest concentrations of TA and T appeared 1–4 h after injection. This information could be useful for limiting the misuse of TA by athletes. We suggest that athletes be aware when using TA injections during a competition period and obtain approval for therapeutic use exemption prior to using TA.

© 2014 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-SA license (http://creativecommons.org/licenses/by-nc-sa/3.0/).

1. Introduction

Triamcinolone acetonide (TA) and triamcinolone (T) are synthetic glucocorticoids that are widely used by athletes to treat articular sprains, pain, and injuries [1,2]. They are both classified as S9 glucocorticoids in the 2014 Prohibited List published by the World Anti-Doping Agency (WADA), which prohibited the use of these glucocorticoids in-competition when administered orally, intravenously, intramuscularly, or rectally. The Minimum Required Performance Level (MRPL) for the detection and identification of glucocorticoids is 30 ng/mL [3].

Glucocorticoids inhibit systemic inflammation and reduce neuropathic pain [4]. Numerous methods of administrating TA were prescribed in Taiwan, including injection, ointment, drip, spray, and tablets. For years athletes have frequently used TA to treat sport-induced injuries, primarily by using local injection routes. The dosage of a local TA injection depends on the site and size of the articulation, inflammation, and the quantity of synovial fluid. The dosage of knee and shoulder joint injections ranges from 40 to 80 mg; small-joint, intratendinous, and intrabursal injections range from 10 to 40 mg; and soft tissue injections range from 10 to 20 mg.

Previous studies [5–11] have reported the pharmacokinetics of TA after oral, intravenous, intramuscular, and inhalation administration, and these studies have primarily focused on investigating the TA concentration in blood instead of urine. One study [12] indicated that the average elimination half-lives of intravenously

* Corresponding author. Tel.: +886 7 3121101x2793; fax: +886 73138359.
E-mail address: meichich@kmu.edu.tw (M.-C. Hsu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.forsciint.2014.07.024
0379-0738/© 2014 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-SA license (http://creativecommons.org/licenses/by-nc-sa/3.0/).

and intraarticularly administrated TA in horses were 6.1 and 23.8 h, respectively, and the maximal plasma concentration of intraarticular and intramuscular was attained at 10 and 13 h, respectively. Additionally, previous study stated that the main metabolite of TA in urine was 6 beta-hydroxy triamcinolone acetonide [9], but it is not a doping substance listed in WADA. However, after administering TA intravenously, T is one of the metabolites of TA [6], and once it exceeded the MRPL would offense the rule still [3].

According to the WADA, adverse results should be reported when the concentrations of TA or T in urine are beyond the MRPL (30 ng/mL). Administering local glucocorticosteroid injections (such as intraarticular, intratendinous, or intrabursal injection) in the treatment of noninfectious local inflammatory conditions is commonly accepted in medical practice and provides symptom relief and often a rapid recovery and subsequent return to sporting activity [13]. However, whether these routes of administration produce positive analytical results is still unclear. The purpose of this study was to determine the possibility of obtaining positive results by using various injection routes, and investigate the metabolism situation between TA and T in urine after a single-dose local injection of TA.

2. Materials and methods

2.1. Materials

Triamcinolone, triamcinolone acetonide standards, β-glucuronidase, and HPLC-grade formic acid were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Betamethasone-D5 standard was obtained from C/D/N Isotopes Inc. (Quebec, Canada). Methanol was purchased from Echo Chemical Co., Ltd. (Miaoli, Taiwan). Acetic acid was purchased from Mallinckrodt Pharmaceuticals (Dublin, Ireland). Reagent-grade potassium phosphate monobasic was obtained from Showa PK Co., Ltd. (Arakawa-Ku, Tokyo, Japan). Diethyl ether was purchased from J.T. Baker (Center Valley, PA, USA). Doubly deionized water was obtained from a Millipore Direct-Q5 system (Bed ford, MA, USA) and used throughout the study.

2.2. Instruments

The LC–MS/MS equipment consisted of an Agilent 1100 Series (Agilent Technologies Italia SpA, Cernusco sul Naviglio, Italy) high-performance liquid chromatograph (HPLC) with a vacuum degasser, binary pump, and an autosampler connected to an Applied Biosystems API 3000 triple-quadrupole mass spectrometer equipped with a TurboIonSpray ionization (electrospray ion [ESI]) source (Applied Biosystems/MDS SCIEX, Canada). Data acquisition was performed using Analyst 1.4.2 software (Applied Biosystems/MDS SCIEX).

2.3. Liquid chromatography–tandem mass spectrometry

Chromatographic separation was performed at room temperature using a Supelco Discovery® HS-C_{18} HPLC column (50 mm × 2.1 mm, 3 μm particle size; PA, USA) and a Supelco Discovery® HS-C_{18} guard column (2 cm × 2.1 mm I.D., 3 μm particle size; PA, USA). A two-solvent linear gradient system was used. Solvents A (deionized distilled water with 0.02% formic acid) and B (methanol) were filtered through a 0.45-μm membrane and degassed before use. A linear gradient was run at 30% solvent B, increased to 70% B from 0 to 4 min (a flow rate of 0.3 mL/min), increased to 90% B from 4 to 10 min (a flow rate of 0.15 mL/min), then decreased to 30% B from 10 to 10.5 min (a flow rate of 0.3 mL/min), and finally reequilibrated at 30% B for 5.5 min (a flow rate of 0.3 mL/min).

The conditions were maintained at an optimal state for the analysis of triamcinolone acetonide and triamcinolone. The turbo gas temperature was 450 °C, and the auxiliary gas flow was 8.0 L/min. Nebulizing gas, curtain gas, and collision gas flows were regulated using the instrument settings of 13, 7, and 11 L/min, respectively. The IonSpray voltage was set at 5500 V. The detection and quantification of analytes was performed in multiple-reaction monitoring (MRM) positive acquisition mode using ion precursor → product ion combinations of m/z 435 → 415/357 for TA and 395 → 357/321. The collision energy used for performing MRM transitions of TA and T was set at 15 and 17 eV, respectively.

2.4. Sample preparation

The sample preparation method was modified from that used by Luca Amendola et al. [14]. Urine samples were collected from 40 Taiwanese patients. A 4 mL aliquot of urine, 1 mL of KH2PO4 buffer (0.1 M, pH 6.0), and 50 μL of internal standard (IS) betamethasone-D5 (5 μg/mL) were mixed. The solid phase extraction columns were washed with methanol (0.4 mL). The samples were then poured into the columns. The samples were washed with doubly deionized water (2.0 mL), acetic acid (2.0 mL, 0.1 M), and then methanol (2.0 mL). The residues were dried under nitrogen at 40 °C and reconstituted in 1 mL of KH2PO4 buffer (0.1 M, pH 6.0). The samples were subsequently incubated with 25 μL of β-glucuronidase for 1 h at 50 °C. After hydrolysis, 250 μL of 7% K_2CO_3 solution was added to the samples. The samples were then extracted with 5 mL of diethyl ether for 5 min. The organic phase was collected and dried under nitrogen at 60 °C. The residues were reconstituted in 350 μL of Solvents A and B (85:15). Finally, the samples were filtered through a 0.45-μm membrane. Aliquots of the samples (20 μL) were injected into the LC–MS/MS system.

2.5. Method validation

Specificity was assessed by analyzing urine samples from six sources. The specimens were prepared using the same sample preparation and analytical procedures to identify interfering peaks at the retention times of analytes or the IS.

Linearity was assessed using eight standard urine solutions with concentrations ranging from 1 to 200 ng/mL. The urine calibration curves were prepared and assayed on 6 separate days. The peak ratios of TA and T were applied to calculate the correlation coefficient, intercept, and slope by using weighted linear regression. A correlation coefficient of ≥0.99 was considered acceptable. The limit of detection (LOD) was defined as the lowest level of concentration at which a compound could be identified in all the urine samples tested using the lowest transition with a signal-to-noise ratio of ≥3. The limit of quantification (LOQ) was defined as the lowest concentration on the calibration curve for which an acceptable accuracy of ±20% and a precision under 20% were exhibited.

The acquisition segments and the relative retention times (RRTs) of the analytes were assessed. Repeatability was estimated using the intraday and interday coefficient of variation in the RRTs, ion ratio interday precision, and the ion ratios of the characteristic transitions at the LOD concentration of the analytes. The intraday precision was achieved by performing analytes (n = 6) of quality control (QC) methanol samples on the same day.

A concentration of 30 ng/mL for each analyte was used for QC and prepared in six replicates. The evaluation of interday precision was conducted by performing analytes over 6 days during the study period, and using QC samples that were newly prepared each day.

Recovery was assessed using the QC samples ($n = 3$). Two sets of urine samples were fortified with analytes before or after sample preparation. The recovery was calculated by comparing the mean peak area of the samples fortified prior to and after sample preparation.

2.6. Drug administration and urine collection

Forty patients with sports injuries or joint pains from Yuan's General Hospital (Kaohsiung, Taiwan), with a mean age (±standard deviation [SD]) of 58.8 (±11.8) years, weight of 63.8 (±8.9) kg, and height of 161.8 (±8.7) cm, participated in the experiment. The study was conducted on 29 participates by administering intratendinous injections, three participates by administering intraarticular injections, three participates by administering intrabursal injections, four participates by administering lumbar epidural injections, and one participate by administering an intramuscular injection.

After administering a single dose of TA, the urine samples of the volunteers were collected. Thirty-eight participants agreed to collect 2–5 times of urine randomly. The other two participants who received intratendinous injections agreed to participate in a 48-h timed urine collection. Thus, the study was divided into two groups: random urine collection ($n = 38$) and timed urine collection ($n = 2$). In the random urine collection, the quantity of water intake, number of collected samples, and the time of the collection were not limited. In the 48-h timed urine collection, blank urine was collected before the injection. Urine specimens were collected in the intervals of 0, 0–1, 1–2, 2–3, 3–4, 4–6, 6–8, 8–10, 10–12, 12–16, 16–20, 20–24, 24–28, 28–32, 32–36, 36–40, 40–44, and 44–48 h after TA administration. To ensure sufficient urine flow during the sampling period, 200 mL of water was ingested after each collection period. The pH, volume, and specific gravity of each urine specimen at room temperature were recorded, and the samples were stored at −20 °C before preparation for chromatographic analysis. The study procedures were approved by the Human Research Ethics Committee of Yuan's General Hospital (Kaohsiung, Taiwan).

3. Results and discussion

3.1. Method validation

The chromatograms of the urine spiked with TA, T, and blank urine are illustrated in Fig. 1. The retention times of TA, T, and betamethasone-D5 were 9.46, 7.23, and 10.96 min, respectively; this is well within the acceptable range of ±2%. No interfering signals from endogenous substances were observed during the retention times of the analytes. For the ion ratio, the acceptable range is ±20%, 29.68–44.52 and 27.84–41.76 for TA and T, respectively.

The correlation coefficients (r^2) of the calibration curves for TA and T were 0.9971 and 0.9944, respectively. The mean calibration curves for TA and T were plotted using the following equation:

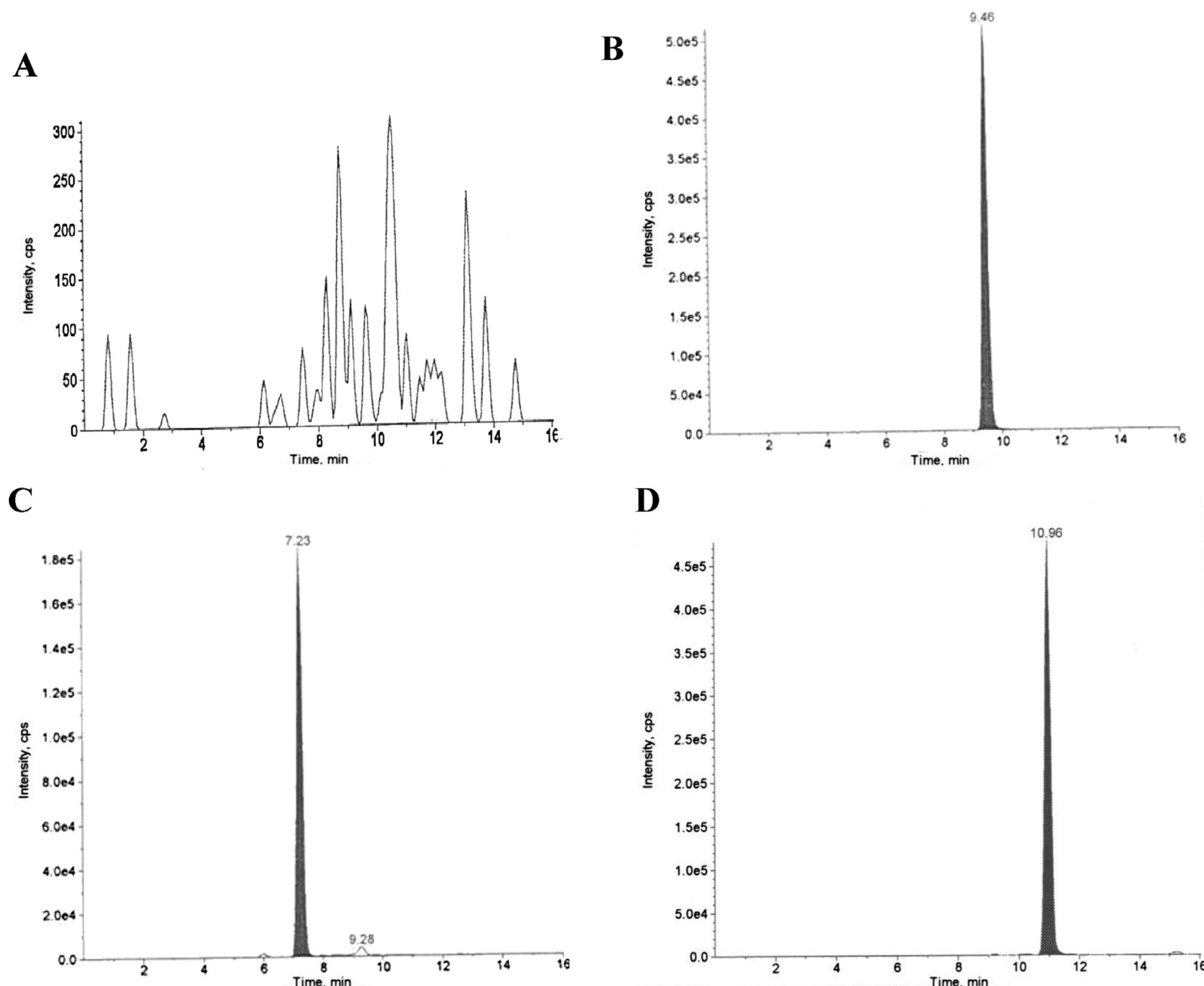


Fig. 1. LC–MS/MS multiple-reaction monitoring chromatograms of blank human urine (A), urine spiked with triamcinolone acetonide (30 ng/mL) (B), triamcinolone (30 ng/mL) (C), and betamethasone-D5 (D).

Table 1
The acquisition segment, RRT, intraday precision during the RRT (CV[a]), interday precision during the RRT (CV[b]), ion ratio interday precision (CV[c]), MRM transition, and recovery for TA and T.

Analyst	Acquisition segment (min)	RRT	CV[a] (%)	CV[b] (%)	CV[c] (%)	CE (V)	MRM transition	Recovery (%)
Triamcinolone acetonide	9–10.5	0.853	0.64	0.20	5.94	17/19	395 → 357/321	88
Triamcinolone	7–8	0.647	0.28	0.07	1.99	15/19	435 → 415/357	79

RRTs, relative retention time; CV[a], intraday precision during the RRTs; CV[b], interday precision during the RRTs; CV[c], ion ratio interday precision.

$y = 0.0036x + 0.0885$ ($n = 8$), $y = 0.0013x + 0.0076$ ($n = 8$). The method exhibited favorable linearity over the concentration ranges of 1–200 ng/mL.

Table 1 shows the acquisition segment, RRT, intraday precision during the RRT (CV[a]), interday precision during the RRT (CV[b]), ion ratio interday precision (CV[c]), MRM transition, and recovery for TA and T. The LOD was 2 ng/mL, which was considerably less than the MRPL of glucocorticoids (30 ng/mL) declared by the WADA. The LOQ was 5 ng/mL.

Pujos et al. [15] compared the analysis of corticosteroids by using various techniques for applying doping tests, and demonstrated that LC–MS and GC–MS were more sensitive and specific than ELISA was. Our results demonstrated that this LC–MS/MS method was suitable for the quantification of TA and T in human urine.

Table 2
Urinary triamcinolone acetonide concentration (ng/mL) following different injection of single-dose triamcinolone acetonide by random collection.

Subject		Time (h)														
		0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–14	14–16	16–20
Tendon (12 mg)	1	–	13.8	–	–	43.4[b]	–	–	–	–	–	–	–	–	–	–
	2	–	12.5	–	–	11.6	–	–	–	–	–	–	–	–	–	–
	3	–	1.2[a]	–	12.4	–	–	–	–	–	–	–	–	–	–	–
	4	2.0[a]	–	–	–	30.8[b]	–	–	–	–	–	–	–	–	–	–
	5	–	5.4	24.5	–	–	1.3[a]	–	4.7[a]	–	–	–	–	–	–	–
	6	ND	–	43.3[b]	–	–	–	–	–	–	–	–	–	48.4[b]	49.0[b]	–
	7	–	2.3[a]	–	–	–	15.4	–	11.7	–	–	–	–	12.1	–	–
	8	–	–	3.7[a]	–	–	–	–	3.6[a]	–	–	–	–	0.7[a]	3.3[a]	–
	9	–	40.4[b]	–	–	35.0[b]	–	–	22.3	–	–	30.9[b]	–	–	–	–
	10	–	2.9[a]	–	7.5	–	–	4.9[a]	–	9.6	–	–	–	–	–	–
	11	–	–	–	76.2[b]	–	–	116.0[b]	–	–	–	–	–	–	84.7[b]	51.4[b]
	12	–	–	–	4.8[a]	4.9[a]	–	12.9	–	11.5	–	–	–	–	–	–
	13	–	9.3	–	7.2	16.0	26.6	–	–	–	–	–	–	–	–	–
Tendon (16 mg)	14	–	–	3.2[a]	–	–	ND	–	–	–	2.4[a]	–	–	–	–	–
Tendon (20 mg)	15	–	–	4.9[a]	–	29.4	–	–	–	–	–	–	–	–	–	–
	16	–	–	–	–	145.7[b]	–	–	204.0[b]	–	–	–	–	–	61.8[b]	22.7
Tendon (24 mg)	17	42.6[b]	–	33.8[b]	–	–	–	–	–	–	–	–	–	–	–	–
Tendon (30 mg)	18	–	10.8	–	–	20.8	–	–	–	–	–	–	–	–	–	–
	19	–	81.3[b]	54.6[b]	61.9[b]	35.5[b]	–	–	–	–	–	–	–	–	–	–
Tendon (40 mg)	20	–	33.1[b]	–	23.7	–	–	–	–	–	–	–	–	–	–	–
	21	–	–	374.2[b]	–	–	155.4[b]	–	–	–	125.5[b]	–	–	–	–	–
	22	ND	–	–	–	–	–	–	59.7[b]	–	–	–	–	11.2	–	–
	23	0.6[a]	–	–	17.9	45.6[b]	–	–	43.4[b]	–	–	–	–	–	–	–
	24	36.0[b]	103.3[b]	101.3[b]	101.6[b]	185.5[b]	–	–	–	–	–	–	–	–	–	–
	25	26.8	11.1	–	13.9	10.0	–	–	–	–	–	–	–	–	–	–
	26	4.7[a]	–	7.3	5.4	–	–	9.3	–	–	–	–	–	–	–	–
	27	–	–	36.3[b]	25.7	–	33.4[b]	–	38.8[b]	–	–	–	–	–	–	–
Articulation (40 mg)	28	0.2[a]	–	20.7	–	30.2[b]	20.7	–	–	–	–	–	–	–	–	–
	29	–	ND	–	ND	–	–	–	ND	–	–	5.3	–	–	–	–
Articulation (80 mg)	30	–	13.5	–	–	20.4	–	–	–	–	–	–	–	–	–	–
Bursa (40 mg)	31	ND	28.1	–	62.1[b]	–	–	–	–	27.6	–	–	–	–	–	–
	32	–	–	–	–	31.4[b]	–	–	–	32.0[b]	–	–	–	29.3	–	25.9
	33	–	–	17.4	–	22.9	–	37.3[b]	–	–	39.7[b]	–	–	–	–	–
Epidural (40 mg)	34	–	–	159.8[b]	–	–	180.4[b]	–	–	–	–	–	–	–	–	–
	35	128.5[b]	–	233.8[b]	–	–	–	–	–	–	–	–	–	–	–	–
Epidural (80 mg)	36	–	–	–	–	–	94.1[b]	–	–	–	48. 9[b]	–	–	–	56.7[b]	92.8[b]
	37	–	–	70.7[b]	–	–	–	–	–	123.0[b]	55.5[b]	–	–	–	–	–
Muscle (80 mg)	38	–	–	–	113.0[b]	–	–	99.0[b]	–	–	–	–	–	–	–	–

ND: sample was not determined; –: no urine collection at the time point.
[a] Indicates concentration below LOQ (5 ng/mL).
[b] A TA-positive result according to WADA's rule (above 30 ng/mL).

3.2. Urine analysis of the triamcinolone acetonide users

The results indicated that the administration of a single dose of triamcinolone acetonide by using both nonprohibited local routes (intratendinous, intraarticular, and intrabursal injections) and prohibited systemic routes (lumbar epidural and intramuscular injections) produced positive TA and T results.

Table 2 shows the positive results of TA in the 14 ($n = 27$) subjects who received intratendinous injections, one ($n = 3$) subject who received an intraarticular injection, three ($n = 3$) subjects who received intrabursal injections, 4 ($n = 4$) subjects who received lumbar epidural injections, and one ($n = 1$) subject who received intramuscular injections in the random urine collection group. The concentration of T in the 38 subjects belonging to the random urine collection group ranged from 0 to 15.2 ng/mL. We did not identify a strong relative connection between the concentrations of TA and T when using various local injection routes. Furthermore, one study [16] revealed that identifying a specific marker for the use of intraarticular or intramuscular injections was not possible.

Fig. 2 shows the urinary TA and T concentration-time profiles after administering intratendinous injections of 12 mg and 20 mg triamcinolone acetonide in two subjects from the timed collection group. The mean urinary pH and specific gravity (mean ± SD) observed in 48 h were 6.1 ± 0.5 and 1.017 ± 0.009, respectively. The time to the peak concentrations (T_{max}) of TA and T were 4 h and 2 h in subject receiving 12 mg injection, and were 3 h and 2 h in subject receiving 20 mg injection. The peak urine levels (C_{max}) of TA and T were 30.3 ng/mL and 22.5 ng/mL in subject receiving 12 mg injection, and were 53.0 ng/mL and 54.5 ng/mL in subject receiving 20 mg injection. The excretion of TA was the highest 20–36 h after intratendinously administering a single dose of TA. The percentage ratios of excretion-to-administration were 0.21% and 0.29% for the 20-mg injection and 12-mg injection, respectively. However, the excretion of T exhibited the highest amount between 1 and 4 h. The percentage ratios of excretion-to-administration were 0.01% and 0.02% for the 20-mg injection and 12-mg injection, respectively. Additionally, the ascending curve between 40 and 48 h in Fig. 2 indicates that TA was not excreted entirely.

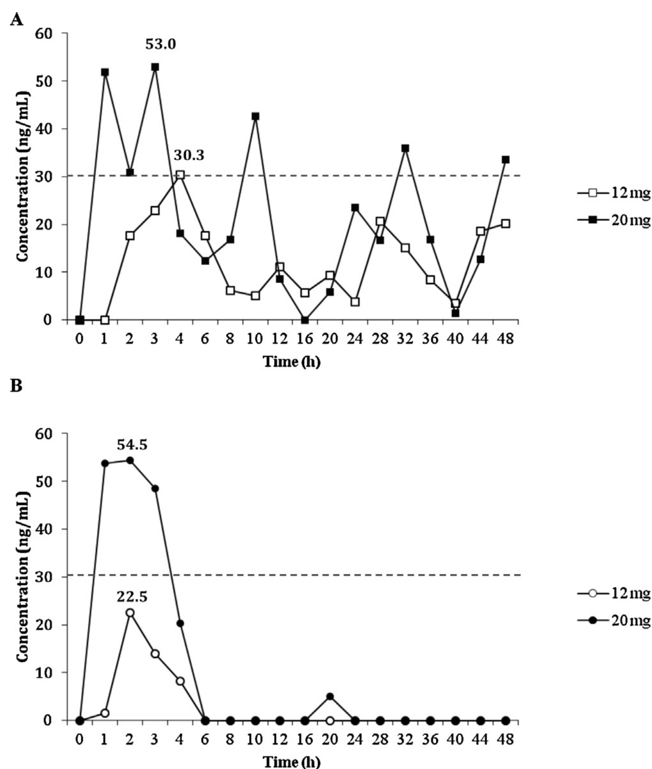


Fig. 2. Urinary concentration–time profile of triamcinolone acetonide (A) and triamcinolone (B) after intra-tendinous injection of 12 and 20 mg triamcinolone acetonide in different subjects by timed collection. Peak urine levels (C_{max}) are showed in the profiles.

Fig. 2 shows that the metabolic situation of TA, a long-acting glucocorticoid, was not an exact linear model. In tendons, joints, and muscles, drugs are absorbed into the bloodstream by simple diffusion through capillaries, the smallest blood vessels in human bodies that connect arterioles and venules. Even a single therapeutic dose administered intraarticularly would be detectable in urine because of the (limited) absorption and systemic distribution [17]. The metabolic situation revealed in the study results was consistent with that observed in previous studies [6,18].

This could be because achieving complete absorption from the site of injection takes a long time because of the low solubility of TA [18], the total body clearance is considerably different at various times of the day [19], or the differences in fluid intake and excretion can substantially dilute urinary drug concentrations [20].

Although T is not the main metabolite of TA [6], an analytically small amount of T was observed in urine after TA administration, despite exceeding 30 ng/mL. Therefore, we may possibly have misinterpreted the legitimate TA local injection users as T oral administration users, which would violate the WADA's rules. TA local injections are used widely for treating sports-related injuries. The study results provide information for limiting the misuse of TA by athletes.

4. Conclusion

Although local injections of glucocorticoids are not prohibited by the WADA, the results revealed that the concentration of both TA and T in urine can exceed the MRPL (30 ng/mL) after a single local injection. Therefore, we suggest that athletes be cautious when considering a TA injection during a competition period and seek approval and exemption for TA use prior to administration.

Acknowledgments

This project was supported by grants from Yuan's General Hospital, Taiwan (RG13-002) and NSC (NSC101-2410-H-037-016-MY3).

References

[1] U. Fredberg, L. Bolvig, Traumatic arthritis in sport, Scand. J. Med. Sci. Sports 11 (2001) 251–254.
[2] B. Gialanella, M. Bertolinelli, Corticosteroids injection in rotator cuff tears in elderly patient: pain outcome prediction, Geriatr. Gerontol. Int. (October) (2013), http://dx.doi.org/10.1111/ggi.12046.
[3] World Anti-Doping Agency, The World Anti-doping Code. The 2014 Prohibited List, International Standard, 2014 Retrieved from: http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-Prohibited-list/2014/WADA-prohibited-list-2014-EN.pdf (accessed 01.01.14).
[4] S. Saunders, Injection Techniques in Orthopedic and Sports Medicine, Elsevier, New York, 2006.
[5] H. Derendorf, G. Hochhaus, S. Rohatagi, H. Möllmann, J. Barth, H. Sourgens, M. Erdmann, Pharmacokinetics of triamcinolone acetonide after intravenous, oral, and inhaled administration, J. Clin. Pharmacol. 35 (3) (1995) 302–305.
[6] H. Möllmann, P. Rohdewald, E.W. Schmidt, V. Salomon, H. Derendorf, Pharmacokinetics of triamcinolone acetonide and its phosphate ester, Eur. J. Clin. Pharmacol. 29 (1) (1985) 85–89.
[7] D. Argenti, B.K. Jensen, R. Hensel, K. Bordeaux, R. Schleimer, C. Bickel, D. Heald, A mass balance study to evaluate the biotransformation and excretion of [^{14}C]-triamcinolone acetonide following oral administration, J. Clin. Pharmacol. 40 (7) (2000) 770–780.
[8] S. Gordon, J. Morrison, The metabolic fate of triamcinolone acetonide in laboratory animals, Steroids 32 (1) (1978) 25–35.

[9] K.J. Kripalani, A.I. Cohen, I. Weliky, E.C. Schreiber, Metabolism of triamcinolone acetonide-21-phosphate in dogs, monkeys, and rats, J. Pharm. Sci. 64 (8) (1975) 1351–1359.

[10] D. Kupfer, R. Partridge, 6 Beta-hydroxylation of triamcinolone acetonide by a hepatic enzyme system. The effect of phenobarbital and 1-benzyl-2-thio-5, 6-dihydrouracil, Arch. Biochem. Biophys. 140 (1) (1970) 23–28.

[11] C.D. Moore, J.K. Roberts, C.R. Orton, T. Murai, T.P. Fidler, C.A. Reilly, R.M. Ward, G.S. Yost, Metabolic pathways of inhaled glucocorticoids by the CYP3A enzymes, Drug Metab. Dispos. (February) (2013), http://dx.doi.org/10.1124/dmd.112.046318.

[12] L.R. Soma, C.E. Uboh, Y. You, F. Guan, R.C. Boston, Pharmacokinetics of intra-articular, intravenous, and intramuscular administration of triamcinolone acetonide and its effect on endogenous plasma hydrocortisone and cortisone concentrations in horses;, Am. J. Vet. Res. (2011 September), http://dx.doi.org/10.2460/ajvr.72.9.1234.

[13] F. Pigozzi, A. Di Gianfrancesco, M. Zorzoli, N. Bachl, D. Mc Donagh, J. Cummiskey, L. Di Luigi, Y. Pitsiladis, P. Borrione, Why glucocorticosteroids should remain in the list of prohibited substances: a sports medicine viewpoint, Int. J. Immunopathol. Pharmacol. 25 (1) (2012) 19–24.

[14] L. Amendola, F. Garribba, F. Botrè, Determination of endogenous and synthetic glucocorticoids in human urine by gas chromatography–mass spectrometry following microwave-assisted derivatization, Anal. Chim. Acta 489 (2) (2003) 233–243.

[15] E. Pujos, M.M. Flament-Waton, O. Paisse, M.F. Grenier-Loustalot, Comparison of the analysis of corticosteroids using different techniques, Anal. Bioanal. Chem. 381 (2005) 244–254.

[16] A. Panusa, L. Regazzoni, G. Aldini, M. Orioli, A. Giombini, P. Minghetti, C. Tranquilli, M. Carini, Urinary profile of methylprednisolone acetate metabolites in patients following intra-articular and intramuscular administration, Anal. Bioanal. Chem. 400 (2011) 255–267.

[17] F. Capolongo, G. Gallina, M. Fidani, F. Baia, C. Montesissa, Endocrine evaluation after an intra-articular therapeutic dosage of dexamethasone in horses, J. Vet. Pharmacol. Ther. 36 (6) (2013) 542–549.

[18] H. Derendorf, H. Möllmann, A. Grüner, D. Haack, G. Gyselby, Pharmacokinetics and pharmacodynamics of glucocorticoid suspensions after intra-articular administration, Clin. Pharmacol. Ther. 39 (3) (1986) 313–317.

[19] J.S. Choi, S.C. Shin, J.P. Burm, Circadian changes in the pharmacokinetics of triamcinolone acetonide in rabbits, Res. Commun. Mol. Pathol. Pharmacol. 107 (3–4) (2000) 233–238.

[20] E.J. Cone, Y.H. Caplan, F. Moser, T. Robert, M.K. Shelby, D.L. Black, Normalization of urinary drug concentrations with specific gravity and creatinine, J. Anal. Toxicol. 33 (1) (2009) 1–7.