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RECENT ADVANCES IN DOPING ANALYSIS (5)

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Screening and confirmation method for the detection of synthetic corticosteroids in human urine.

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Introduction.

Corticosteroids are high potency drugs. According to the rules of racing, their use is prohibited. In human doping control, corticosteroids which are not administered locally, are prohibited (IOC, 1997), (Off. J, 1994). For many years, a variety of methodologies have been studied for detecting synthetic corticosteroids in biological fluids and tissues (Girault *et al*, 1990), (Mc Laughlin and Henion, 1990). In horse doping control, ELISA (Chui *et al*, 1992), RIA and HPLC have been described as screening methods and GC/MS NICI (Stanley *et al*, 1993), (Houghton *et al*, 1982) and LC/MS (Houghton *et al*, 1981), as confirmatory methods for synthetic corticosteroid drugs. Metabolism of these such as dexamethasone (Dumasia *et al*,1986), betamethasone (Skrabalak and Henion, 1986), methylprednisolone (Gallicano *et al*, 1985) have been also proposed in the literature. In human field, Park *et al*, (1990) have proposed an HPLC and thermospray LC/MS methods for the separation and identification of corticosteroids.

The purpose of the present study was:
-to investigate if the screening and confirmatory methods available in our laboratory for the control of synthetic corticosteroid in the horse are accurate for human corticosteroids antidoping purpose.
-to evaluate the use of corticosteroids in human sport

Material and methods.

Material.

Five healthy volunteers participated in the following experiments: betamethasone (1 mg orally), dexamethasone (1 mg orally), prednisone (20 mg orally), prednisolone (20 mg orally), and triamcinolone acetonide (40 mg IM). Post-administration samples were collected for 48 hours after dosing or twelve days in the case of triamcinolone acetonide administration. 177 samples obtained from LNDD* were screened. The description of the population is presented in Table 1

Method.

ELISA tests using two specific kits (i.e. dexamethasone, triamcinolone acetonide kits from Neogen) and two generic corticosteroid kits (i.e. Corticosteroid group kit from IDS, St Joseph, USA, Corticosteroid group kit from CER, Marloie, Belgium) were carried out on these samples.

HPLC/APCI/MS method on a Finnigan Mat TSQ 700 instrument was used to confirm the presence of synthetic corticosteroid drugs in post-administration samples and in routine. Ten millilitres of urine were adjusted to pH 9.5 with diluted ammonia solution. In the case of a smaller initial volume, it is made up to 10ml using water. The solution was poured onto a Chemelut C1010 column. Corticosteroids were extracted using 40ml dichloromethane-ethanol (99:1, v/v). The residue was evaporated and the dry residue dissolved in the mobile phase. Analysis was performed in the scan or SIM mode on TSQ 700 in the following conditions.

The APCI source is linked with a HPLC system from TSP. The APCI source was optimised for maximum response at about 450°C. The heated capillary temperature was maintained at 200°C. The HPLC column is a Colochrom Nucleosil 3μ C18 (150 X 4.6mm) and the mobile phase is a mixture of methanol and water (59:41, v/v).

Results and discussion.

An ELISA preliminary study has been conducted to select a kit for each corticosteroid of interest. Using the kits presented in Table 2, ELISA test has been applied on post-administration samples. It was possible to detect dexamethasone and betamethasone 36 hours after administration, prednisone and prednisolone 10 hours after administration and triamcinolone acetonide more than 12 days after administration. By HPLC/APCI/MS, in the SIM mode, dexamethasone and betamethasone were detectable 36 hours after administration, prednisone and prednisolone 24 hours after administration. Triamcinolone acetonide was detectable in the 8 day-post administration sample.

In the population study (Table 1), various synthetic corticosteroids (Table 3) such as triamcinolone acetonide, dexamethasone, betamethasone, prednisone, and prednisolone have been detected by ELISA and confirmed by HPLC/APCI/MS scan mode. As shown in table 3, one or two of these corticosteroids have been found in many cycling sport samples. In the samples provided from the other sports (n = 115), only three have been reported positive; one contained prednisone and prednisolone.

Conclusion

The results confirm that ELISA, HPLC/APCI/MS are suitable techniques in terms of specificity and sensitivity for the screening and confirmation of synthetic corticosteroid in human urine. It also indicates at least in France the frequent use of corticosteroids in cycling. However as legislation allows the use of corticosteroids administered locally, it is not easy to differentiate doping cases from permissive medication.

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SPORT		Number
Water sports	Canoeing:12 Underwater sport:8 Sailing:6 Swimming:6 Rowing:4	36
Others	Athletics:8 Archery:8 Jockey:8 Basket-ball:6 Triathlon:6 Unknown:6 Golf:5 Motocycling:4 Motoring:4 Fencing:2 Shooting:2 Badminton:1	62
Cycling		79

Table1: Population description (n = 177).

method **molecule**	**ELISA** **Test**	**Detection limit**
dexamethasone	dexamethasone Neogen kit	1ng/ml
betamethasone	corticosteroid IDS kit	1ng/ml
triamcinolone acetonide	triamcinolone Neogen kit	<10ng/ml
prednisone	corticosteroid CER kit	10ng/ml
prednisolone	corticosteroid CER kit	10ng/ml

Table2: ELISA preliminary results: Kit selection for the drug of interest.

molecule detected	number of detection	sport
dexamethasone	5	cycling
betamethasone	5	
dexamethasone or betamethasone	1	
Triamcinolone ace	13	
dexamethasone + Triamcinolone ace	2	
betamethasone + Triamcinolone ace	4	
dexamethasone or betamethasone + prednisone+ prednisolone	1 1	
prednisone	1	
prednisone+ prednisolone	1	basket-ball
prednisone	1	
Triamcinolone ace	1	motoring

Legend: ace:acetonide

Table3: Routine samples (n=177).