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Lack of increased diuresis after topical administration of the carbonic anhydrase inhibitor dorzolamide

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INTRODUCTION

Diuretics are classified according either to their chemical structure and/or their mechanism of action. Carbonic anhydrase (CA) inhibitors act in the proximal tubule: although their efficacy is relatively low, especially when compared to more recent drugs, they are still included in the Olympic Movement Antidoping Code (OMAC) list of forbidden substances and methods. Due to their diuretic effect, the presence in the urine of the athletes of CA inhibitors and/or their metabolites constitutes an offence and may lead to sanctions by the Sport Authorities.

The recent development and commercialization of novel, potent CA inhibitors, to be administered topically for the pharmacological control of intraocular pressure, raised the question whether the use of these compounds should be forbidden to athletes, and, if so, whether they would be detectable in the urine, after topical administration, by the antidoping laboratory. The question was specifically - and officially - formulated in the summer of 1999, by the medical director of a major Italian soccer team, enquiring on the possibility of using dorzolamide (Dorz), a topically effective CA inhibitor, for the topical treatment of open angle glaucoma.

The key questions that activated this study were:

1. Is the topical administration of a CA inhibitor able to cause diuresis (this being the only pharmacological effect which CA inhibitors are banned for), and:
2. Are the low amount of drug/metabolite excreted in the urine following topical administration, detectable by the routine technique followed by the antidoping laboratory?

We have preliminarly evaluated in this study the possibility of detecting Dorz and/or its main metabolite N-desethyl dorzolamide (NDED) (Figure 1) in human urine following both acute and chronic administration, checking at the same time for a possible increase in the diuresis. The study was carried out on healthy volunteers and on 14 patients treated with Dorz for periods ranging from 7 days to 3 years. Urine samples where assayed by the GC-MS method of analysis developed by the antidoping laboratory of Rome for the detection of diuretics, their metabolites, and related substances in human urine.

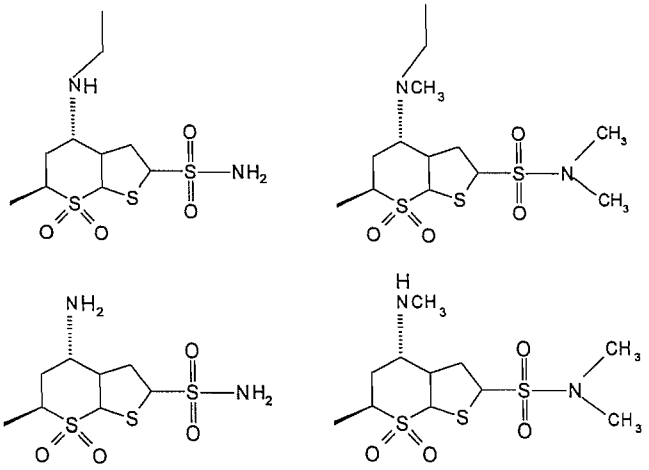

Figure 1

Molecular structure of dorzolamide (above) and N-desethyl-dorzolamide (below) prior (left) and after (right) derivatization by CH_3I.

EXPERIMENTAL SECTION

a) Dorzolamide administration

Acute: Ocular drops (two drops, bilaterally) containing 2% dorzolamide (Trusopt) were administered to 12 healty volunteers (6 male & 6 female, age 29-45) and urine was collected 2-12 hours after administration.

Chronic: Urines of 14 patients (see Table 1 for details) under chronic topical Dorz therapy were collected 2-4 hours after the last administration. All patients were constantly followed to monitor basic ocular hydrodynamic parameters and diuresis.

b) Instrumental apparatus and reagents

All GC-MS-EI assays were performed on a Hewlett Packard 6890-5973 GC-MS system. Determination of Dorz fragmentation pattern was carried out by MS-MS studies performed on a

Thermoquest GCQ GC-MS-MS system. GC-HRMS assays were also carried out, on a Fisons Autospec, for confirmation of the exact mass. All standards and reagents were supplied by Sigma Chemical Co., St Louis (MO, USA). All solvents were analytical grade.

c) Urine pretreatment/calibration samples

Urine pretreatment was the same for both GC-MS and GC-MS-MS studies. 5.0 mL of urine were passed on preactivated C18 cartridges and derivatized with CH_3I:acetone 1:10 (3 h at T=70 °C, pH= 9.0). Internal standard (50 µL of a 20 µg/ml methanolic solution of indomethacine) was added to quantitate all samples which resulted positive after the first screening. Calibration solutions (in MeOH), and calibration spiked urines (containing 400 ng/ml each of acetazolamide, althiazide, bendroflumethiazide, bumetanide, canrenone, clopamide, clortalidone, clothiazide, diclophenamide, ethacrinic acid, furosemide, hydroclorthiazide, hydroflumethiazide, indapamide, spironolactone) were used to simulate the same conditions of the screening analysis of diuretics.

d) Screening GC-MS assays

Carrier gas: He; column: HP5 (5% phenylmethylsilicone 18m, 0.20 mm i.d., 0.33 µm film thickness); injector: T=280 °C, constant pressure, injection type: split 1:10. Oven temperature program: starting T=190 °C, 15 °C/min to 280 °C, then 5 °C/min to 290 °C. Volume injected: 1 µL. GC-MS-SIM acquisition was carried out on ions m/z 152, 135, 118, for both Dorz and its metabolite.

e) GC conditions: GC-MS-MS-EI study

Carrier gas: He; column: HP1 (cross linked methyl siloxane, 17 m, 0.20 mm i.d., 0.11 µm film thickness); injector: T=240 °C, constant flow rate = 0.6 mL/min, injection type: split 1:10. Oven temperature program: 100 °C 1 min, 8 °C/min to 182 °C, 11 min at 182 °C, then 18 °C/min to 305 °C. The sequential MS-MS fragmentation profile, based on precursor ions m/z 324, 260, 152, 135, 118, 108, has been carried out for Dorz standard. For samples obtained from patients under chronic Dorz therapy the most abundant fragment (m/z 152) was selected as precursor ion for MS-MS experiments; ion m/z 152 was also used for quantitation of both Dorz and NDED.

RESULTS AND DISCUSSION

1. Neither Dorz nor NDED were detected in the urine following acute administration, possibly owing to specific interaction with and selective binding to circulating CA isoenzymes I-III

(1-2 g/L in blood).

2. The presence of Dorz and NDED was detectable in all samples collected from patients under chronic therapy (more than 1 week, see Table 1), regardless the concurrent use of other medications and/or the presence of other pathologies;
3. Dorz and NDED were detected jointly in 12 out of 14 cases, although the urinary ratio unchanged/metabolite depended mostly on individual parameters (Table 2);
4. Levels of Dorz and NDED were correlated to the duration of the chronic therapy, with minor fluctuations due to individual parameters (i.e. concurrent administration of other drugs);
5. Side peaks were detected in some samples, but they did not interfere with the identification/quantitation of Dorz and/or NDED.
6. Apart from the absence of other relevant side effects, ***none of the patients reported an increase of diuresis*** during the period of treatment, thus confirming that the use of Dorz in the form of ocular drops is not in contrast with the Olympic Movement Antidoping Code.

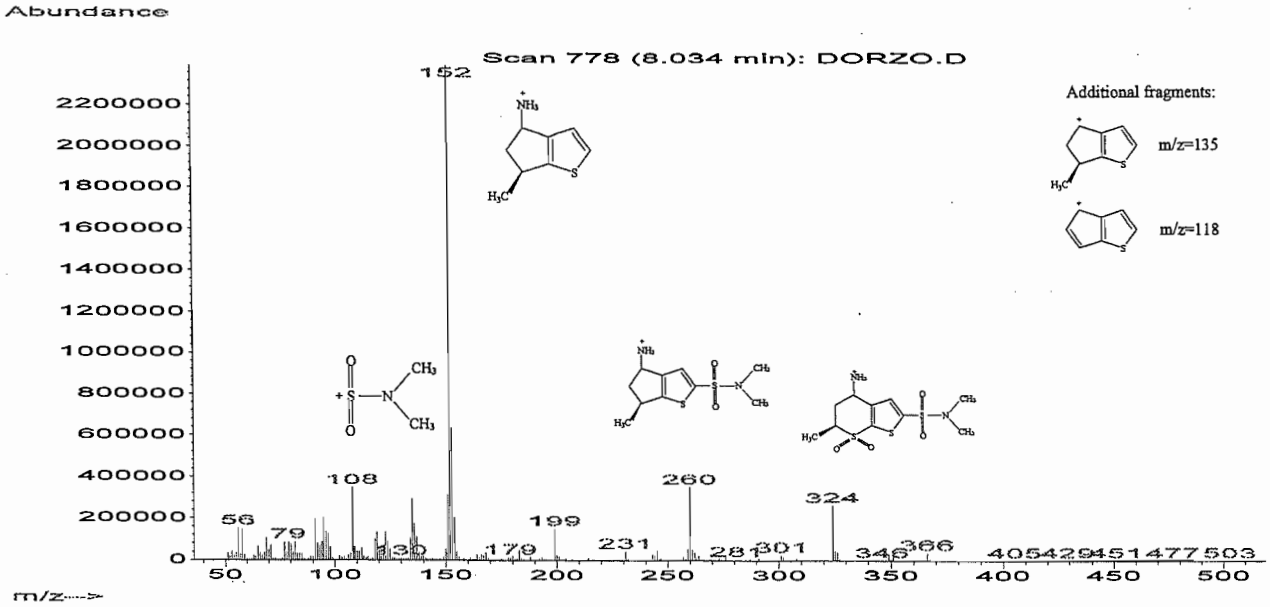

Figure 2

GC-MS-EI spectrum of dorzolamide CH_3I (trimethyl-derivative), reporting also the fragmentation pattern, as evaluated and confirmed by additional GC-MS-MS and GC-HRMS assays.

Table 1

Patient	Sex	Age	Period of treatment	Dosage (per day)	Other medications	s. e.	Diuresis
1	M	41	5 months	2	Atenolol (1/4)	n.r.	Normal
2	M	44	3 years	2	Topical beta blockers	n.r.	Normal
3	F	50	7 days	2	Enalapril (1/2)	n.r.	Normal
4	F	66	1 year	3	Enalapril, Aspirin, Latanoprost	n.r.	Normal
5	M	60	1 month	3	Captopril + Hydrochlordiazide, L-Tiroxine	n.r.	Normal
6	F	65	7 days	2	Glibenclamide, Sodium alendronate	n.r.	Normal
7	F	75	1 month	2	Amiloride + Hydrochlordiazide, Lisinopril	n.r.	Normal
8	M	68	1 month	3	Enalapril	n.r.	Normal
9	M	60	1 year	2	None	n.r.	Normal
10	F	55	1 year	2	Nifedipine, Spironolactone Gliclazide	n.r.	Normal
11	F	67	1 month	2	None	n.r.	Normal
12	F	64	1 month	2	Enlapril	n.r.	Normal
13	F	55	1 year	2	None	n.r.	Normal
14	M	44	3 years	2	None	n.r.	Normal

Table 2

Patient	pH	s.g.	Dorz (µg/mL) (RT 7.7 min)	NDED (µg/mL) (RT 8.3 min)	Met/Unch ratio
1	5.3	1.032	0.20	0.15	0.75
2	5.0	1.018	1.80	1.06	0.59
3	5.8	1.016	0.21	0.11	0.52
4	9.0	1.022	3.10	not quant.	--
5	5.0	1.012	0.26	0.15	0.58
6	5.8	1.016	2.47	1.0	0.40
7	6.5	1.018	2.1	not quant.	--
8	5.5	1.012	1.16	0.40	0.34
9	5.5	1.028	2.20	0.20	0.10
10	5.5	1.016	0.52	0.52	1.0
11	5.3	1.026	0.57	0.46	0.81
12	5.3	1.016	0.55	0.20	0.36
13	6.0	1.012	0.59	0.27	0.46
14	5.0	1.018	1.80	1.06	0.59

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