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Short report

Cyclosporine A increases serum cortisol levels in rabbits

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P-glycoprotein (P-gp), a membrane protein that was originally found to be involved in the efflux of cytotoxic drugs out of the tumor cells, is also present in a variety of normal human and animal tissues, such as the adrenal cortex. The function of P-gp in the adrenal cortex has not been defined yet. The aim of our study was to determine whether the blockade of P-gp by cyclosporine A (CsA) dissolved in Cremophor EL (Crem) inhibits cortisol secretion in rabbits. In 14 rabbits, the baseline and ACTH stimulated serum cortisol levels were measured before and after CsA treatment. Seven rabbits were treated with 2 × 30 mg/kg CsA and seven with 2 × 90 mg/kg CsA injected s.c. Serum cortisol levels were determined by radioimmunoassay adjusted for expected values. The whole blood CsA levels were determined by a commercially available fluorescence polarization immunoassay. Serum cortisol levels, both baseline and ACTH stimulated, significantly increased after both low and high dose CsA treatment. The increase was dose dependent. The mean baseline cortisol levels increased from 5.7 (SD = 6.3) to 15.0 nmol/l (SD = 7.2) in the low dose group and from 7.7 (SD = 4.9) to 44.9 nmol/l (SD = 13.8) in the high dose group. The mean cortisol levels 8 h after ACTH stimulation increased from 53.3 (SD = 34.5) to 106.0 nmol/l (SD = 33.0) in the low dose group and from 47.7 (SD = 12.2) to 153.0 nmol/l (SD = 55.1) in the high dose group. The mean whole blood CsA levels measured at that time were 1782 μg/l (SD = 634) in the low dose group and 2428 μg/l (SD = 483) in the high dose group. Contrary to our expectations, CsA treatment increased serum cortisol levels, both baseline and ACTH stimulated, in rabbits. The increase may or may not be related to the drug's interaction with P-gp. Further studies to explore our surprising finding and adrenal as well as pituitary gland function under P-gp inhibitors are warranted.

Key words: Cortisol, cyclosporine A, P-glycoprotein.

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Introduction

P-glycoprotein (P-gp) is a membrane glycoprotein that was originally found to be involved in the efflux of anti-cancer drugs out of tumor cells.[1] P-gp functions as an energy-dependent efflux pump and is considered to be one of the most important factors involved in the multidrug resistance (MDR) of tumor cells.[2] The function of P-gp can be modified by a number of drugs (modulators) that bind competitively to P-gp.[3] Cyclosporine A (CsA) is one of the most potent P-gp modulators.[4] CsA is water-insoluble; in order to be given parenterally it is dissolved in vehicle containing Cremophor EL (Crem), which was also shown to be a P-gp modulator.[5] P-gp is present in a variety of normal human and animal tissues.[6] The normal physiological roles of P-gp have not yet been explored. It was found to be expressed on the luminal surfaces of the epithelial cells in the gastrointestinal tract, liver and kidneys,[7,8] where it is supposed to be involved in the excretion of toxic substances.[9] P-gp is also present in the endothelial cells of the central nervous system and testis where it is thought to take part in maintaining the blood–brain and blood–testicular barrier.[10] P-gp is also highly expressed in the adrenal cortex of humans and other mammalians, such as rabbits.[11,12] Although the function of P-gp in the adrenal cortex has not been defined yet, *in vitro* studies suggest a possible interaction between P-gp and steroid hormones. Steroid hormones interact with P-gp and are able to reverse drug resistance in resistant tumor cells.[13] In addition, some steroid hormones can be transported across the cell membranes by P-gp.[14–16] Steroid hormones regulate the expression of the

mdr gene encoding P-gp.[17,18] Furthermore P-gp modulators block steroid hormone secretion from the adrenal cells *in vitro*.[19] Because high dose CsA has been used to inhibit P-gp function in clinical trials to modulate MDR,[9] we hypothesized that this approach might result in an inhibition of adrenal cortisol secretion *in vivo*. To examine this hypothesis we performed the study in rabbits. The aim of our study was to determine the effect of CsA treatment on serum cortisol levels in rabbits.

Materials and methods

New Zealand White adult male rabbits were housed in laboratory conditions on standard pellet diet and tap water. In 14 rabbits, baseline and ACTH stimulated serum cortisol were measured on day 1. Baseline serum cortisol levels were measured at 8 a.m.; then an ACTH stimulation by tetracosactide 0.5 mg i.m. was performed and cortisol levels were measured 1 and 8 h after stimulation. After the rabbits had rested for 12 days we treated them with CsA (Sandimmun injectable concentrate containing per ml: 50 mg CsA and 650 mg Crem, Sandoz) and repeated the baseline and ACTH stimulated cortisol level measurements on day 14, using the same procedure as on day 1. CsA treatment consisted of two s.c. injections, the first at 6 p.m. on day 13 and the second at 6 a.m. on day 14. Seven rabbits were treated with 2 × 30 mg/kg CsA and seven with

Table 1. Serum cortisol (nmol/l) before and after low dose CsA treatment in rabbits

Animal no.	Before any treatment			After 2 × 30 mg/kg CsA		
	Baseline	1 h after ACTH	8 h after ACTH	Baseline ACTH	1 h after ACTH	8 h after
1	1	20	20	4	42	80
2	1	13	28	12	80	159
3	12	42	80	12	90	90
4	1	17	40	22	52	90
5	18	18	105	26	60	125
6	1	15	20	13	26	65
7	6	27	80	16	95	134
Mean	5.7	21.7	53.3	15.0	63.6	106.0
SD	6-.3	10.0	34.5	7.2	25.8	33.0
t test[a]				$p=0.0110$	$p=0.0021$	$p=0.0116$

Table 2. Serum cortisol (nmol/l) before and after high dose CsA, and after placebo treatment in rabbits

Animal no.	Before any treatment			After 2 × 90 mg/kg CsA			After placebo treatment		
	Baseline	1 h after ACTH	8 h after ACTH	Baseline	1 h after ACTH	8 h after ACTH	Baseline	1 h after ACTH	8 h after ACTH
1	9	32	70	41	86	175	2	45	55
2	14	34	41	67	70	90	9	30	59
3	4	42	41	26	72	139	1	27	47
4	1	52	51	31	42	91	2	25	56
5	14	35	54	52	114	250	2	48	50
6	6	34	32	50	49	154	12	40	44
7	6	39	45	47	73	170	5	47	50
Mean	7.7	38.3	47.7	44.9	72.3	153.0	4.7	37.4	54.9
SD	4.9	6.9	12.2	13.8	23.8	55.1	4.2	9.9	9.6
t test				$p=0.0001$	$p=0.0186$	$p=0.0018$	$p=0.2203$	$p=0.8869$	$p=0.2294$

[a]Matched pair *t* test.

2 × 90 mg/kg CsA. Whole blood CsA levels were measured at 8 a.m. and 4 p.m. on day 14. In seven rabbits, initially treated with high dose CsA, after a 12 day rest period, placebo treatment with normal saline was initiated. Placebo treatment was performed at the same time intervals and with the same volume of the injected substance as CsA treatment was. Blood samples were collected by heart puncture. Serum cortisol levels were determined by radioimmunoassay (CORT-CT; CIS Biointernational, Gif-sur-Yvette, France) adjusted for expected values. Whole blood CsA levels were determined by a commercially available fluorescence polarization immunoassay (TDX; Abbott Laboratories, North Chicago, IL). At the end of the study, seven rabbits treated with low dose CsA were sacrificed and P-gp expression in the adrenal cortex was determined on paraffin sections using alkaline phosphatase immunohistochemistry with monoclonal antibody C 219 (Centocor, Malvern, PA). The sections were examined with a light microscope.

Results

Serum cortisol levels, both baseline and ACTH stimulated, significantly increased after both low (Table 1) and high dose CsA treatment (Table 2). The increase was dose dependent. Serum cortisol levels were not affected after placebo treatment (Table 2). As expected, CsA whole blood levels were higher after high dose treatment. In the low dose group the mean whole blood CsA levels measured at 8 a.m. and 4 p.m. were 1177 (SD = 234) and 1995 μg/l (SD = 232), whereas in the high dose group the levels were 1782 (SD = 634) and 2428 μg/l (SD = 483), respectively. Strong P-gp immunoreactivity on plasma membranes of cortical cells was found uniformly in the adrenal glands.

Discussion

To study the effect of CsA treatment on serum cortisol an experiment in rabbits was performed. Contrary to our expectations from *in vitro* studies, CsA treatment increases serum cortisol levels, both baseline and ACTH stimulated. The increase is dose dependent. CsA blood levels in the high dose group are associated with significant but not complete inhibition of P-gp function *in vitro*.[20] Although we were unable to demonstrate it, we cannot exclude the possibility that higher levels of inhibition of P-gp might block cortisol secretion. We plan additional experiments with more specific inhibitors of P-gp, such as PSC 833,[21] to further examine the mechanism. The dramatic increase in cortisol secretion after CsA treatment may or may not be related to the drug's interaction with P-gp. Possible mechanisms for increased cortisol levels after CsA treatment include an induction of cortisol biosynthesis, an inhibition of cortisol catabolism or simulation of cortisol secretion. However, due to the regulatory feedback mechanisms between the adrenal gland and pituitary gland, we do not believe that increased biosynthesis or decreased cortisol catabolism could be the reason for such a high increase of serum cortisol levels. We consider stimulation of cortisol secretion either through increased ACTH production or through another not yet defined regulatory mechanism in which P-gp might be involved, the most reasonable.

In conclusion, treatment withe the P-gp modulator CsA in the doses known to be able to interact with P-gp function *in vitro* does not block cortisol secretion in rabbits. In contrast, it causes an increase in both baseline and ACTH stimulated cortisol levels which may or may not be related to drug interaction with P-gp. Therefore further studies to explore our surprising finding, and adrenal as well as pituitary gland function, under P-gp inhibitors are warranted.

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ADDENDUM

Katherine J Martin, Cornelia D Vassallo, Beverly A Teicher and Rima Kaddurah-Daouk. Microtubule stabilization and potentiation activity by the creatine analog cyclocreatine. *Anti-Cancer Drugs* 1995; 6: 419–426.

Due to time constraints, the publishers were unable to incorporate a change of address for the corresponding author of the above paper. Please be advised that the corresponding author is now Dr R Kaddurah-Daouk **not** Dr K J Martin and can be contacted at the following address: Avicena Group, One Kendall Square, Bldg. 200 Cambridge MA 02139, USA. Tel: +1 617 621 7126; fax: +1 617 489 0927.

ERRATUM

Allan T van Oosterom and Dirk Schrijvers. Docetaxel (Taxotere®), a review of preclinical and clinical experience. Part II: clinical experience. *Anti-Cancer Drugs* 1995; 6: 356–368

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