Another point of view on side effects of antifungal compounds used in veterinary medicine

Un alt punct de vedere asupra efectelor secundare ale compusilor antifungici utilizati in medicina veterinara

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Abstract

The release of pharmaceuticals into environment has become an increasing concern in recent years. Fungi are part of the microbial flora of many animals, humans and foodstuffs, and some species can cause disease [10]. An antimycotic or antifungal product is one that is used in the treatment of fungal infections. Even at low concentrations, antifungals exert an action against micro-organisms and exhibit selective toxicity towards them. The use of antimicrobials selects for resistant populations of micro-organisms. Development of resistance to antifungals is an increasing problem in veterinary and human medicine.

Key word*: antifungal compound, drug residues, fungal resistance mechanism, invasive aspergilloses. cytochrome P450.*

Rezumat

Patrunderea produselor farmaceutice in mediul inconjurator a devenit o problema ingrijoratoare in ultimii ani. Fungii fac perte din microflora naturala a animalelor a oamenilor si produselor alimentare insa in anumite specii pot produce imbolnaviri [10]. Un produs antimicotic este acela care este folosit pentru tratarea infectiilor provocate de fungi. Chiar si la concentratii mici compusii antifmicotici isi exercita actiunea asupra microorganismelor. Utilizarea antibioticelor da nastere la aparitia de populatii rezistente de microorganisme. Dezvoltarea rezistentei la antifungice este o problema in crestere in medicina veterinara si umana.

Cuvinte cheie*: compus antimicotic, rezistenta la medicamente, mecanismul rezistentei la fungi, aspergiloza invaziva,. citocrom P450.*

If the presence of veterinary drug residues in food is regulated through Council Regulation 2377/90/EC and Council Directive 96/23/EC, also the regulation authorities for environmental concern, was sensitized by use on a large scale of veterinary medicinal products. In 1996 Committee for veterinary medicinal product (CVMP) of EMEA adopt o Note for Guidance: EMEA/CVMP/055/96 to assist the applicant in their evaluation environmental risk assessment for veterinary products.

In accordance with Directive 81/852/EEC as amended by Directive 2001/82/EC and Directive 2004/28/EC, revised in 2008 by Revised Guideline on Environmental Impact Assessment for Veterinary Medicinal Products, EMEA/CVMP/ERA/ 418282/2005, published on 23 June, 2008, this guidance consist of two phases: first assesses the potential of exposure of the environment to the product and its metabolites and second, investigated the effects on particular ecosystems: direct toxicity on aquatic and terestrum medium, contamination of plants, natural microbiological balance changes, the emergence of antibiotic resistance in bacteria and fungi, etc. The use of antibiotics in livestock production is really a potential source of environmental contamination.

Antibiotics and antifungal compounds are used in commercial feed supplemented and in therapeutic treatment. Veterinary pharmaceuticals are excreted unchanged or as metabolites and may enter in the environment via agricultural runoff (1, 14).

Over use of this substances in this type of system often occur, causing surface water and soil antibiotic contamination.

Antifungals are used in veterinary medicine for the treatment of dermatophytoses, mycotic mastitis, mycotic abortions, aspergillosis, and other mycotic infections.

Antifungals are used topically, orally or injected. Topical application results in higher emissions of active ingredients, due to relatively small absorption, and may lead to residues in the environment (14).

According to the European Consultation Conference on the Availability of Veterinary Medicinal Products (July 1999), only two substance – enilconazole and natamycin are left to treat ringworm, parconazol is left for candidoses, thiabendazole, griseofulvin and enilconazole are left for aspergilloses. But a lot of human products with different active substances are used in antimycotic treatment (13), as it show in table nr 1.

As can it be seen in Table nr. 1, there are more classes of antifungal used: polygene antifungal (nystatin and natamycin), azoles antifungal (ketonazole, miconazole, enilconazole, fluconazole, clotrimazole, and itraconazole), benzimidazole antifungals (thiabendazole), and mycotic inhibitors (griseofulvin).

Azolic substances are widely used as active ingredients in biocides and agricultural fungicides too. Chemical, physical and pharmakinetical proprieties are shown in table 2.

Mode of action of antifungals

Is different according with their structures (4, 11).

Polyenes (amphotericin B, natamycin and nystatin) intercalate of a ring of 8–10 polyene molecules into the fungal membrane, and in association with ergosterol, are thought to form membrane spanning channels with hydrophilic interiors that allow the leakage of essential components especially potassium ions, which ultimately results in fungal cell death.

Azolic-substances are the second class of antifungals that include imidazole (clotrimazole, miconazole, and ketoconazole) and triazoles (fluconazole and itraconazole).

Azoles inhibit ergosterol biosynthesis by blocking the enzyme lanosterol 14α-demetilaze (14-DM, or CYP51), a cytochrome P450 enzyme, which catalyzes the conversion of lanosterol into ergosterol in the fungal cell membrane.

This has the effect depletion of membrane ergosterol level, leading to increase membrane fluidity and permeability and inhibit of fungal cell growth and replication. Also this leads to accumulation of lanosterol and other toxic 14-α methylated sterols.

Triazoles bind to lanosterol 14-demethylase that is encoded by the ERG11 gene. Under the selection imposed by antifungal drugs, drug-sensitive fungal pathogens frequently evolve **resistance** (9).

Different **resistance mechanisms** towards the antimycotics have been identified (3, 11).

- exclusion or even active efflux from the fungi. Azoles resistance is related to increased export from the fungal cell.

Efflux pumps from the CDR family (members of the ATP binding cassette transporters) as well as MDR1 (a major facilitator) may be active. Several different CDR1 genes have been found in a fungal cell whereby some are involved in azole resistance.

- resistance mechanisms may be based on structural alterations in the target fungal enzyme: alteration of the cytochrome P450 Erg11 protein, which is responsible for the demethylation of lanosterol and is an important enzyme of ergosterol biosynthesis.

- resistance may stem from over production of the target fungal enzyme.

- a downstream mutation in the ergosterol pathway (defective-5,6-desaturase, encoded by the gene ERG3) that allows the accumulation of less toxic sterols (for example 14-methyl fecosterol) (6).

- decreased sterol component of cell membrane (polyene resistance) (6).

An interesting alternative for developing azole resistance has been recently described (3). It uses the ability of fungal pathogens to build biofilms on synthetic or natural surfaces.

Biofilms are organized as a dense network of differentiated cells onto which a layer of extracellular matrix can form.

Biofilms can constitute a physical barrier for the efficient penetration of antifungals.

Multidrug resistance is frequently observed (7). Also, it is known that through exposure to azoles residues (fungicides or pharmaceutical azoles used in veterinary practice), fungi becoming cross-resistant to the medical triazoles (15).

There are few reported incidences on antifungal resistance both in veterinary and medical field (2):

Jadhav, (2001) reported fluconazole, nystatin and clotrimazole resistance exhibited by *C. albicans* isolated from man, animals and birds.

Similarly, Thomas, (2004), reported antifungal drug resistance from *Cryptococcus neoformans* isolated from man, animals birds and environment.

Among the fungal isolates from canine cystitis, *C. albicans* and *C. tropicalis* were resistant to fluconazole and Amphotericin B.

It is well known invasive aspergillosis due to multi-azole-resistant *Aspergillus fumigatus* emerged in the Netherlands since 1999.

The presence of a single resistance mechanism in 90% of epidemiologically unrelated patients is consistent with a route of resistance development through exposure of *Aspergillus fumigatus* to azole compounds in the environment (12), because is unlikely that the development of resistance to medical

triazoles to be caused by azole residues in foods.

Fungi do not have any mechanism comparable to bacteria for the horizontal transfer of genes encoding resistance from one isolate to another.

Antifungal resistance is not encoded in extrachromosomal DNA and fungal cells do not readily take up exogenous DNA.

Even infectious extra chromosomal elements in fungi, such mitochondrial DNA elements and mycoviruses, require cell fusion and cytoplasmic contact before they can spread.

Experimental studies (2005) with C. albicans show that, the fungus probably can transfer the genes by mating, but does so naturally at a low frequency (5).

Asexual reproduction seems to be essential for phenotypic expression of mutations, including those predisposing for resistance. In patients with acute invasive aspergillosis, asexual reproduction by sporulation does not happen, and the infection progresses through hyphal elongation (12).

This strengthens the hypothesis that azole resistance appeared and sent out the patient's body, in the environment.

Toxicological Profiles of Fungicides

Toxic effects described for azole compounds as medical agents are similar to those reported for azoles used agriculturally e.g. disturbances in the liver function.

A rather seldom occurring but very severe adverse effect is acute liver failure that can be fatal. Due to the described teratogenicity the use of azole compounds during pregnancy and lactation is contraindicated.

Azole compounds are lipophilic and penetrate to the cytochrome P450 enzymes within the endoplasmatic reticulum.

This lipophilic character in part is held responsable for their toxicological profile.

Inhibition of non-target cytochrome P450 enzymes leads to toxicologically relevant changes in the liver and endocrine system.

In mammals, sterol 14α-demethylase (CYP 51) is part of the pathway leading to the biosynthesis of cholesterol.

Cholesterol in turn is the substrate for the production of the sex steroid hormones.

The ability of the azoles to inhibit the cytochrome P450 enzymes involved in the biosynthesis of steroid hormones in mammalian cells has lead to endocrine-related side effects.

Furthermore, the specificity of the enzyme inhibition of several of these compounds is poor, both with respect to fungal versus nonfungal sterol 14α-demethylases and versus other P450 enzymes including aromatase (8).

Aromatase (CYP19) is expressed in a wide variety of tissues such as ovaries, fat, muscle and mamma carcinoma.

Inhibition of aromatase can lead to disruption and imbalance between androgens and estrogens.

Fungicides, applied agriculturally in veterinary or in humans medicines, inhibition of CYP19 is considered a severe side-effect.

Results of in vivo studies of some fungicides are summarized in Table 3, with special focus on effects putatively connected to disturbed steroidogenesis. In the allocation procedure for an "Acceptable Daily Intake" (ADI) by the Joint Food and Agriculture Organisation/World Health Organisation Meetin on Pesticide Residues (JMPR), FAO/WHO Expert Committee on Food Additives these effects are included.

Studies on medical antifungals in the environment are so far limited.

High concentrations of azole pharmaceuticals in sewage sludge suggest their strong tendency to be absorbed onto and persist in solids.

Continuous monitoring is required to record variations in concentrations of these substances in the environment and also, developing models to predict the minimum concentration that exert selection for resistance development.

But how will be that information used in decisions of risk management, knowing that this also applies to antimicrobial products used as drugs, agriculture fungicides and biocides also?

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Bibliography:

1. A. B. A Boxall, L. Fogg, P. A. Blackwell, P. Kay, E. J. Pemberton (2002). Review of Veterinary Medicines in the Environment, R&D Technical Report P6-012/8/TR, *Environment Agency* 2002.

2. B. B. Bhanderi, M. M. Yadav, A. Roy (2009). Antifungal Drug Resistance - Concerns for Veterinarians, *Veterinary World*, Vol.2(5): 204-207.

3. D. Sanglard (2003). Resistance and tolerance mechanisms to antifungal drugs in fungal pathogens, *Mycologist,* Volume 17, Part 2, May 2003, 74-78.

4. E.S. Dodds Ashley, R. Lewis, J. S. Lewis, C. Martin, D. Andes (2006). Pharmacology of Systemic Antifungal Agents Clinical, *Infectious Diseases*, 43:S28–39.

5. F.C. Odds (2005). Antifungal Agents: Resistance and Rational Use Antibiotic, Policies: Theory and Practice, Edited by Gould and van der Meer Kluwer Academic / Plenum Publishers, New York.

6. G. Chamilos, D.P. Kontoyiannis (2005). Update on antifungal drug resistance mechanisms of Aspergillus fumigatus, *Drug Resistance Updates*, 8 (2005) 344–358.

7. H. Hof, (2001) Critical Annotations to the Use of Azole Antifungals for Plant Protection, Antimicrobial *Agents and Chemotherapy*, Nov. 2001, DOI: 10.1128, p. 2987–2990.

8. J.A. Zarn, B.J. Brüschweiler, J.R. Schlatter (2003) Azole Fungicides Affect Mammalian Steroidogenesis by Inhibiting Sterol 14α-Demethylase and Aromatase, *Environmental Health Perspectives* , volume 111, nr. 3, March 2003, 255-261.

9. J. B. Anderson (2005). Evolution of antifungal drug resistance: mechanisms and pathogen fitness, *Nature Reviews* | *Microbiology*, vol 3, july 2005, 547-556.

10. K.L. Goodyeara, E.J. Threlfallb (2004). Use of veterinary antimycotic products in the UK, 1998–2002, *International Journal of Antimicrobial Agents* 24 (2004) 311–314

11. M.A., Ghannoum, L., B., Rice, (1999). Antifungal Agents: Mode of Action, Mechanisms of Resistance, and Correlation of These Mechanisms with Bacterial Resistance, *Clinical Microbiology Reviews*, Vol. 12, No. 4, p. 501–517.

12. P.E., Verweij, E.Snelders, G., H Kema, E., Mellado (2009). Azole resistance in Aspergillus fumigatus: a side effect of environmntal fungicide use?, *Lancet Infect Dis* 2009;9:789-95.

13. Rochette, F., Engelen, M., Vanden Bossche, H. (2005). Antifungal agents of use in animal health – practical applications. *J. vet. Pharmacol. Therap.* 26, 31–53.

14. Q. Huang, Y. Yu, C. Tang, X. Peng (2010). Determination of commonly used azole antifungals in various waters and sewage sludge using ultra-high performance liquid chromatography–tandem mass spectrometry, *Journal of Chromatography A*, 1217 (2010) 3481–3488.

15. ***Azole resistance in aspergillus species, consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands. 2010.

Antifungal drugs used in veterinary medicine Table nr 1

Antifungal	Animal	Use and formulation	Dosage	Officially licensed as veterinary products
Nystatin (polyene)	cats, dogs,	Topical use		Officially licensed in US for chicken, turkey, cats, and dogs.
	turkey,	Oral powder, tablet.	50-100g/t	Officially licensed in EU for human
	poultry, exotic birds	Oral solution		
Natamicin (polyene)	Cattle, horses	spray suspension for topical use		Officially licensed in EU for cattle and horses
Enilconazole (imzalil)	cattle, horses	Solution for topical use		Officially licensed in EU for birds, cattle and horses
(azole antifungal)	poultry	Disinfectant for environment Spray		
Ketoconazole	Poultry	Oral tablet	30mg/kg	Officially licensed in EU for human
(azole antifungal)	Exotic birds			Officially licensed in US for dogs
Fluconazole	Poultry,	Oral tablet or solution	75-120mg/kg	Officially licensed in EU for human
(azole antifungal)	exotic birds		2-5mg/kg	
Clotrimazole	Birds, dogs	Solution for topical use		Officially licensed in EU for human
(azole antifungal)				Officially licensed in US for dogs.
Miconazole	Birds	Solution for topical use	10-20mg/kg	Officially licensed in EU for human
(azole antifungal)	Exotic birds	Solution for injection		
Itraconazole	Horses, poultry,	Oral solution	10mg/kg	Officially licensed in EU for human
(azole antifungal)	Cats, dogs			Officially licensed in US for dogs, cats, horses
Parconazole	Poultry, guinea fowl	Powder in feed	Prophylactic:	Officially licensed in EU for guinea fowl.
(azole antifungal)			30 mg/kg;	
			therapeutic:	
			60 mg/kg	
Griseofulvin	cattle, horses	7.5% food additive	10mg/kg	Officially licensed in EU for horses
	dogs, cats			Officially licensed in US for cats and dogs and horses.
Thiabendazole	Cattle, swine, horse, seep	4%solution for topical use,	40-100g/t	Officially licensed in US for seep, swine, cattle, horse.
(benzimidazole)	birds	oral solution, medicated feed		Officially licensed in EU for cats, dogs.
		Smoke tablet		Officially licensed in EU for birds

Table nr 2

Physico-chemical and pharmacological proprieties for some antifungal compounds

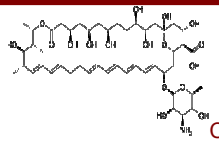
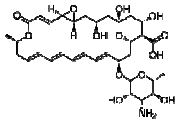
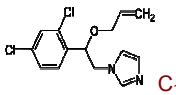
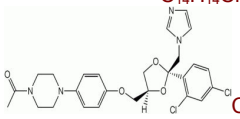
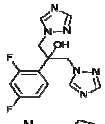
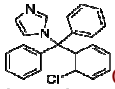
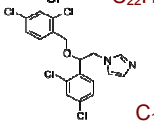
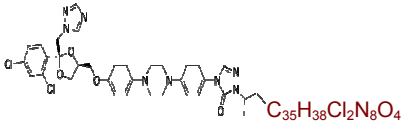
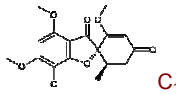
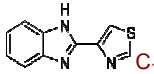
Antifungal	Class	CAS nr	M Mol/kg	Formula and chemical structure	pka	log P	Water solubility	Bioav. %	Half life h	Removal
Nystatin	Poliene antifungal	1400.61.9	926.09	 $C_{47}H_{75}NO_{17}$	4.5	2.81	360 mg/l water	Gastro-intestinal abs. is insignificant	-	Most orally administered nystatin is passed unchanged in the faces
Natamycin	Poliene antifungal	7681.93.8	665.725	 $C_{33}H_{47}NO_{17}$		3.45	4100 mg/L water	Gastro-intestinal abs. is insignificant	-	-
Enilconazole (Imazalil)	Azole antifungal	35554.4.0	297.18	 $C_{14}H_{14}Cl_2N_2O$	6.5	3.82	180 mg/l water at pH 7.6	-	2	90%
Ketoconazole	Azole antifungal	65277.42.1	531.431	 $C_{23}H_{29}Cl_3N_5O_3$	4.6	4.4	0.29 mg/l water	84-99	Biphasic Initial phase: 2h. Terminal phase: 8h	75% in faces 13% in urine
Fluconazole	Azole antifungal	86386.73.4	306.271	 $C_{13}H_{12}F_2N_8O$	2.0	0.58	1 mg/L water	>90	30	61-88
Clotrimazole	Azole antifungal	23593.75.1	344.837	 $C_{22}H_{17}ClN_2$	6.1	6.1	30 mg/ml water	90% Poorly absorbed oraly	2	
Miconazole	Azole antifungal	22916.47.8	416.127	 $C_{18}H_{14}Cl_4N_2O$	6.5	6.1	100mg/100ml water	25-30%	24	50% unchanged in faces 20% in urine (metabolits)
Itraconazole	Azole antifungal	84625.61.6	705.64	 $C_{35}H_{38}Cl_2N_8O_4$	3.7	6.5	Insol in water	55	21	~3% - 18% in feces; ~0.03% of parent drug in urine 40% as inactive metabolites in urine
Griseofulvin	Micotic inhibitors	126.07.8	352.766	 $C_{17}H_{17}Cl_8$	4.4	2.2	Insol in water	25-70	9-21	<1% unchanged in urine metabolits in urine, faces
Thiabendazole	Benzimidazole antifungal	148.79.8	201.249	 $C_{10}H_7N_3S$	4.6	2.2	Insol in water	>90	8	90%urine 5% feces

Table nr 3

***In vivo* toxicity study**

Compound	Toxicological effects observed in animal studies
Natamicin	increased number of fetuses born dead in rats (100 mg/kg)
Enilconazole (imzalil)	fenotoxic in rats, mice and rabits (5-20mg/kg.bw) likely to be carcinogenic to humans
Ketoconazol	ketoconazole is classified as FDA pregnancy category C. teratogenicity (ie, syndactylia and oligodactylia), embryotoxicity, maternal toxicity, and dystocia have been observed in rats given oral, possibly due to the sensitivity of female rats ketoconazole has been shown to be present in human milk.
Fluconazole	dose-dependent cytotoxicity (in rats)
Clotrimazole	embryotoxic in rats and mice (50 to 120 mg/kg.bw.)
Itraconazole	maternal dosedependent toxicity, embryotoxicity, and teratogenicity in dogs (40 - 160 mg/kg.bw.) increased weight of adrenals and ovaries in rats at 40mg/kg.bw. induced bone defects at < 20 mg/kg/day in rats.
Parconazole	reduction in body weight, colestasis and hepatocellular at dogs.
Griseofulvin	congenital malformations in kittens (500-1000 mg griseofulvin weekly, to pregnant cats) embryotoxic and teratogenic in rats
Thiabendazole	chronic inflammatory degenerative renal changes in dog (0-125 mg/kg b.w)a two-year study in rats showed heavier thyroid glands at 10 mg/kg body-weight/day in the male animals
Hexaconazole	induced cell tumors of testis in rats (2 yars, 4.7mg/kg b.w) histopathologic changes in adrenal glands of rats (>2.5mg/kg.bw) fenotoxic (> 25mg/kg.bw.)
Propioconazole	reduced testes and epididymis weights in pups (reproduction study, rat, 21mg/kg b.w) increase in the incidence of benign and malignant liver cell tumors in males mice at 2500 ppm
Cyproconazole	increased incidences of resorbtion and dead foetuses (>50mg/kg b.w.)
Penconazole	reduced testes weight with a atrophic changes (1 ear, dog study, >17mg/kg.bw.)
Tebuxconazole	histopathologic changes in adrenal glands (1 ear, dog study, 4.5mg/kg)
Myclobutanil	effects on male reproductive system including reduced testes and atrophy (2 years study, .10mg/kg.bw.)
Flucilazole	reduced testosterone and estradiol level in rats (14days>20mg/kg.bw.) induced cell tumors of testis in rats (2 years, 31mg/kg b.w) fenotoxic and embryotoxic (> 9mg/kg.bw.)
Pachloraz	reduced testes and prostate wight (90days, dog study, >7mg/kg b.w) Increasing the incidence of adenomas and adenocarcinomas of the liver in both males and females mices, at 1300 ppm