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Study on the common teratogenic pathway elicited by the fungicides triazole-derivatives

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Abstract

Triazole-derivatives alter the pharyngeal apparatus morphogenesis of rodent embryos cultured in vitro. The hindbrain segmentation and the rhombencephalic neural crest cell (NCCs) migration are altered by Fluconazole exposure in vitro. The aim of the present work is to identify if a common pathogenic pathway is detectable also for other molecules of this class of compounds.

9.5 days *post coitum* (d.*p.c.*) old rat embryos were exposed in vitro to the teratogenic concentrations of Flusilazole, Triadimefon and Triadimenol and cultured for 24, 48 or 60 h. The expression and localisation of Hox-b1 and Krox-20 proteins (used as markers for hindbrain segmentation) were evaluated after 24 h of culture. The localisation and distribution of NCC was evaluated after 24, 30 and 48 h of culture. The morphology of the embryos was analysed after 48 h, while the branchial nerve structures were evaluated after 60 h of culture.

Hindbrain segmentation and NCC migration alteration as well as pharyngeal arch and cranial nerve abnormalities were detected after exposure of the tested molecules.

A common severe teratogenic intrinsic property for the tested molecules of this chemical class has been found, acting through alteration of the normal hindbrain developmental pattern.

Keywords: Facial malformations; Flusilazole; Neural crest cells; Triadimefon; Triadimenol

1. Introduction

In toxicological research, the study of pathogenic pathways and mechanisms inducing toxic effects is a topical subject classifying xenobiotics on the basis of their specific biological activity. Teratogenic molecules exert their toxic effects on the complex of cells and tissues that co-operate to permit the embryo development. At present, the pathological pathway for only a few developmental toxicants has been clearly identified (Giavini and Menegola, 2004), so the risk assessment of teratogens is usually made on the basis of the hazard evaluation of each molecule rather than on the overall evaluation of the intrinsic teratogenic properties of their specific chemical class.

On the basis of our previous data, indicating that some antifungals derived from triazole have common teratogenic effects in rodent embryos developing in vitro, the aim of the present work is to identify a common pathogenic pathway for this class of compounds.

Triazole-derivatives are effective antifungals widely used clinically and as systemic agriculture fungicides (Fig. 1).

Abbreviations: FON, triadimefon; NOL, triadimenol; FLUSI, flusilazole; FLUCO, fluconazole; NOAELs, no observed effect level; BAs, branchial arches; NCCs, neural crest cells; rhombomere, r; NCFZs, neural crest free zones; CRABP, cellular retinoic acid binding protein; RA, retinoic acid.

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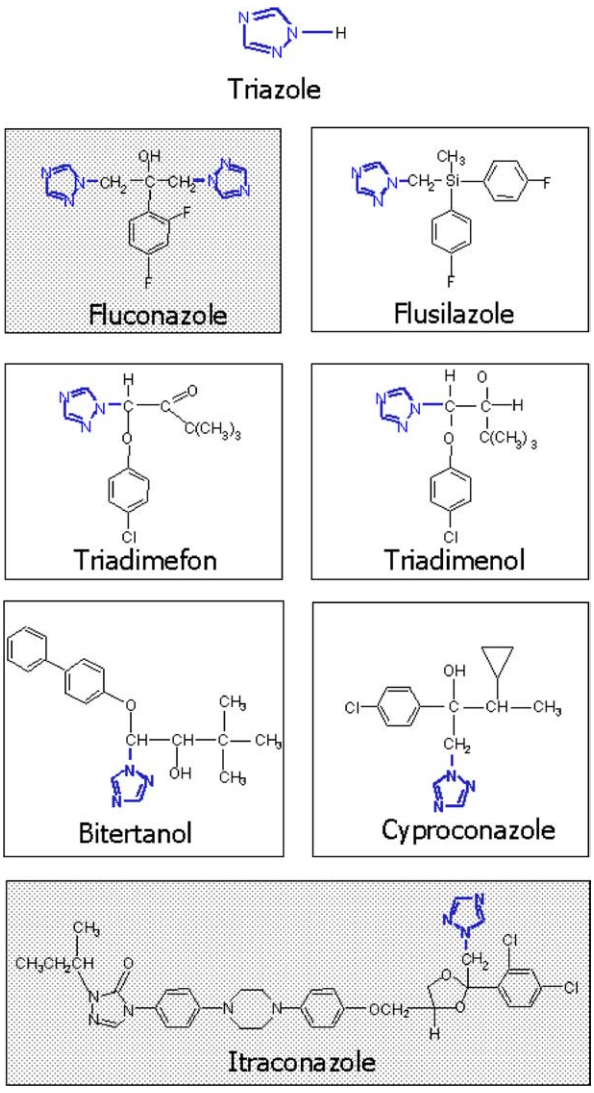

Fig. 1. Chemical structures of the triazole and some triazole-derivatives. ▨ Drugs used in the clinical treatment of fungal infections.

In vitro studies, using the rodent post-implantation whole embryo culture method (WEC), have shown a specific dysmorphogenetic activity of some triazoles on mouse (Tiboni, 1993) and rat embryos (Menegola et al., 2000, 2001, 2003). The triazole-derivatives (Triadimefon (FON), Triadimenol (NOL), Flusilazole (FLUSI) and Fluconazole (FLUCO)) demonstrated specific teratogenic effects at the pharyngeal apparatus level, with reduction, agenesis and fusion between pharyngeal arches (BAs), while the triazole itself was unable to induce these kinds of malformations, even at very high concentrations (5000 μM). The comparison of effective concentrations showed the differing teratogenic potential of the four molecules, with greater activity for the monoderivatives (FLUSI, FON, NOL) compared to the bistriazole (FLUCO). The NOAELs (No Observed Adverse Effect Levels), in fact, were: 3.125 μM for FLUSI, 6.25 μM for NOL, 12.5 μM for FON and 62.5 μM for FLUCO. In mammals, BAs are five iterative, metameric structures giving rise to the masticatory, pharyngeal, and laryngeal apparatuses (Barghusen and Hopson, 1979; Langille and Hall, 1989) (Fig. 2). BAs are composed of epithelially-covered mesenchymal bars of paraxial mesoderm and neural crest cells (NCCs) that originate from the developing hindbrain, to migrate and arch around the ventro-lateral wall of the primordial oral cavity and pharyngeal foregut (Le Douarin, 1982; Bronner-Fraser, 1993).

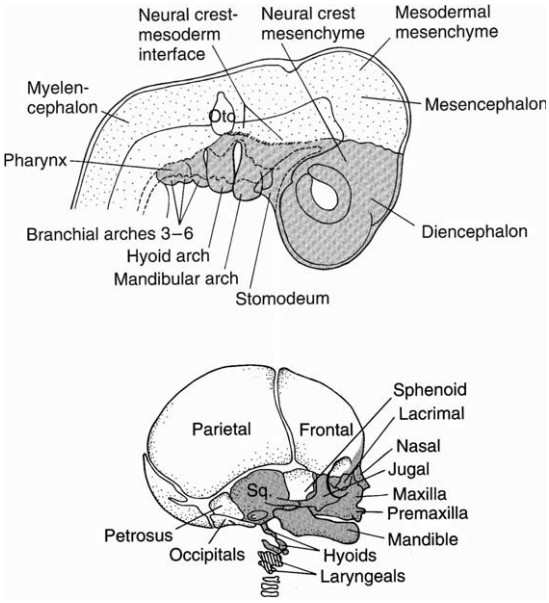

Fig. 2. Neural crest contribution to the skull in mammals.

The hindbrain (rhomboencephalon, the embryonic more caudal encephalic vesicle) is transiently subdivided into 7–8 neuroepithelial compartments (rhombomeres, r) (Vaage and Weiss, 1969; Lumsden and Keynes, 1989).

The migration of the rhombencephalic NCCs into BAs is coincident with their craniocaudal origin: cells originating from rhombomere 1 (r1) and 2 (r2) contribute, together with mesencephalic NCCs, to BA1; BA2 is colonized by r4 cells, and r6-7-8 contribute to BA3–6 (Kulesa et al., 2000; Kulesa and Fraser, 2000; Lumsden et al., 1991; Osumi-Yamashita et al., 1994; Sadaghiani and Theibaud, 1987; Serbedzija et al., 1992; Trainor and Tam, 1995). During the migration processes, three basic streams of NCC are visible from the rhombencephalic vesicles, entering respectively BA1, BA2 and BA3–6 (Hunt et al., 1991; Lumsden et al., 1991; Serbedzija et al., 1992; Trainor and Tam, 1995). Although small numbers of r3 and r5 NCCs appear to join up with the even-numbered rhombomeric streams and make a limited contribution to the arches, in rodents the three NCC streams are separated from each other by crest-free zones in regions adjacent to r3 and r5 (neural crest