Breckling, B. & Verhoeven, R. (2013) GM-Crop Cultivation – Ecological Effects on a Landscape Scale.
Theorie in der Ökologie 17. Frankfurt, Peter Lang.

Teratogenesis by glyphosate based herbicides and other pesticides. Relationship with the retinoic acid pathway

Andrés Carrasco

Lab. Molecular Embryology, School of Medicine UBA, Conicet, Argentina

In South America, the incorporation of genetically modified organisms (GMO) engineered to be resistant to pesticides changed the agricultural model into one dependent on the massive use of agrochemicals (Teubal et al. 2005; Teubal 2009). Different pesticides are used in response to the demands of the global consuming market to control weeds, herbivorous arthropods, and crop diseases.

A recent study using a commercial formulation of glyphosate based herbicides (GBH) showed that treatments with a 1/5000 dilution (430 μM of glyphosate) were sufficient to induce reproducible malformations in embryos of the South African clawed frog Xenopus laevis, a widely used vertebrate model for embryological studies (Paganelli et al. 2010). The phenotypes observed include shortening of the trunk, cephalic reduction, microphthalmy, cyclopia, reduction of the neural crest territory at neurula stages and craniofacial malformations at tadpole stages. In addition GBH inhibits the anterior expression domain of the morphogen Sonic Hedgehog (shh) and reduces the domain of the cephalic marker otx2, prevents the subdivision of the eye field and impairs craniofacial development. Moreover, in recent experiments with another commercial formulation of GBH, the malformations observed before were reproduced in a dose-dependent manner, even at dilutions of 1/500000, which produced developmental abnormalities in 17% of the embryos, without lethality (unpublished results).

It is known that glyphosate penetration through the cell membrane and subsequent intracellular action is greatly facilitated by adjuvants such as surfactants. For this reason, the active principle was also tested by injecting frog embryos with glyphosate alone (between 8 and 12 μM per injected cell). The calculated intracellular concentration for glyphosate injected into embryos was 60 times lower that the glyphosate concentration present in the 1/5000 dilution of the GBH which was used to culture whole embryos. The injection of glyphosate produced similar phenotypes and changes in gene expression, suggesting that the effects are attributable to the active principle of the herbicide.

It is very well known that acute or chronic increase of retinoic acid (RA) levels leads to teratogenic effects during human pregnancy and in experimental models. The characteristic features displayed by RA embryopathy in humans include brain abnormalities such us microcephaly, microphtalmia and impairment of hindbrain development; abnormal

external and middle ears (microtia or anotia), mandibular and midfacial underdevelopment, and clefts palate. These craniofacial malformations can be attributed to defects in cranial neural crest cells. An excessive cell death in regions where apoptosis normally takes place may underlie a general mechanism for craniofacial malformations associated to teratogens (Sulik et al. 1988, Clotman et al. 1998).

In fact, an excess of RA signaling is able to down-regulate the expression of shh in the embryonic dorsal midline in Xenopus (Franco et al. 1999, Sharpe & Goldstone 2000). Shh deficiency is associated to the holoprosencephaly syndrome (HPE), a CNS malformation with a frequency of 1 / 250 of pregnancies and 1 / 10000 of live births. The HPE is a defect generated by the deficiency of the embryonic dorsal midline, which results in a failure in the division of the brain hemispheres, leading to different grades of craniofacial malformations. Moreover, Shh signaling is also necessary for the development of the cranial neural crest derivatives. In mouse, specific removal of the Shh responsiveness in the neural crest cells that give rise to skeleton and connective tissue in the head, increases apoptosis and decreases proliferation in the branchial arches, leading to facial truncations. In addition Shh signaling from the ventral midline is necessary, as an anti-apoptotic agent, for the survival of the neural epithelium and it is also essential for the rapid and extensive expansion of the early vesicles of the developing midbrain and forebrain (Charrier et al. 2001)

An excess of RA signaling also down-regulates otx2 expression in Xenopus, chicken and mouse embryos (Clotman et al. 1998). Knock-out mice for otx2 lack all the brain structures anterior to rhombomere 3. Interestingly, heterozygous mutants showed craniofacial malformations including loss of the eyes and lower jaw (agnathia). These phenotypes are reminiscent of otocephaly reported in humans and other animals and suggest that otx2 plays an essential role in the development of cranial skeletons of mesencephalic neural crest origin (Matsou et al. 1995, Kimura et al. 1997, Erlich et al. 2000).

All this evidence indicates that RA, otx2 and shh are part of a genetic cascade critical for the development of the brain and craniofacial skeleton of neural crest origin. Glyphosate inhibits the anterior expression of shh, reduces the domain of otx2, prevents the subdivision of the eye field and impairs craniofacial development, resembling aspects of the holoprensecephalic and otocephalic syndromes (Geng & Oliver 2009). Indeed, assays using a RA-dependent gene reporter revealed that GBH treatment increases the endogenous RA activity in Xenopus embryos. Moreover, an antagonist of RA rescued the morphological phenotype produced by GBH. This lead to the conclusion that at least some of the teratogenic effects of GBH were mediated by increased endogenous RA activity in the embryos (Paganelli et al. 2010). This is consistent with the very well known syndrome produced by excess of RA, as described by the epidemiological study of Lammer et al. in humans (Lammer et al. 1985) and in vertebrate embryos (Durston et al. 1989, López & Carrasco 1992, López et al. 1995, Padmanabhan 1998).

In Xenopus embryos, the endogenous activity of retinoids gradually increases during early embryogenesis and is finely regulated in space. Therefore maintaining a normal endogenous distribution of RA is important for axial patterning and organogenesis in vertebrates (Chen et al. 1994, López et al. 1995)

It has been reported that triadimefon, a systemic fungicide with teratogenic effects in rodent models, produces craniofacial malformations in Xenopus laevis by altering endogenous RA signalling (Papis et al. 2007). Arsenic, another endocrine disruptor, also increases RA signaling at low, non-cytotoxic doses, in human embryonic NT2 cells (Davey et al. 2008). In addition, atrazine produces teratogenic effects and decreases the levels of cyp26 transcripts in Xenopus tadpoles, suggesting that this herbicide also disrupts the RA signaling pathway (Lenkowski et al 2008, Lenkowski & McLaughlin 2010). RA signaling is one of the finest pathways to tune up gene regulation during development, and all this evidence raises the possibility that disturbances in RA distribution may be a more general mechanism underlying the teratogenic effects of xenobiotics in vertebrates. Since mechanisms of development are highly conserved in evolution among vertebrates, we would like to stress that they could be useful as very sensitive biosensors to detect undesirable effects of new molecules.

The evidence that links GBH (and potentially other chemicals) to increased activity of the RA signaling pathway might explain the higher incidence of embryonic malformations and spontaneous abortions observed in populations exposed to pesticides.

An important evidence came from the epidemiological study carried out by Benitez-Leite et al. in Paraguay identified 52 cases of malformations in the offspring of women exposed during pregnancy to agrochemicals. The congenital malformations observed include anencephaly, microcephaly, facial defects, myelomeningocele, cleft palate, ear malformations, polydactily, syndactily (Benítez-Leite et al. 2009). These defects are indeed consistent with the well-known and expected syndrome caused by misregulation of the RA pathway.

These conclusions should be taken into account together with the incidence of malformations and cancer in Chaco, an Argentine province with soybean harvest and massive use of glyphosate. Official records reveal a 4-fold increase in developmental malformations in the province and a 3-fold increase of cancer in the locality of La Leonesa in the last decade (Comisión Investigadora 2010).

All this information is extremely worrying because the risk of environmental-induced disruptions in human development is highest during the critical period of gestation (2 to 8 weeks). Moreover, the mature human placenta has been shown to be permeable to glyphosate. After 2.5 hr of perfusion, 15 % of administered glyphosate is transferred to the fetal compartment (Poulsen et al. 2009). Indeed, a two-compartment model study suggested that a considerable diffusion of glyphosate into the tissue is reached after intravenous administration in rats. These authors conclude that direct blood concentration is only an average indicator of the presence of the chemical and does not provide

evidence about its tissue distribution (Anadón et al. 2009). It is necessary to consider the possibility that very low concentrations (pg/cell and not necessarily evenly distributed to all cells) may be sufficient to cause embryonic lethality (which is consistent with increased frequency of embryonic death and spontaneous abortions) or to modify normal embryonic pattern formation (Antoniou et al. 2011).

References

Anadón, A., Martínez-Larrañaga, M.R., Martínez, M.A., Castellano, V.J., Martínez, M., Martin, M.T. et al. (2009) Toxicokinetics of glyphosate and its metabolite aminomethyl phosphonic acid in rats. Toxicology letters, 190(1): 91–95.

Antoniou, M., Habib, M.E.E.-D.M., Howard, C.V., Jennings, R.C., Leifert, C., Nodari, R.O. et al. (2011) Roundup and birth defects. Is the public being kept in the dark?, Earth Open Source Org. (June 2011). http://www.earthopensource.org/index.php/reports/17-roundup-and-birth-defects-is-the-public-being-kept-in-the-dark

Benítez-Leite, S., Macchi, M.L., Acosta, M. (2009) Malformaciones congénitas asociadas a agrotóxicos, Archivos de Pediatría del Uruguay, 80: 237–247.

Charrier, J.B., Lapointe, F., Le Douarin, N.M., a Teillet, M. (2001) Anti-apoptotic role of Sonic hedgehog protein at the early stages of nervous system organogenesis. Development. 128: 4011–4020.

Chen, Y., Huang, L., Solursh, M. (1994) A concentration gradient of retinoids in the early Xenopus laevis embryo. Developmental Biology 161: 70–76.

Clotman, F., van Maele-Fabry, G., Chu-Wu, L., Picard, J.J (1098) Structural and gene expression abnormalities induced by retinoic acid in the forebrain. Reproductive Toxicology. 12: 169–176.

Comisión Investigadora de contaminantes del agua de la Provincia del Chaco (2010) Informe de la Comisión Investigadora de contaminantes del agua de la Provincia del Chaco, Resistencia, Chaco. Argentina.

Davey, J.C., Nomikos, A.P., Wungjiranirun, M., Sherman, J.R., Ingram, L., Batki, C. et al. (2008) Arsenic as an endocrine disruptor: arsenic disrupts retinoic acid receptor-and thyroid hormone receptor-mediated gene regulation and thyroid hormone-mediated amphibian tail metamorphosis. Environmental Health Perspectives. 116: 165–172.

Durston, A.J., Timmermans, J.P., Hage, W.J., Hendriks, H.F., de Vries, N.J., Heideveld, M. et al. (1989) Retinoic acid causes an anteroposterior transformation in the developing central nervous system. Nature. 340: 140–144.

Erlich, M.S., Cunningham, M.L., Hudgins, L. (2000) Transmission of the dysgnathia complex from mother to daughter. American Journal of Medical Genetics. 95: 269–274.

Franco, P.G., Paganelli, A.R., López, S.L., Carrasco, A.E. (1999) Functional association of retinoic acid and hedgehog signaling in Xenopus primary neurogenesis. Development. 126: 4257–4265.

Geng, X., Oliver, G. (2009) Pathogenesis of holoprosencephaly, J. Clin. Invest. 119 1403-1413.

Kimura, C., Takeda, N., Suzuki, M., Oshimura, M., Aizawa, S., Matsuo, I. (1997) Cis-acting elements conserved between mouse and pufferfish Otx2 genes govern the expression in mesencephalic neural crest cells. Development. 124: 3929–3941.

Lammer, E.J., Chen, D.T., Hoar, R.M., Agnish, N.D., Benke, P.J., Braun, J.T. et al. (1985) Retinoic acid embryopathy. New England Journal of Medicine 313: 837–841.

Lenkowski, J.R., McLaughlin, K.A. (2010) Acute atrazine exposure disrupts matrix metalloproteinases and retinoid signaling during organ morphogenesis in Xenopus laevis, Journal of Applied Toxicology. 30: 582–589.

Lenkowski, J.R., Reed, J.M., Deininger, L., McLaughlin, K.A. (2008) Perturbation of organogenesis by the herbicide atrazine in the amphibian Xenopus laevis. Environmental Health Perspectives. 116: 223–230.

López, S.L., Carrasco, A.E. (1992) Retinoic acid induces changes in the localization of homeobox proteins in the antero-posterior axis of Xenopus laevis embryos. Mechanisms of Development. 36: 153–164.

López, S.L., Dono, R. Zeller, R., Carrasco, A.E. (1995) Differential effects of retinoic acid and a retinoid antagonist on the spatial distribution of the homeoprotein Hoxb-7 in vertebrate embryos. Developmental Dynamics. 204: 457–471.

Matsuo, I., Kuratani, S., Kimura, C., Takeda, N., Aizawa, S. (1995) Mouse Otx2 functions in the formation and patterning of rostral head. Genes & development. 9: 2646–2658.

Padmanabhan, R. (1998) Retinoic acid-induced caudal regression syndrome in the mouse fetus. Reproductive Toxicology 12: 139–151.

Paganelli, A., Gnazzo, V., Acosta, H., López, S.L., Carrasco, A.E. (2010) Glyphosate-Based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signaling. Chemical Research in Toxicology 23: 1586–1595.

Papis, E., Bernardini, G., Gornati, R., Menegola, E., Prati, M. (2007) Gene expression in Xenopus laevis embryos after Triadimefon exposure. Gene Expression Patterns. 7: 137142.

Poulsen, M.S., Rytting, E., Mose, T., Knudsen, L.E. (2009) Modeling placental transport: correlation of in vitro BeWo cell permeability and ex vivo human placental perfusion. Toxicology in Vitro. 23: 1380–1386.

Sharpe, C., Goldstone, K., (2000) Retinoid signalling acts during the gastrula stages to promote primary neurogenesis. International Journal of Developmental Biology 44: 463–470.

Sulik, K.K., Cook, C.S., Webster, W.S. (1988) Teratogens and craniofacial malformations: relationships to cell death. Development. 103 Suppl: 213–231.

Teubal, M. (2009) Expansión del modelo sojero en la Argentina. De la producción de alimentos a los commodities. In: Lizarraga, P., Vacaflores, C. (Eds.) La persistencia del campesinado en América Latina, Comunidad de Estudios JAINA, Tarija: pp. 161–197.

Teubal, M., Domínguez, D., Sabatino, P. (2005) Transformaciones agrarias en la Argentina. Agricultura industrial y sistema agroalimentario. In: Giarracca, N., Teubal, M. (Eds.) El campo argentino en la encrucijada. Estrategias y resistencias sociales, Ecos en la ciudad, Alianza Editorial, Buenos Aires: pp. 37–78.