

Mycophenolic acid impairs growth and differentiation of rat embryos *in vitro* at subtherapeutic concentrations

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Introduction

Mycophenolate mofetil is a widely used immunosuppressive drug which recently has been categorized as a human teratogen. A number of case reports have been published describing malformations after prenatal exposure to mycophenolate mofetil. However, human data are not unequivocal, because pregnant women had been treated with combinations of several immunosuppressive agents.

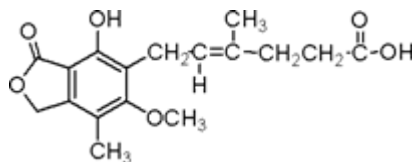
In addition, the interpretation of teratogenicity studies performed in rats and rabbits during the preclinical development of the drug, is hampered due to species differences of the pharmacokinetics of mycophenolate mofetil in animals and humans. So far, the drug has not been studied in embryotoxicity *in vitro* assays.

The aim of this study was to evaluate the *in vitro* embryotoxic potential of mycophenolic acid, the active moiety of mycophenolate mofetil, with the rat whole-embryo-culture system.

Material and Methods

Test compound:

Mycophenolic acid was purchased from Alexis Biochemicals. The substance was dissolved in DMSO and added to the culture medium to reach a final DMSO-concentration of 0.1%.



Animals:

Wistar rats (Wistar unilever / spf; Harlan-Winkelmann GmbH)

Whole embryo culture:

We cultured rat embryos (n = 58) from gestational day 9.5 for 48 hours and exposed them to mycophenolic acid at six concentrations ranging from 0.1 to 2.0 mg/l. Control embryos were cultured in medium with 0.1% DMSO. Four embryos were cultivated in one bottle containing 6 ml heat-inactivated and sterile-filtered serum mixture for WEC (Biochrom AG), composed of 85% serum (90% bovine serum and 10% rat serum), 15% Hanks balanced salt solution, 75 µg/ml methionine and 1.57 mg/ml glucose. The bottles were gased initially with 10% O₂, 5% CO₂ and 85% N₂ and after 36 hours of culture they were re-gased with 50% O₂, 5% CO₂ and 45% N₂. At the end of the culture period, growth and differentiation of each embryo was evaluated by means of a morphological scoring system and analyzed with a statistic program (SPSS Version 16.0).

Results

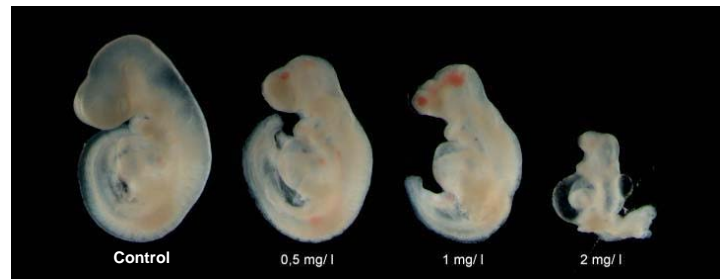


Fig. 1: Rat embryos exposed *in vitro* for 48 hours to increasing concentrations of mycophenolic acid

Mycophenolic acid caused dysmorphogenic development at a concentration as low as 0.25 mg/l. At concentrations of 0.75 mg/l and more mycophenolic acid caused abnormalities in all embryos. The main effects were defective yolk sac blood circulation, neural tube defects (open cranial neural pore), malformations of the head with missing eye anlagen and heart defects. Moreover, the exposed embryos showed a concentration-dependent decrease in protein content, crown-rump-length, number of somites and morphological score.

Table 1: Effects of mycophenolic acid on growth and development of rat embryos after 48 hours of culture

Concentration [mg/l]	N	Yolk sac blood circulation	Crown rump length [mm]	Protein [µg/embryo]	Somites [#] [n]	Score	Dysmorphogenesis N [%]
0	10	3.0±0.0	3.04±0.11	126.2±15.4	25.4±1.3	35.2±3.7	0 0
0.10	8	3.0±0.0	2.90±0.15	116.5±21.1	24.5±2.3	34.4±3.1	0 0
0.25	8	2.8±0.7	3.10±0.21	127.4±23.8	25.5±0.9	35.1±2.8	3 38
0.50	8	2.9±0.4	3.14±0.25	138.5±31.9	25.3±2.2	34.9±3.2	4 50*
0.75	8	2.4±0.9	2.80±0.39	108.0±39.4	22.1±2.5*	28.8±5.3**	8 100**
1.00	8	2.3±0.9*	2.77±0.28	95.0±26.6	18.9±3.1**	27.9±3.6**	8 100**
2.00	8	1.0±0.0**	1.50±0.15**	28.5±4.5**	n.d.	13.6±0.7**	8 100**

* N = 6 to 10

Legend: Data are shown as mean ± standard deviation, N = Number of analyzed embryos at each concentration, n.d. = not determined, statistical significance compared to the control group: * - p ≤ 0.05, ** - p ≤ 0.01

Discussion

In humans, peak concentrations achieved in the plasma of the patients are approximately ten times higher than the maximum concentration tested in our experiments. Using the rat whole-embryo-culture system we showed that mycophenolic acid causes dose-dependent dysmorphogenic effects on the developing rat embryo *in vitro* at subtherapeutic concentrations.