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The different effects on cranial and trunk neural crest cell behaviour following exposure to a low concentration of alcohol *in vitro*

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ABSTRACT

Embryonic neural crest cells give rise to large regions of the face and peripheral nervous system. Exposure of these cells to high alcohol concentrations leads to cell death in the craniofacial region resulting in facial defects. However, the effects of low concentrations of alcohol on neural crest cells are not clear. In this study, cranial neural crest cells from Xenopus laevis were cultured in an ethanol concentration approximately equivalent to one drink. Techniques were developed to study various aspects of neural crest cell behaviour and a number of cellular parameters were quantified. In the presence of alcohol, a significant number of cranial neural crest cells emigrated from the explant on fibronectin but the liberation of individual cells was delayed. The cells also remained close to the explant and their morphology changed. Cranial neural crest cells did not grow on Type 1 collagen. For the purposes of comparison, the behaviour of trunk neural crest cells was also studied. The presence of alcohol correlated with increased retention of single cells on fibronectin but left other parameters unchanged. The behaviour of trunk neural crest cells growing on Type 1 collagen in the presence of alcohol did not differ from controls. Low concentrations of alcohol therefore significantly affected both cranial and trunk neural crest cells, with a wider variety of effects on cells from the cranial as opposed to the trunk region. The results suggest that low concentrations of alcohol may be more detrimental to early events in organ formation than currently suspected.

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1. Introduction

Fetal Alcohol Spectrum Disorder (FASD) is a collection of birth defects thought to result from prenatal alcohol exposure[1] (Barr and Steissguth, 2001). While cognitive impairment is a major characteristic of FASD[2,3,4] (Mattson and Riley, 1998) facial anomalies may also develop and constitute a major feature.[5]

A large contribution to the face is made by embryonic neural crest cells which arise along the length of the neural tube but with regional differences in their appearance and behaviour.[6] Cranial neural crest cells give rise to the facial skeleton as well as sensory neurons, supporting cells and parts of the teeth[7,8] while, in contrast, trunk neural crest cells form neurons but also form large numbers of pigment cells.[9,10] Recent reviews of the neural crest include Strobl-Mazzulla and Bronner,[11] Dupin and Coeljo-Agular,[12] Nitzan and Kalcheim[13] and McKeown et al.[14]

The differences between cranial and trunk neural crest are important in FASD because facial abnormalities are common and presumably associated with faulty cranial neural crest development while there is an apparent absence of trunk neural crest abnormalities. Therefore there might be significant differences between the two cell populations in their responses to alcohol and knowledge of these differences would provide a better understanding of FASD.

High concentrations of alcohol *in vivo* (corresponding to binge drinking or chronic alcohol use) correlate with neural crest cell death in the craniofacial region and facial malformations.[6,15,16] In contrast, the effects of low alcohol concentrations are less clear because they have been less well studied.[17,18] This is surprising because understanding the effects of low alcohol concentrations is important as many pregnant women may feel that moderate alcohol use will not harm the embryo or foetus.

In general, there has also been limited work using tissue culture techniques. Both high and low alcohol levels have been used in tissue culture studies[19–27] but low alcohol concentrations have received less attention.[19,21] This is again surprising because the *in vivo* work has shown that low alcohol concentrations can cause intracellular changes within neural crest cells and morphological changes of embryos.[17,18] While tissue culture techniques can be criticized for being far removed from the *in vivo* environment, the results show that significant changes can be observed and quantified for specific groups of cells.

Accordingly, this investigation used cranial and trunk neural crest cells from the amphibian *Xenopus laevis*, a well-recognized and accepted model for studying neural crest cell behaviour.[28,29] The effects on the cells of a low concentration of alcohol (0.5 mg/ml or 10.9 mM) were evaluated using two different substrates (Type 1 collagen and fibronectin). This alcohol concentration was chosen because this blood alcohol level can be reached in a 100 lb woman after one drink[30] (http://oade.nd.edu/educate-yourself-alcohol/blood-alcohol-concentration/). While the primary focus was on cranial neural crest, trunk neural crest cells were also studied for comparison. The results suggest that neural crest cells from both regions can be affected by alcohol but significant differences in cell behaviour between the two populations of cells were also found. Interestingly, there were also differences in the responses of the two different populations to the different substrates.

2. Materials and methods

2.1. Animals

This research was carried out under a license from the Animal Care and Use Committee, Faculty of Medicine and Dentistry, University of Alberta.

Amplexus was induced in adult *Xenopus laevis* with subcutaneous chorionic gonadotropin injections (Sigma). The NASCO (company which supplies the frogs) protocol was used: Day 1 – 9AM – male – 150 IU; Day 2 – 9 AM – male – 150 IU, female – 250 IU; Day 2 – 5 PM – female – 500 IU. Embryos were also obtained from the Department of Biological Sciences, University of Alberta breeding colony. Stage 19–21 embryos were collected.[31–33] At this stage cranial neural crest cells have moved part way down the head in several easily identifiable groups while trunk neural crest cells have not yet started migration.

2.2. Neural crest isolation

2.2.1. Cranial

Each embryo was placed on its side in a depression cut into a 2% agar operating base. The embryo was bathed in Danilchik's medium without serum [NaCl – 53 mM; Na_2CO_3 – 10 mM; K-Gluconate – 4.25 mM; $MgSO_4$ – 1 mM; $CaCl_2{\cdot}2H_2O$ – 1 mM; Bicine 20 mM; Gentamicin – 10 mg; dH_2O – 200 ml[27] – pH 7.4]. Cactus spikes (Echinopsis and Mammillaria species) attached to holders were used for dissections.

The small bulge made by the cranial neural crest under the ectoderm was always clearly visible using a dissecting microscope for viewing. The ectoderm over the neural crest was easily removed revealing the well-defined grey-coloured neural crest cell masses on top of the pale yellow mesoderm. The neural crest masses posterior to the eye were teased off of the mesoderm and cut away from the sides of the brain. Other investigators have used and described this technique previously, possibly because the neural crest and mesoderm can be distinguished from one another.[34–36] In particular, DeSimeone et al.[35] and Theveneau et al.[36] have also commented on the distinct difference in colouration between the neural crest and underlying mesoderm. DeSimeone et al.[35] note that the difference in colouration can help the investigator to distinguish clearly the ventral edge of the tissue.

2.2.2. Trunk

Embryos were placed dorsal side uppermost. The ectoderm was removed and the grey neural crest tissue was easily identified and removed as a continuous cell strand.

2.3. Media and substrates

Two different substrates (Type I collagen- rat tail or calf skin or bovine plasma fibronectin) were used. Separate plastic Falcon petri dishes (10 mm × 35 mm) were coated with one of these substrates at concentrations of 0.26 μg/mm^2 and 0.06 μg/mm^2