Can Gene Therapy For Alcoholism Lead to Gastrointestinal Cancer?

Nengding Wang*
Department of Biology
Lake Forest College
Lake Forest, Illinois 60045

Introduction

Alcoholism is defined as physical dependence on alcohol and loss of control over alcohol intake (Retrieved from MayoClinic). This addiction can have severe health, economic, and work-related consequences (Retrieved from MayoClinic). Many factors such as mental health and social relationships have been found to affect the likelihood of an individual becoming alcohol-dependent, and genetics is one of them (Retrieved from MayoClinic). Three decades ago scientists noticed a strange phenomenon in certain Asian populations: after consuming a small amount of alcohol, they begin to experience facial flushing, chest palpitations, and dizziness (Mizoi *et al.*, 1979). Research later revealed a mutation in a specific gene-aldehyde dehydrogenase 2 (ALDH2) that leads to the inactive form of the enzyme which causes these symptoms which are collectively known as the alcohol flush reaction (Yoshida *et al.*, 1984). Normal ALDH2 can break down acetaldehyde, the byproduct of ethanol metabolism; however, inactive ALDH2 cannot (Zakhari, 2006). Scientists have now made use of the inactive ALDH2 phenotype to treat alcoholism; that is, they can elicit the alcohol flush reaction in patients so as to discourage them from further drinking.

Ocaranza *et al.* (2008) found that a single administration of an anti-*Aldh2* antisense gene can significantly inhibit ethanol intake in alcohol-dependent rats. This gene therapy works by inactivating ALDH2 which consequently elevates acetaldehyde levels (Ocaranza *et al.*, 2008). Though this treatment has been shown to be more effective than disulfiram (Antabuse®), a drug currently on the market, Ocaranza *et al.* (2008) have yet to look at the consequences of acetaldehyde buildup in the treated rats. It is important to carry out a follow up because acetaldehyde is a known carcinogen that can lead to the formation of cancer-causing DNA adducts such as $1,N^2$-propanodeoxyguanosine (Zakhari, 2006). People with the defective ALDH2 phenotype have exhibited higher risks for gastrointestinal cancer than people with the normal phenotype (Yokoyama *et al.*, 1998), thus it is critical to assess the effects of inducing acetaldehyde accumulation by inactivating ALDH2 in rats treated with anti-*Aldh2* antisense gene.

Specific Aims

The first goal is to determine whether administering anti-*Aldh2* antisense gene induces a high rate of cell differentiation (hyperproliferation) and an increase in the number of cells (hyperplasia) in the gastrointestinal tract of alcohol-dependent rats. Abnormal cell differentiation and proliferation are characteristic signs of cancer (Homann *et al.*, 1997). Thus, if the experiment is able to detect both characteristics in the treated rats but not the untreated rats, it can be concluded that cancer has developed after administering the gene therapy. The second goal is to determine whether the reason behind altered differentiation

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and proliferation is elevated acetaldehyde level in the gastrointestinal tract. The hypothesis is that the tissues exhibiting hyperproliferation and hyperplasia in the rats that received the antisense gene treatment have increased acetaldehyde buildup compared to the animals that did not receive the antisense gene treatment.

Experimental Proposal

Two experiments will be conducted to assess the consequences of genetically inactivating ALDH2 in alcohol-dependent rats that are consuming small amounts of ethanol regularly. Both will be extensions of the study done by Ocaranza *et al.* (2008) where they first discovered and tested the efficacy of the anti-*Aldh2* antisense gene treatment. Following the methods used in the Ocaranza *et al.* (2008) study, anti-*Aldh2* adenoviral vector (AdV-AS) that generates the antisense RNA will be prepared. Twenty alcohol-preferring rats bred selectively for their high alcohol intake will either be administered the AdV-AS or the control vector (AdV-control) not carrying the transgene (Ocaranza *et al.*, 2008). From the morning of day 1 onwards, the animals will be allowed access to only 0.6 g/kg of 10% ethanol solution every day for 8 months (Homann *et al.*, 1997; Ocaranza *et al.*, 2008). The amount of alcohol fed to the rats is determined from the Ocaranza *et al.* (2008) study where they found that when the rats treated with AdV-AS were allowed free access to ethanol, they consumed only the aforementioned amount each day. If alcohol intake is not controlled, the rats not treated with the antisense gene will consume significantly more alcohol than the treated rats because the animals are originally alcohol-dependent (Ocaranza *et al.*, 2008).

After the 8 month period, the rats will be killed under ether anesthesia, and tissue specimens will be dissected from the oral cavity, the esophagus, and the stomach. These specific locations from the gastrointestinal tract are chosen because cancers of these areas have been associated with the inactive ALDH2 phenotype (Yokoyama *et al.*, 1998). To look for signs of cancer, the tissue samples will be immunohistochemically stained for markers of cellular differentiation or proliferation (Homann *et al.*, 1997). Light microscopy with an eyepiece containing grid lines will then be used to measure the average epithelial thickness of each sample (Homann *et al.*, 1997). The staining patterns and the epithelial thickness measurements of the rats treated with the antisense gene and the rats treated with the control vector will be compared using the unpaired Student's *t* test (Homann *et al.*, 1997).

If hyperproliferation and hyperplasia can be found in the tissue specimens, a second experiment will be conducted to determine if they are caused by increased levels of acetaldehyde. Tissue samples will be dissected from the same two groups of rats and again from the oral cavity, the esophagus, and the stomach. Using the methods described in Ohata *et al.* (1996), the tissues will be homogenized, and the acetaldehyde present will be derivatized to 2,4-dinitrophenylhydrazone. This substance will then be analyzed with a GC14A gas chromatograph, equipped with a [63]Ni electron-capture detector. This assay can determine minute amounts of acetaldehyde in biological samples (Ohata *et al.*, 1996). Differences between the experimental and control groups will be analyzed by one-way analysis of variance, followed by the Turkey-Kramer multiple comparison test (Visapaa *et al.*, 2002).

For the proposed experiments, there could be two possible outcomes. One is that hyperproliferation and hyperplasia are seen in the tissue samples of the rats treated with the anti-*Aldh2* antisense gene compared to the untreated rats; acetaldehyde level is higher in these tissues as well, which provides evidence that acetaldehyde plays a role in the development of cancerous cells. These results would show that the gene therapy for alcoholism developed by Ocaranza *et al.* (2008) can induce cancer development in the animal model. Thus the proposed treatment would not be safe for human use.

The other possibility is that neither hyperproliferation nor hyperplasia in the rats treated with the anti-*Aldh2* antisense gene can be detected, and there is no significant increase in acetaldehyde level. This would indicate that the newly discovered gene therapy does not increase the risks of gastrointestinal cancer in the animal model. Additionally, there are possible negative consequences associated with applying this treatment to human use that must be considered. An adenoviral vector is used to transport the anti-*Aldh2* antisense gene into the alcohol-dependent rats. The major disadvantage of using such vectors is the possibility of the host mounting an immune response upon vector delivery as adenoviral vectors are based on an extremely common human pathogen (Reece, 2004). Also, Ocaranza *et al.* (2008) did not check for side effects in the alcohol-dependent rats after treatment with the antisense gene. Similar to the anti-*Aldh2* antisense gene treatment, disulfiram discourages alcoholic patients from further drinking by eliciting the inactive ALDH2 phenotype (Garver *et al.*, 2001). However, this drug has many severe side effects such as sensory and motor neuropathies (Garver *et al.*, 2001). Therefore, it would be important to determine whether there are serious side effects associated with this new form of treatment. Further research on both the safety issues of using adenoviral vectors and the possibility of side effects should be included in the next steps taken to determine whether the anti-*Aldh2* antisense gene treatment is safe for clinical use.

Conclusion

The proposed research will determine whether the anti-*Aldh2* antisense gene, which has previously shown to effectively protect against alcoholism, can cause hyperproliferation and hyperplasia (both indicative of cancer) in the gastrointestinal tract when administered to an animal model. The results from these experiments can potentially protect patients from therapies that could lead to cancer, and can also be helpful in the development of better treatments for alcoholism.

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