

Hepatology **1986**, 6, 502–510; Yoshida et al., *Prog. Nucleic Acid Res. Mol. Biol.* **1991**, 40, 255–287). The ADH2*2 allele is associated with the accumulation of acetaldehyde (Crabb et al., *Proc. Nutr. Soc.* **2004**, 63(1), 49-63).

[0006] Also prevalent in East Asian populations is a variant allele of aldehyde dehydrogenase subtype 2 (ALDH2*2) that encodes for an ALDH enzyme with an E487K amino acid substitution (Chen et al., *Am. J. Hum. Genet.* **1999**, 65(3), 795-807). The K487 ALDH enzyme exhibits reduced activity that results in a 40%-90% reduction in the rate of acetaldehyde removal when compared to the more active E487 ALDH2 enzyme encoded by the “typical” allele (ALDH2*1), such that persons who express the variant allele display reduced or absent ALDH2 activity.

[0007] Acetaldehyde is linked to acute symptoms such as facial flushing, tachycardia, shortness of breath, dizziness, nausea, vomiting and headache, as well as to increased long-term health risks for cancers of the upper digestive tract, breast cancer, liver disease, Alzheimer’s disease, hypertension and myocardial infarction (see Visapää et al., *Gut* **2004**, 53, 871-876; Yokoyama et al., *Jpn. J. Clin. Oncol.* **2003**, 33(3), 111-121; Ohsawa et al., *J. Hum. Genet.* **2003**, 48, 404-409; and references cited therein). People who express the ALDH2*2 allele having reduced or absent ALDH2 activity exhibit alcohol-related sensitivity, for example, facial flushing, tachycardia, etc., when drinking small portions of ethanol (Goedde et al., *Hum. Genet.* **1992**, 88, 344-346; Xiao et al., *J. Clin. Invest.* **1995**, 96, 2180-2186). Therefore, reducing acetaldehyde levels in people with reduced or absent ALDH2 activity may be helpful in reducing the acute systems and long-term health risks experienced by these people when they consume ethanol.

[0008] Administration of 4-methylpyrazole (also known as fomepizole or 4-MP) has been shown to ameliorate symptoms of elevated acetaldehyde levels. See, e.g., WO 2005/085392 and Japanese Unexamined Patent Application Publication No. S57-106620. However, it is becoming increasingly evident that 4-MP treatment is not suitable for all human genetic subpopulations. Additional information regarding individualized genetic factors that contribute to effective treatments with 4-MP is sought.

4. SUMMARY OF THE INVENTION

[0008a] In one aspect, there is provided the use of 0.1 milligram to 4 milligrams of 4-methylpyrazole (4-MP) per kilogram of a subject's body mass in the manufacture of a medicament for oral administration for preventing or ameliorating a symptom of ethanol intolerance in a subject with reduced or absent aldehyde dehydrogenase subtype 2 (ALDH2) activity, wherein the subject is homozygous for alcohol dehydrogenase subtype 2*1 (ADH2*1/ADH2*1) or is heterozygous for alcohol dehydrogenase subtypes 2*1 and 2*2 (ADH2*1/ADH2*2).

[0009] In one aspectexample, methods are provided for preventing, reducing or ameliorating a symptom of ethanol intolerance, or acetaldehyde accumulation, in a subject with reduced or absent aldehyde dehydrogenase subtype 2 (ALDH2) activity. In certain ~~embodiments~~examples, the

subject is also homozygous for alcohol dehydrogenase subtype 2*1 (ADH2*1/ADH2*1) or is heterozygous for alcohol dehydrogenase subtypes 2*1 and 2*2 (ADH2*1/ADH2*2).

[0009a] In another aspect, there is provided the use of 4-MP that reduces acetaldehyde accumulation by 50% to 60% as compared to a subject not administered 4-MP in the manufacture of a medicament for preventing or reducing a symptom associated with acetaldehyde accumulation accompanying ethanol consumption in a subject with reduced or absent aldehyde dehydrogenase subtype 2 (ALDH2) activity, wherein the subject is homozygous for alcohol dehydrogenase subtype 2*1 (ADH2*1/ADH2*1) or is heterozygous for alcohol dehydrogenase subtypes 2*1 and 2*2 (ADH2*1/ADH2*2).

[0010] In certain aspectsexamples, methods are provided for preventing or ameliorating a symptom of ethanol intolerance in a subject with reduced or absent aldehyde dehydrogenase subtype 2 (ALDH2) activity comprising administering 4-MP, or a physiologically acceptable salt of 4-MP, to the subject. In certain embodimentsexamples, the subject is also homozygous for alcohol dehydrogenase subtype 2*1 (ADH2*1/ADH2*1) or is heterozygous for alcohol dehydrogenase subtypes 2*1 and 2*2 (ADH2*1/ADH2*2).

[0011] In certain aspectsexamples, methods are provided for preventing, reducing or ameliorating a symptom associated with acetaldehyde accumulation in a subject with reduced or absent aldehyde dehydrogenase subtype 2 (ALDH2) activity comprising administering 4-MP, or a physiologically acceptable salt of 4-MP, to the subject. In certain embodimentsexamples, the subject is also homozygous for alcohol dehydrogenase subtype 2*1 (ADH2*1/ADH2*1) or is heterozygous for alcohol dehydrogenase subtypes 2*1 and 2*2 (ADH2*1/ADH2*2). In certain embodimentsexamples, the acetaldehyde accumulation accompanies ethanol consumption.

[0012] A symptom of ethanol intolerance, or acetaldehyde accumulation, in a subject can be, for example, selected from the group consisting of flushing, elevated heart rate, palpitations, hypotension, nausea, dizziness, headache, vomiting, diarrhea, upset stomach, ataxia, and confused consciousness.

[0013] In another aspectexample, methods are provided for reducing a likelihood or risk in a subject with reduced or absent aldehyde dehydrogenase subtype 2 (ALDH2) for a disease or disorder caused by consumption of ethanol. In certain embodimentsexamples, the subject is also homozygous for alcohol dehydrogenase subtype 2*1 (ADH2*1/ADH2*1) or heterozygous for alcohol dehydrogenase subtypes 2*1 and 2*2 (ADH2*1/ADH2*2). Conditions or diseases associated with the consumption of ethanol can include, for example and without

limitation, hangover, alcoholic gastritis, alcohol-induced liver damage, upper aerodigestive tract cancers, digestive tract cancers, breast cancer, late-onset Alzheimer's disease, hypertension, myocardial infarction, Parkinson's disease, amyotrophic lateral sclerosis, and cerebral ischemia. In preferred embodiments, diseases associated with the consumption of ethanol include upper aerodigestive tract cancers, digestive tract cancers, breast cancer, late-onset Alzheimer's disease, hypertension, myocardial infarction, Parkinson's disease, amyotrophic lateral sclerosis, and cerebral ischemia.

[0014] In certain embodimentsexamples, methods are provided for reducing a likelihood or risk in a subject for a disease or disorder caused by consumption of ethanol comprising administering to a subject in need thereof an amount of a 4-MP, or a physiologically acceptable salt of 4-MP, effective to increase catabolism of acetaldehyde in the subject, thereby reducing a likelihood or risk in for a disease or disorder caused by exposure to acetaldehyde in the subject. In certain embodimentsexamples, the acetaldehyde is a product of ethanol consumption.

[0015] In certain embodimentsexamples, the methods provided comprise administering to the subject about 0.1 mg to about 5 mg 4-MP, or the equivalent mass of 4-MP in a physiologically acceptable salt form, per kilogram of the subject's body mass. In certain embodimentsexamples, the methods provided comprise administering to the subject about 0.1 mg to about 4 mg 4-MP, or the equivalent mass of 4-MP in a physiologically acceptable salt form, per kilogram of the subject's body mass. In certain embodimentsexamples, the methods provided comprise administering to the subject about 1 mg to about 4 mg 4-MP, or the equivalent mass of 4-MP in a physiologically acceptable salt form, per kilogram of the subject's body mass.

[0016] In certain embodimentsexamples, the methods provided comprise administering to the subject about 0.1 mg to about 5 mg, about 0.1 mg to about 4.5 mg, about 0.1 mg to about 4 mg, about 0.1 mg to about 3.5 mg, about 0.1 mg to about 3 mg, about 0.1 mg to about 2.5 mg, about 0.1 mg to about 2 mg, about 0.1 mg to about 1.5 mg, about 0.1 mg to about 1 mg, about 0.1 mg to about 0.5 mg, about 1 mg to about 4.5 mg, about 1 mg to about 4 mg, about 1 mg to about 3.5 mg, about 1 mg to about 3 mg, about 1 mg to about 2.5 mg, about 1 mg to about 2 mg, about 1 mg to about 1.5 mg, about 0.1 mg to about 3 mg, about 0.5 mg to about 2 mg, or about 2 mg to about 4 mg of 4-MP, or the equivalent mass of 4-MP in a physiologically acceptable salt form, per kilogram of the subject's body mass.

[0017] In certain embodimentsexamples, the methods provided comprise administering to the subject about 0.1 mg, about 0.5 mg, about 1 mg, about 1.5 mg, about 2 mg, about 2.5 mg, about 3 mg, about 3.5 mg, about 4 mg, about 4.5 mg, or about 5 mg 4-MP, or the equivalent mass of 4-MP in a physiologically acceptable salt form, per kilogram of the subject's body mass.

[0018] In certain embodimentsexamples, 4-MP is orally administered.

[0019] In certain embodimentsexamples, 4-MP is orally administered before the subject consumes ethanol.

[0020] In certain ~~embodiment~~examples, 4-MP is orally administered about two hours to about fifteen minutes before the subject consumes ethanol.

[0021] In other ~~embodiment~~examples, 4-MP is orally administered concurrently with the subject's consumption of ethanol or after the subject has consumed ethanol.

[0022] In certain ~~embodiment~~examples, the percent reduction in the subject's ethanol elimination rate is no more than about 10% in comparison to the ethanol elimination rate of a subject not administered 4-MP.

[0023] In certain ~~embodiment~~examples, the methods comprise administering an effective amount of 4-MP that reduces acetaldehyde accumulation by about 25% to about 60% as compared to a subject not administered 4-MP.

[0024] In certain ~~embodiment~~examples, the methods comprise administering an amount of 4-MP or a physiological acceptable salt of 4-MP effective to reduce or inhibit ethanol-oxidizing activity of alcohol dehydrogenase in the subject.

[0025] In certain ~~embodiment~~examples, an effective amount of a hydrochloride salt of 4-MP is administered.

[0026] In certain ~~embodiment~~examples, the ALDH2 and ADH2 genotypes of the subject are determined prior to administration of 4-MP.

[0027] In another ~~aspect~~example, compositions are provided which comprise 4-MP, or a physiologically acceptable salt thereof, and a physiologically acceptable excipient, suitable for oral administration to a subject.

[0028] In certain ~~embodiment~~examples, the compositions may be used for preventing, reducing or ameliorating a symptom of ethanol intolerance, or a symptom associated with acetaldehyde accumulation accompanying ethanol consumption, in a subject with reduced or absent ALDH2 activity. In certain ~~embodiment~~examples, the compositions may be used for reducing a risk for a disease or disorder caused by consumption of ethanol in a subject with reduced or absent ALDH2 activity.

[0028a] In another aspect, there is provided the use of 4-MP or a physiologically acceptable salt of 4-MP effective to reduce or inhibit ethanol-

oxidizing activity of alcohol dehydrogenase in a subject in the manufacture of a medicament for ameliorating a symptom of acetaldehyde accumulation accompanying ethanol consumption in a subject with reduced or absent aldehyde dehydrogenase subtype 2 (ALDH2) activity, wherein the subject is homozygous for alcohol dehydrogenase subtype 2*1 (ADH2*1/ADH2*1) or is heterozygous for alcohol dehydrogenase subtypes 2*1 and 2*2 (ADH2*1/ADH2*2).

[0029] Also disclosed are ~~In a further aspect,~~ uses of 4-MP, or a pharmaceutically acceptable salt thereof, optionally in the manufacture of a medicament, are provided for preventing, reducing or ameliorating a symptom of ethanol intolerance, or a symptom associated with acetaldehyde accumulation accompanying ethanol consumption, in a subject with reduced or absent ALDH2 activity.

[0029a] In another aspect, there is provided the use of 4-MP or a physiologically acceptable salt of 4-MP effective to increase catabolism of acetaldehyde in a subject in the manufacture of a medicament for reducing a risk in a subject for a disease or disorder caused by consumption of ethanol in a subject with reduced or absent aldehyde dehydrogenase subtype 2 (ALDH2) activity, wherein the acetaldehyde is a product of ethanol consumption by the subject and wherein increasing catabolism of acetaldehyde reduces a risk for a disease or disorder in the subject caused by consumption of ethanol, wherein the subject is homozygous for alcohol dehydrogenase subtype 2*1 (ADH2*1/ADH2*1) or is heterozygous for alcohol dehydrogenase subtypes 2*1 and 2*2 (ADH2*1/ADH2*2).

[0030] Also disclosed are ~~In a still further aspect,~~ uses of 4-MP, or a pharmaceutically acceptable salt thereof, optionally in the manufacture of a medicament, are provided for reducing a risk for a disease or disorder caused by consumption of ethanol in a subject with reduced or absent ALDH2 activity.

[0030a] In yet another aspect, there is provided a composition for use in preventing or ameliorating a symptom of ethanol intolerance in a subject with reduced or absent aldehyde dehydrogenase subtype 2 (ALDH2) activity, comprising methylpyrazole (4-MP), wherein when the composition is to be orally administered to the subject the dosage of 4-MP is 0.1 milligram to 4 milligrams 4-MP per kilogram of the subject's body mass, and wherein the subject is homozygous for alcohol dehydrogenase subtype 2*1 (ADH2*1/ADH2*1) or is heterozygous for alcohol dehydrogenase subtypes 2*1 and 2*2 (ADH2*1/ADH2*2).

[0030b] In yet another aspect, there is provided a composition for use in preventing or reducing a symptom associated with acetaldehyde accumulation accompanying ethanol consumption in a subject with reduced or absent aldehyde dehydrogenase subtype 2 (ALDH2) activity, comprising methylpyrazole (4-MP), wherein when the composition is to be administered to the subject the amount of 4-MP is effective to reduce acetaldehyde accumulation by 50% to 60% as compared to a subject not administered the composition, and wherein the subject is homozygous for alcohol dehydrogenase subtype 2*1 (ADH2*1/ADH2*1) or is heterozygous for alcohol dehydrogenase subtypes 2*1 and 2*2 (ADH2*1/ADH2*2).

[0030c] In yet another aspect, there is provided a composition for use in ameliorating a symptom of acetaldehyde accumulation accompanying ethanol consumption in a subject with reduced or absent aldehyde dehydrogenase subtype 2 (ALDH2) activity, comprising 4-MP or a physiologically acceptable salt of 4-MP, wherein when the composition is to be administered to the subject the amount of 4-MP or physiologically acceptable salt of 4-MP is effective to reduce or inhibit ethanol-oxidizing activity of alcohol dehydrogenase in the subject, and wherein the subject is homozygous for alcohol dehydrogenase subtype 2*1 (ADH2*1/ADH2*1) or is heterozygous for alcohol dehydrogenase subtypes 2*1 and 2*2 (ADH2*1/ADH2*2).

[0030d] In yet another aspect, there is provided a composition for use in reducing a risk in a subject for a disease or disorder caused by consumption of ethanol in a subject with reduced or absent aldehyde dehydrogenase subtype 2 (ALDH2) activity, wherein the composition comprises 4-MP or a physiologically acceptable salt of 4-MP, wherein when the composition is to be administered to the subject the amount of 4-MP or physiologically acceptable salt of 4-MP is effective to increase catabolism of acetaldehyde in the subject, wherein the acetaldehyde is a product of ethanol consumption by the subject and wherein increasing catabolism of acetaldehyde reduces a risk for a disease or disorder in the subject caused by consumption of ethanol, and wherein the subject is homozygous for alcohol dehydrogenase subtype 2*1 (ADH2*1/ADH2*1) or is heterozygous for alcohol dehydrogenase subtypes 2*1 and 2*2 (ADH2*1/ADH2*2).

5. DESCRIPTION OF FIGURES

[0031] **Figure 1.** Metabolism of ethanol.

[0032] **Figure 2.** Graph of mean serum acetaldehyde concentration measured in subjects with reduced ALDH2 activity, who are also ADH2*1/ADH2*1 homozygous or ADH2*1/ADH2*2 heterozygous, following dosage with 1 mg/kg 4-MP (filled circles); comparison with placebo (open circles).

[0033] **Figure 3.** Graph of mean serum acetaldehyde concentration measured in subjects with reduced or absent ALDH2 activity, who are also ADH2*2/ADH2*2 homozygous, following dosage with 1 mg/kg 4-MP (filled circles); comparison with placebo (open circles).

[0034] **Figure 4.** Graph of mean serum acetaldehyde concentration across all subjects with reduced or absent ALDH2 activity, following dosage with 4-MP (filled circles); comparison with placebo (open circles).

6. TERMINOLOGY

[0035] Generally, the nomenclature used herein and the laboratory procedures in medicinal chemistry, biochemistry, and pharmacology described herein are those well known and commonly employed in the art. Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In the event that there is a plurality of definitions for a term used herein, those in this section prevail unless stated otherwise.

[0036] As used herein, "about" indicates a range of $\pm 10\%$. For example, "about 4 mg 4-MP" means a range of from 3.6 mg to 4.4 mg 4-MP.

[0037] The term "subject" refers to animals such as mammals, including, but not limited to, primates (*e.g.*, humans), monkeys, cattle, sheep, goats, horses, dogs, cats, rabbits, pigs, deer, bear, rats, mice and the like. In preferred embodiments, the subject is a human.

[0038] The terms "treat," "treating" or "treatment," as used herein, refers to a method of alleviating or abrogating a disorder and/or its attendant symptoms.