Chronic alcohol exposure induces genome damage measured using the cytokinesis-block micronucleus cytome assay and aneuploidy in human B lymphoblastoid cell lines

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Excessive alcohol consumption is associated with an increased risk of a variety of cancers. The specific association between alcohol consumption and increased risk of breast cancer has been a consistent finding in numerous studies to date; however, the biological mechanism remains unknown. One possibility is that alcohol induces chromosome instability and aneuploidy events commonly seen in cancer. The cytokinesis-block micronucleus cytome assay was used to assess the ability of alcohol to induce DNA damage and aneuploidy in two human B lymphoblastoid cell lines—WIL2-NS and GM13705. The cells were treated chronically with physiologically relevant levels of alcohol (0.36 and 1.35% v/v) for a period of 6 weeks. Results demonstrate that in these cell lines, chronic treatment with alcohol induces micronuclei, nucleoplasmic bridges and nuclear buds, indicative of the various genome damaging events of chromosome loss and breakage, asymmetric chromosome rearrangement and gene amplification, respectively. Using chromogenic in situ hybridisation, we measured chromosome 17 aneuploidy in these cell lines. Results from this assay indicate that chronic treatment of alcohol induces aneuploidy (measured as chromosome 17 aneuploidy) in both cell lines. The results from this study support the hypothesis that alcohol is a probable cause of cancer initiation by inducing chromosomal instability and aneuploidy, which may be a result of multiple indirect mechanisms.

Introduction
Alcohol, when consumed in excess, is clearly a toxic substance to the human body. Chronic consumption of alcohol can damage a number of organs, resulting in a variety of pathological effects and is also associated with an increased risk of a variety of cancers (1,2). Williams and Horn (3) in 1977 through the Third National Cancer Survey were the first to report, a small, yet significant association between alcohol and cancer of the breast. A further study by La Vecchia et al. (4) in 1985 supported the association between alcohol and breast cancer risk; however suggested that although there is an association, alcohol alone is not necessarily the cause of breast cancer. Further reviews (5,6) confirmed that, overall, published studies that explored the relationship of alcohol consumption and breast cancer have consistently reported a statistically significant, although modest increasing risk with increasing alcohol consumption. However, although the toxic effects of alcohol are known, there is still no established biological mechanism to explain the risk of increased risk of cancer due to alcohol (2,7,8).

The toxic effects of alcohol are believed to be mediated by damage to DNA via several mechanisms (1,2). However, as there is no evidence to support that alcohol itself is a direct carcinogen, this suggests that the mechanism by which alcohol damages DNA is indirect (1,9). There are several proposed mechanisms in which alcohol (ethanol) can damage DNA indirectly, such as via induction of oxidative damage, DNA adducts, DNA crosslinks and DNA strand breaks (1,2,9). These mechanisms involve the generation of a number DNA damaging molecules, such as reactive oxygen species, lipid peroxidation products and acetaldehyde (a toxic metabolite of ethanol metabolism) (1).

DNA damage can induce genomic instability via a number of mechanisms, which leads to a hypermutable cell, a central step for the induction of the cancer phenotype (10). This process can occur in any cell and any individual; however, a normal DNA repair system should be able to repair most of the damage from low levels of alcohol exposure such that there is a minimal effect on the genome. However, a system that is either exposed to excessive and chronic levels of alcohol or a system where the activity of DNA repair is impaired may be more susceptible to the DNA damage from alcohol and hence more susceptible to the induction of genomic instability. The breast cancer-associated gene 1 (BRCA1), which is associated with a predisposition to breast and ovarian cancer, has been found to play a role in DNA damage repair and the maintenance of genome integrity (8). Individuals carrying a BRCA1 mutation are found to have a higher sensitivity to mutagens such as hydrogen peroxide and gamma radiation (11) and therefore may also have a higher sensitivity to the DNA damaging effects of alcohol, increasing the likelihood of genomic damage occurring. It is possible that DNA damage from alcohol specifically induces the same initiating events of genomic instability that are also seen in breast and other cancers.

Aneuploidy, meaning an abnormal number of chromosomes, is common in cancer cells, and the specific aneuploidy of chromosome 17 is commonly detected in breast cancer (12). The association of BRCA1 mutants with dysfunctional homologous recombination repair of DNA strand breaks (13,14), defective G2 to M cell cycle checkpoint and abnormal centrosome amplification (15), can all result in abnormal chromosome arrangements and unequal segregation of chromosomes into the daughter nuclei during cell division (non-disjunction) and/or chromosome loss. This abnormal chromosomal segregation or loss consequently leads to the production of aneuploid cells, a common cause of cancer (16).
Ethanol has been shown to induce aneuploidy in *Aspergillus nidulans* (17). Acetaldehyde has also been shown to induce chromosome aberrations and aneuploidy in cultured mammalian cells at a significantly high frequency when compared to a known aneuploidy-inducing agent diethylstilboestrol (18). The evaluation of lymphocytes of alcoholics detected a change in the number of chromosomes, due to chromosome loss, providing evidence for a possible aneuploidy effect of ethanol consumption (2). The ability of alcohol to initiate aneuploidy is important for the association of alcohol with cancer in general. In this study, we have assessed the ability of alcohol to induce genome damage in two cell lines using the cytogenics-block micronucleus cytome (CBMN-Cyt) assay (19–21). This assay is an important tool in the measurement of genomic instability from exposure to a toxic agent as it provides a comprehensive measure of chromosome damage, as well as rates of apoptosis and necrosis (22). Using the techniques of chromogenic *in situ* hybridisation (CISH), we also measured the occurrence of chromosome 17 aneuploidy in these cell lines.

**Materials and methods**

**Cell lines**

The WIL2-NS cell line is a human B lymphoblastoid cell line with an inactivating mutation in the p53 gene and was originally derived from the spleen of a 5-year-old Caucasian male free from malignancy (23,24). The mutation in the p53 gene allows cells with DNA damage to survive as apoptosis is inhibited when p53 is inactivated (25,26). The second cell line GM13705 is a human B lymphoblast cell line derived from a 38-year-old Caucasian female with a positive family history of breast cancer and contains a germline mutation in the coding region of the BRCA1 gene consisting of a four base pair deletion at nucleotide number 3875 (exon 11, codon 1252). This was shown to result in the production of a stop codon 29 base pairs downstream from the deletion (27) resulting in a truncated protein (28).

**Alcohol dosage level selection**

Two physiological alcohol dosage levels were tested. Absolute ethanol was diluted in medium to obtain final *in vitro* concentrations equal to 1.35 and 0.36%. The 0.36% concentration is equivalent to the theoretical average concentration of ethanol found in the blood of a 60 kg person within 1 h of consuming 300 ml of white wine, assuming that 50% was absorbed and not rapidly excreted from the blood (29). The 1.35% alcohol concentration is therefore equivalent to the expected blood concentration after drinking approximately four times as much white wine, in effect, the equivalent of 125 ml white wine.

**Experimental design**

In both cell lines, 0.36 and 1.35% alcohol dosage levels and a control (0% alcohol) were tested in duplicate. Cells were subcultured every 3.5 days at a seeding density of 0.25 × 10^6 viable cells/ml for a total of 2 ml in RPMI 1640 culture medium (Thermo Electron, Melbourne, Australia) containing 10% foetal bovine serum (Thermo Electron), 1% penicillin-streptomycin solution (Thermo Electron), 1% 1-glutamine (Sigma, Sydney, Australia) with or without ethanol (Aldrich Chemical Company, Milwaukee, WI, USA) for a total of 6 weeks. Cell counts and viability were recorded at each subculture. Each week, cell samples were taken for CBMN-Cyt analysis and in Weeks 1 and 6 of the cycle separate CBMN-Cyt slides were also prepared for use in CISH analysis as per the method in the protocol given from Spot-Light® Chromosome 17 centromeric Probe and Spot-light® CISH centromere detection kit both from Zymed Laboratories Inc. (South San Francisco, CA, USA). To perform the CBMN-Cyt assay, a 500 μl subculture of the cultured cells were seeded at 0.25 × 10^6 viable cells/ml in a 24-well plate; cytchalasin-B (Cyto-B) (Sigma Chemical Company, St Louis, MO, USA) was added to each sample to give a final concentration in the cell sample of 4.5 μg/ml and the plate returned to the incubator. Twenty-four hours after the addition of Cyto-B, the cells were harvested by cytocentrifugation using a Shandon Cytospin Cytocentrifuge (Shandon Scientific, Cheshire, UK). Slides were air dried for 10 min. The CBMN-Cyt slides were fixed and stained using the commercial kit ‘Diff Quik’ (Lab Aid, Narberth, Australia) and CISH slides fixed in a 3:1 solution of ethanol (BDH Laboratory Supplies, Poole, UK) and acetic acid (BDH Laboratory Supplies), respectively, and left to air dry completely for at least 30 min. They were then stored in an airtight slide box at −20°C, with ~2 g of silica to remove moisture, until required. All slides were scored by one person using a Leica DMLB light microscope on ×1000 magnification to avoid any inter-individual variation in interpretation or variation in equipment, which may have influenced the results obtained. Criteria for scoring CBMN-Cyt slides are described extensively by Fenech et al. (21,30). Briefly, 1000 binucleated (BN) cells per slide were scored for frequency of BN cells with micronuclei (MNI), nucleoplasmic bridges (NPBs) and nuclear buds (NBuds). Five hundred cells were scored for rate of necrosis, apoptosis and nuclear division cytotoxicity index (NDCI). The NDCI gives an estimated measure of the nuclear division status and cell division kinetics in viable cells in relation to the total number of viable and non-viable cells. The proportion of mononucleated, BN, multinucleated, necrotic and apoptotic cells scored for a total of 500 cells is used for the equation: NDCI = (Apop + Nec + M1 + 2(M2) + 3(M3) + 4(M4))/T, where Apop = number of apoptotic cells, Nec = number of necrotic cells, M1–M4 = number of viable cells with 1–4 nuclei and T = total number of cells (viable and non-viable) scored. Scoring criteria for CISH slides are based on descriptions by Van Staden and King (31); being: (i) signals were visualised as brown colourless deposits of similar size, in the nuclei of cells; (ii) each brown deposit is counted as one signal; (iii) signals directly alongside each other (split signal) are counted as one signal; (iv) only cells with visible nuclear borders are scored and (v) overlapping nuclei are not scored to ensure all signals are counted. A minimum of 1000 mononucleates were scored for chromosome 17 signal per slide. Signals from the 1000 mononucleates were counted under one of the following categories: no signal, 1 signal, 2 signals, 3 signals and 4–7 signals. A minimum of 1000 binucleates were scored for chromosome 17 signal per slide. The ratio of signals from both nuclei of 1000 binucleates was classified under one of the following categories: 1:1 signals (1 signal in each nuclei), 2:2 signals, 3:3 signals, 4:4 signals, 2:1 signals, 3:1 signals and 3:2 signals.

**Statistical treatment of all data**

MNI, NPB, NBud and cell growth data were log transformed and all exhibited normal (Gaussian) distribution after log transformation. One-way analysis of variance (ANOVA) was used to compare results of various concentrations of alcohol. Bonferroni’s multiple comparison post-test was used to determine any differences between the alcohol concentrations. Test for linear trend post-test was also used to determine any trends in the data with dose and time over the 6-week testing period. Linear regression post-test was also used for cell growth log data to determine any difference in the slope for dose-related cell growth. A correlation matrix of all data was performed using Pearson’s test. Chromosome 17 aneuploidy data were analysed using the chi-squared test. Significance for all tests was accepted at P < 0.05. All calculations were performed using GraphPad Prism version 4.00 software (GraphPad, San Diego, CA, USA).

**Results**

**Effect of chronic ethanol exposure on cell growth and genome damage rate in WIL2-NS cell line**

The results shown in Figure 1A indicate an increase in the number of MNI, NPBs and NBuds with increasing alcohol dose; however, this effect did not increase with time. The combined average results of the weekly measurements are shown in Figure 1B. One-way ANOVA results were highly significant (P < 0.0001) when comparing total MNI frequencies of the three alcohol concentrations. Bonferroni’s multiple comparison test indicated that both the 0.36 and the 1.35% v/v alcohol concentrations were significantly different (P < 0.001) from the control and each other (P < 0.05). Test for linear trend was significant (P < 0.0001) indicating that MNI frequency increased with alcohol dose in the WIL2-NS cell line.

One-way ANOVA indicated a very significant (P < 0.0001) difference in NBp between alcohol groups. Bonferroni’s multiple comparison test revealed that both 0.36 and 1.35% v/v alcohol concentrations were significantly different (P < 0.001) from the control sample but were not significantly different from each other (P > 0.05). The test for linear trend was significant (P < 0.0001) suggesting that NBp frequency is in fact alcohol dose dependent, increasing 2.5-fold and 3.0-fold relative to the control in 0.36 and 1.35% alcohol, respectively.
Fig. 1. (A) Effect of chronic exposure to ethanol at concentrations of 0.00, 0.36 and 1.35% v/v on MNi, NPB and NBUD induction in the WIL2-NS cell line. The results are shown as frequency of MNi, BNs containing NPB and BNs contain NBuds per 1000 BN after each week of culture up to 6 weeks. The results represent the mean ± 1 SE from two replicate cultures. (B) Average of the effect of chronic exposure to ethanol at concentrations of 0.00, 0.36 and 1.35% v/v on total MN, NPB and NBud frequencies in the WIL2-NS cell line. Results represent the mean ± 1 SE of the weekly measurements performed over 6 weeks.
The frequency of Nbuds in response to alcohol did not achieve statistical significance (ANOVA \( P = 0.0611 \)); however, the test for linear trend did achieve statistical significance (\( P = 0.0291 \)), suggesting that nuclear budding is marginally associated with alcohol in this cell line.

A linear regression test on the WIL2-NS cell growth data obtained each time the cells were subcultured during the testing period is shown in Figure 2. This indicates that the slope of the cell growth of the control and 0.36% v/v alcohol is significantly different from the slope of 1.35% v/v alcohol (\( P < 0.0001 \)). This suggests that cell growth is retarded at higher levels of alcohol. The NDCI was also calculated from the proportion of mononucleated, BN, multinucleated, necrotic and apoptotic cells in a total of 500 cells and results are also shown in Figure 2. There was no significance (\( P > 0.05 \)) found between the NDCIs of any of the groups.

**Fig. 2.** Effect of chronic exposure to ethanol at concentrations of 0.00, 0.36 and 1.35% v/v on viable cell growth and NDCI of WIL2-NS cell line. Cell growth data were obtained each time the cells were subcultured during the testing period. Results represent the mean ± 1 SE of six assays each done in duplicate over the 6-week period. The slope of the cell growth data for each ethanol concentration was determined using a linear regression test. Each week cell samples were taken and analysed using the CBMN-Cyt assay. For each cell sample, the proportion of mononucleated, BN, multinucleated, necrotic and apoptotic cells in a total of 500 cells was scored. From this, the NDCI was calculated to give an estimated measure of the nuclear division status of the cell line. Results represent the mean ± 1 SE of the weekly measurements performed over 6 weeks.

**Effect of chronic ethanol exposure on cell growth and genome damage rate in GM13705 cell line**

Results shown in Figure 3A indicate a marginal increase in the number of MNi and a steady increase in NPBs and NBuds in the alcohol-treated groups in comparison to the control from Week 3 onwards. MNi and NPBs did not appear to be increasing with time or number of treatments and there appeared to be a downward trend in the frequency of NBuds from Weeks 5–6. The combined average results of the weekly measurements for each biomarker are shown in Figure 3B. One-way ANOVA was significant (\( P = 0.024 \)) for total MNi frequencies. Bonferroni’s multiple comparison test indicated that the MNi frequency at the 0.36% v/v alcohol concentration significantly increased 1.6-fold relative to the control; however, it was not statistically different from 1.35% v/v. The test for linear trend was significant suggesting that MNi frequency is alcohol dose dependent. Significant differences between the alcohol-treated cells and controls became evident only after 2–3 weeks of culturing in the GM13705 cell line, which differs from the WIL2-NS cell line in which genotoxic effects of alcohol were evident after 1 week of culture.

One-way ANOVA was significant (\( P = 0.0024 \)) for NPB frequency confirming a significant difference between the alcohol dose treatment groups and the control. Bonferroni’s multiple comparisons test indicated that both 0.36 and 1.35% v/v were significantly different from the control (\( P < 0.01 \)), however not significantly different from each other (\( P > 0.05 \)). Test for linear trend however was significant (\( P = 0.002 \)) suggesting that NPB frequency is alcohol dose dependent.

One-way ANOVA was significant (\( P = 0.016 \)) for NBuds frequency, indicating that increments in this biomarker were alcohol treatment related. Bonferroni’s multiple comparison test indicated that both 0.36 and 1.35% v/v were significantly different from the control (\( P < 0.01 \)), however not significantly different from each other (\( P > 0.05 \)). Test for linear trend however was significant (\( P = 0.009 \)) suggesting that NBud frequency is alcohol dose dependent, increasing by 2.8- and 3.5-fold relative to control in 0.36 and 1.35% alcohol, respectively.

A linear regression test on the GM13705 cell growth data obtained each time the cells were subcultured during the testing period is shown in Figure 4. This indicates that the slope of the cell growth regression line of the control and 0.36% v/v alcohol is significantly greater from the slope of 1.35% v/v alcohol (\( P < 0.0001 \)), which means that cell growth is retarded at higher levels of alcohol. The NDCI was also calculated from the proportion of mononucleated, BN, multinucleated, necrotic and apoptotic cells in a total of 500 cells. These results are also shown in Figure 4. There was no significance (\( P = 0.8026 \)) found between the NDCI’s of any of the groups.

**Effect of chronic ethanol exposure on chromosome 17 aneuploidy in WIL2-NS cell line**

These results are shown as column graphs in Figure 5A. Chi-squared test was significant (\( P < 0.0001 \)) for chromosome 17 aneuploidy of MONO cells in control and alcohol-treated WIL2-NS cells. In the presence of alcohol, the number of MONO cells with 2 signals (normal) decreased by 1.1-fold and the number of aneuploid MONO cells [monosomy (1), trisomy (3) and polysomy (4–7) signals] increased by 1.3-, 2.4- and 12.0-fold, respectively. This is supported by data from the BN cells, which also showed a significant shift in aneuploidy
(chi-square $P < 0.0001$), where the number of normal BN cells with 2 signals in each nuclei (i.e. 2:2) decreased by 1.1-fold in the presence of alcohol and the number of aneuploid cells (1:1, 3:3, 4:4, 2:1, 3:1 and 3:2) increased. The most noticeable increased aneuploid ratio due to alcohol in BN cells was 2:1 signals, which increased 3.0-fold relative to the control.

**Effect of chronic ethanol exposure on chromosome 17 aneuploidy in GM13705 (BRCA1 mutant) cell line**

These results are shown as column graphs in Figure 5B. Chi-squared test was significant ($P < 0.0001$) for comparison of chromosome 17 aneuploidy in MONO cells in control and alcohol-treated GM13705 cells. In the presence of alcohol, the
number of MONO cells with 2 signals (normal) decreased 1.3-fold and the number of aneuploid MONO cells [monosomy (1), trisomy (3) and polysomy (4–7 signals)] increased 1.3-, 3.6- and 2.0-fold, respectively. This is supported by the results in the BN cells, which also showed a significant shift in aneuploidy (chi-square \( P < 0.0001 \)), where the number of normal BN cells with 2 signals in each nuclei (i.e. 2:2) decreased 1.2-fold in the presence of alcohol and the number of aneuploid cells (1:1, 3:3, 4:4, 2:1, 3:1 and 3:2) increased 1.0-, 4.2-, 10.0-, 2.0-, 1.7- and 2.2-fold, respectively. It is important to note that the percentage of MONO and BN cells in the control group that exhibited normal chromosome 17 signals was lower in the GM13705 cell line (71 and 75\%, respectively) than in WIL2-NS cell line (78 and 87\%, respectively) indicating that the spontaneous aneuploidy rate may be higher in GM13705 than in WIL2-NS.

Discussion

The association between alcohol and risk of cancer, including breast cancer, has been a consistent finding in numerous studies to date; however, a biological mechanism to explain this relationship has yet to be established (2,3,5–7,32). The toxic effects of ethanol are thought to be mediated by DNA damage (1) and these damaging events may induce genetic instability and cancer. The CBMN-Cyt assay was used in this study to assess the effect of alcohol on the induction of MNI, NBuds and NPBs, markers of genome instability that are often seen in cancer (10,33). These markers provide a measure of genomic instability at the chromosome level detecting chromosome breakage, chromosome loss, chromosome rearrangement, gene amplification, necrosis and apoptosis (10,33). These biomarkers have been associated with increased cancer risk in case–control and prospective cohort studies (34–36).

In the WIL2-NS cell line, both the 0.36\% v/v ethanol [equivalent to the expected concentration of alcohol in the blood within 1 h of consuming ~300 ml of white wine (29)] and the 1.35\% v/v ethanol (four times as much as 0.36\% v/v, an effect relevant to ‘binge’ drinking) increased the frequency of MNI, NBuds and NPBs when compared with the control. This supports the hypothesis that chronic alcohol exposure can induce MNI, NBuds and NPB, markers of genome instability often seen in cancer. The detection of MNI as a result of alcohol exposure is supported by the study of Maffei et al. (2), who demonstrated that the frequency of MNI is significantly greater in the lymphocytes of alcoholics compared with the controls. An in vitro study by Greenrod and Fenech (29) also determined that ethanol tended to increase the formation of MNI when compared with other wine components and increased susceptibility to radiation-induced DNA damage (37). This indicates that genome damage associated with alcohol can be detected by the micronucleus assay. MNI originate from acentric chromosome fragments or whole chromosomes that lag at anaphase during nuclear division and are therefore evidence for chromosome breakage or loss (38). There is evidence in the literature to support the ability of ethanol (and its primary metabolite acetaldehyde) to induce chromosome breakage (9,39). Furthermore, another study illustrated an association between habitual alcohol drinking and genotype for deficiency in acetaldehyde dehydrogenase (ALDH2) with increased MNI frequency (40). This deficiency in ALDH2 activity results in increased levels of acetaldehyde accumulating in the blood after ethanol consumption which is associated with increased frequency of DNA strand breaks (39,41,42). As there is no ALDH2 activity detected in cultured lymphocytes (43), it seems unlikely that chromosome damage observed in the present study is a result of acetaldehyde exposure; however, this was not measured. In the present study, an increase in the frequency of MNI is correlated positively with a simultaneous increase in NPBs, which originate from dicentric chromosomes produced by mis-repair of DNA strand breaks, leading also to the generation of an acentric fragment from which MNI are derived (44,45). As in this study there was an increase in both NPBs and MNI with the addition of alcohol, it indicates that acentric chromosome fragments (expressed as MNI) and dicentric chromosomes (expressed as NPBs) are being expressed simultaneously in the cells which suggests that that alcohol may induce double strand breaks in DNA (9,41).

The occurrence of MNI, NBuds and NPBs in the WIL2-NS cell line was evident from Week 1 of the testing period and was maintained at a relatively steady state in comparison to the

Fig. 4. Effect of chronic exposure to ethanol at concentrations of 0.00, 0.36 and 1.35\% v/v on viable cell growth and NDICI of GM13705 cell line. Cell growth data were obtained each time the cells were subcultured during the testing period. Results represent the mean ± 1 SE of six assays each done in duplicate over the 6-week period. The slope of the cell growth data for each ethanol concentration was determined using a linear regression test. Each week cell samples were taken and analysed using the CBMN-Cyt assay. For each cell sample, the proportion of mononucleated, BN, multinucleated, necrotic and apoptotic cells in a total of 500 cells was scored. From this, the NDICI was calculated to give an estimated measure of the nuclear division status of the cell line. Results represent the mean ± 1 SE of the weekly measurements performed over 6 weeks.

Linear regression of log transformed cell growth

Average Nuclear Division Cytotoxicity Index per 500 cells

ANOVA \( P = 0.8026 \)

control vs 0.36\%: \( P > 0.05 \)
control vs 1.35\%: \( P > 0.05 \)
0.36\% vs 1.35\%: \( P > 0.05 \)

* slope of control & 0.36\% is significantly different from 1.35\% (\( P < 0.0001 \))

slope values:
control: 0.9500 ± 0.006859
0.36\%: 0.9593 ± 0.004533
1.35\%: 0.9081 ± 0.007054

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control right for the 6 weeks of the experiment. Viable cell number also increased over the testing period whereas the rate of both necrosis and apoptosis did not increase. This suggests that the cells became permissive to the DNA damaging effect of alcohol, allowing induction of the genome instability phenotype, which was maintained over the testing period. Whether the acquisition of the genome instability phenotype was a direct and permanent effect of the alcohol or whether the continual presence of alcohol is what maintained the elevated damage rate over time cannot be determined from this study as the effect of exposure and subsequent removal of alcohol in the cell line was not assessed.

A trend for the ability of alcohol to induce MNi, NBuds and NPB in the GM13705 (BRCA1 mutant) cell line was less obvious than for the WIL2-NS cell line, with increases in the frequency of MNi, NBuds and NPB becoming apparent from Week 3 onwards. This suggests that there was an initial resistance of the cells to alcohol. However, over time, the cells became permissive to the effects of alcohol, thus maintaining an increased alcohol-induced damage rate for the remainder of the experimental period. This may explain why the mean results from GM13705 did not report a clear linear trend with increasing alcohol dose. To determine more clearly the relationship between alcohol dose and MNi, NBud and NPB frequency, exposure to alcohol for a longer period of time may need to be tested in this cell line.

Cell growth of both cell lines appeared to be inhibited by a higher (1.35% v/v) level of alcohol. This result may be expected to correlate with the NDCI, which gives an estimate of the nuclear division status and cell division kinetics in viable cells in relation to the total number of viable and non-viable cells. However, no significant difference was found between the NDCI of the alcohol-treated and control groups in both cell lines. This suggests that alcohol is not affecting the proportion of viable cells that are performing nuclear division; however, alcohol may be marginally reducing the number of cell cycles occurring by increasing cell cycle time which would be expected to result in a lowered cell growth rate over the 6-week period.

The ability of ethanol to induce aneuploidy (an abnormal number of chromosomes) was first demonstrated by Kafer (17) in A. nidulans in 1984. A possible aneuploidy effect of alcohol in humans was first shown by Maffei et al. (2), who analysed the lymphocytes of alcoholics. The assessment of MNi present in the lymphocytes using fluorescent in situ hybridisation in the study provided evidence for the occurrence of whole chromosome loss (present within the MNi). Breast cancer was specifically shown to contain a high proportion of chromosome 17 aneuploidy (monosomy and polysomy) in a study conducted by Watters et al. (12). Thus, it was postulated that if alcohol increases the risk of breast cancer, then it may induce genome instability events commonly seen in breast cancer, such as chromosome 17 aneuploidy. In this study, the use of CISH with a chromosome 17 alpha satellite centromeric probe in combination with the CBMN-Cyt assay indicated that both WIL2-NS and GM13705 cell lines exhibited an increase in the number of chromosome 17 aneuploid cells in the presence of alcohol. This was supported by results from both mononucleate and binucleate cell data. It cannot be determined from this study whether alcohol induces aneuploidy of solely chromosome 17 or of a number of chromosomes (including chromosome 17) as no other chromosomes were assessed. However, the results of this study support the hypothesis that alcohol may be involved in the induction of chromosome 17 aneuploidy, an event commonly seen in specific cancers, such as breast cancer.
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Chronic ethanol exposure may promote chromosome damage by (i) production of acetaldehyde, which is a weak mutagen and carcinogen, although the evidence that this happens in cultured lymphocytes is not strong (43); (ii) induction of cytochrome P450 2E1 and associated oxidative stress, which could cause chromosome breaks leading to MN formation; (iii) depletion of S-adenosylmethionine and, consequently, induction of global DNA hypomethylation and centromere dysfunction which may lead to MN as a result of chromosome loss; (iv) accumulation of iron and associated oxidative stress which could also lead to MN formation from chromosome breaks; (v) inactivation of the tumour-suppressor gene BRCA1 which results in higher baseline frequencies of MN probably due to impaired homologous recombination repair (46,47). Consequently, there could be an accumulation of DNA double strand breaks and for correct chromosome segregation and gene amplification.

In summary, the results of this study support the hypothesis that chronic exposure to alcohol induces Mn, Nbsuds and Npbs, markers of genome instability that are commonly seen in cancer. This means that alcohol has the ability to induce various genome damage events, such as chromosome loss and breakage, chromosome rearrangement and gene amplification. Furthermore, chronic exposure to alcohol was shown to induce aneuploidy, an event that is commonly seen in cancers, which are epidemiologically associated with increased alcohol consumption, such as breast cancer.

References