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CC/08/10

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Mechanisms contributing to the synergism of alcohol and tobacco in human cancers

Following the initial discussion on mixtures at the last meeting, it was decided to consider a well-known carcinogenic interaction of significance to public health and to review the literature for information on potential mechanisms which explain the interaction. The synergistic interaction between alcohol and smoking on certain cancer endpoints was selected. The Department of Health Toxicology Unit has produced the attached paper which presents some of the studies which describe the combined effect of alcohol and smoking, and considers some of the mechanisms which have been proposed in the literature to explain the synergism.

The committee is asked to consider the attached paper and to answer the following questions:

1. What is members' opinion of the mechanisms which have been proposed to explain the synergistic effect of alcohol and tobacco?
2. Do members consider that any other mechanisms may be of importance?
3. Are there any other known interactions which it would be useful to consider in this way?

Secretariat

July 2008

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Mechanisms contributing to the synergism of alcohol and tobacco in human cancers

1. Following on from the initial discussion paper on the carcinogenicity of chemical mixtures presented at the COC meeting in April 2008, it was decided to examine interactions which may be of importance to public health. One such example is the known potentiation of some tobacco-induced cancers by the consumption of alcohol. This paper attempts to examine the extent of this interaction and the mechanisms which underpin this synergism.

2. Tobacco and alcohol are well established risk factors for cancers of the head and neck, larynx, and oesophagus and these are the cancers which have most commonly been investigated with respect to the combined impact of these life-style factors. A few studies have been designed to look specifically for the effects of combined use and in other larger studies, it is possible to extract the relevant data to address this end-point.

3. A study which specifically aimed to investigate the combined effects of alcohol and tobacco smoking on the incidence of cancer of the larynx collected data on tobacco smoking (g/day) and alcohol intake (g/week) from 326 cases and 1134 control subjects (Olsen et al 1985a). Age and sex adjusted risk ratios indicated, in general, dose related increases in OR for laryngeal cancer for both intakes as follows

Alcohol g/week	Tobacco g/day				Adjusted for tobacco
	0-10	11-20	21+	unknown	
0-100	1.0	1.5	1.7	0.7	1.0
101-200	1.2	1.6	3.2	-	1.5
201-300	1.5	6.0	15.5	-	3.2
301+	5.4	4.4	7.5	-	4.1
Unknown	0.5	2.8	-	0.5	0.8
Adjusted for alcohol	1.0	1.7	2.3	0.5	

4. These analyses showed a synergistic effect for all intakes with the exception of moderate alcohol and tobacco use. The authors conclude that even if the collection of data on residual confounding factors is flawed, this would be unlikely to account for the entirety of the synergistic response seen.

5. The same group also examined the impact of alcohol and tobacco exposure on the risk of cancer of the hypopharynx, although the numbers of patients evaluated was much smaller (Olsen et al 1985b). Assessment of a total of 32 case of cancer of the hypopharynx and 321 with laryngeal cancer and 1141 controls similarly demonstrated a more than additive effect of combined intakes of tobacco and alcohol (maximum OR=5.1).

6. Talamini et al (2002) report a case-control study in which 527 cases of squamous –cell carcinoma of the larynx were age, sex and area of residency matched with 1297 controls. ORs for current smokers

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compared to never smokers was 19.8 (95% CI 11.9-32.9) and for drinkers 1.8 (95% CI 1.0-3.3). Their assessment was detailed, including stratification across those with low, moderate and high intakes of both alcohol and tobacco. The data clearly demonstrate that both alcohol and tobacco are independent risk factors for laryngeal cancer and that heavy alcohol consumption and tobacco use produced a remarkable multiplicative effect.

Smoking habit no cigs/day	Alcohol intake drinks/week				OR (95% CI)
	Never and <14	14-27	28-55	>56	
Never	1 ^a	0.8 (0.19-2.95)	1.7 (0.42-7.01)	8.5 (2.39-30.17)	1 ^a
1-14	9.7 (3.56-26.13)	8.0 (2.82-22.80)	18.7 (6.99-50.22)	63.7 (20.97-193.42)	9.2 (5.15-16.38)
15-24	27.6 (10.19-74.73)	31.5 (11.96-82.94)	66.5 (26.83-165.03)	139.9 (54.73-357.86)	28.0 (16.01-48.96)
>25	18.9 (5.69-62.70)	52.5 (18.28-150.62)	83.9 (30.92-227.52)	177.2 (64.99-483.28)	34.3 (18.57-63.47)
OR (95% CI)	1 ^a	1.2 (0.73-2.03)	2.5 (1.54-4.08)	6.1 (3.58-10.43)	

a: reference category

7. Laryngeal cancer is substantially less common in women than in men (sex ratio of 10-30 fold) and thus there is a paucity of data assessing risk factors in women. This was addressed in two pooled case-control studies, although only 78 cases were collected (compared to 340 controls) (Gallus et al 2003). The fact that alcohol and tobacco usage could not explain the marked sex difference was also explored. The ORs for <3 drinks/day increased with the number of cigarettes smoked to 19.1 (95% CI 6.0-60.3) for 0-15 cigarettes and 88.4 (95% CI 22.5-347.2) for >15 cigarettes/day. In those that consumed >3 drinks/day the ORs were 1.3 for never or ex-smokers and 20.4 (95% CI 4.7-88.8) and 317.9 (95% CI 70.5-1434.0) for those smoking 0-15 and >15 cigarettes/day respectively. Cigarette smoking was shown conclusively to be the most prominent risk factor and again, the data clearly demonstrate the multiplicative interaction of alcohol and tobacco on risk for this kind of cancer.

8. A large study in an Indian population investigated tobacco smoking and chewing and alcohol drinking on the risk of oral (lip, tongue and mouth), pharyngeal and oesophageal cancer incidence (Znaor et al 2003). Data were collected from 1563 oral, 636 pharyngeal and 566 oesophageal male cancer patients and compared to 1711 controls. A large number of analyses were undertaken including the combinations of smoking, chewing and drinking. The OR data for smoking and alcohol drinking only are presented:

Smoke	Drink	Oral		Pharynx		Oesophagus	
		OR	95% CI	OR	95% CI	OR	95% CI
No	No	1		1		1	
Yes	No	2.45	1.94-3.10	3.54	2.54-4.94	3.57	2.51-5.06
No	Yes	2.56	1.42-4.64	No cases		3.41	1.46-7.99
Yes	Yes	4.81	3.74-6.19	8.41	5.94-11.90	7.33	5.06-10.62

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9. Chewing tobacco further increased the odd ratios particularly for the oral cancers.

10. Oesophageal cancer is broadly classified into two histological types; squamous cell carcinoma (SCC) which arises principally in the upper and middle thirds of the oesophagus, and oesophageal adenocarcinoma (EAC) which occurs in the lower third of the oesophagus. There are strong associations with SCC and alcohol and tobacco consumption but these are considered to be of less importance in the etiology of EAC. Whereas the SCC cancer is more closely related to the head and neck cancers such as pharyngeal, EAC is associated with (and included within?) the gastric cancers. This was also discussed in the COC paper reviewing oesophageal cancer (CC/04/14)

11. The studies described previously did not specify from which part of the oesophagus the cancer had arisen. However, Lagergren (2000) compared the incidences of the two oesophageal cancer types. For alcohol alone, high consumption of hard liquor was associated with an increased risk of SCC (OR 5.0, 95% CI 2.8-9.0) for >30g/week ethanol, although there was not a clear association trend across dose. For tobacco the incidence of SCC was strongly associated with smoking; the adjusted OR was 9.3 (95% CI 5.1-17.) among current smokers vs those never smoked. Assessment of combined alcohol and tobacco use (>35y + >70g week) was 23.1 (95%CI, 9.6-56.0) compared with never users for SCC, but was not significant for risk of EAC (2.3 95% 0.9-5.7) (Lagergren *et al.*, 2000).

12. Lee et al (2007) specifically assessed the impact of tobacco, alcohol and combined exposure on the incidence of SCC. Statistical models were applied to analyse the multiplicative nature of the interaction. Tobacco was shown to interact with light to moderate alcohol (0.1-30g/day) in a supra-multiplicative manner (OR 5.5-5.7) whereas it interacted with heavy alcohol consumption in a simple multiplicative model (OR 1.7-2.3). Regular alcohol intake was most strongly associated with the increased risk of tumour development,

13. A Scandinavian cohort study collected data from 69,962 individuals and a total of 1,117,648 person-years between 1984 and 2002 (Sjodahl et al 2006). Cardia and non-cardia gastric cancers were analysed separately. The following relevant data were retrieved:

Smoking	Alcohol ^a	Gastric cancer	Noncardia gastric cancer
		OR(95% CI)	OR(95% CI)
Never	Never	Reference	Reference
<20	Never	1.34 (0.40-4.51)	1.40 (0.41-4.75)
>20	Never	-	-
Never	<4	1.08 (0.66-1.78)	1.06(0.64-1.78)
Never	>5	1.57 (0.47-5.28)	1.69 (0.50-5.73)
<20	<4	1.79 (1.07-2.99)	1.57 (0.91-2.68)
>20	<4	1.78 (0.85-3.73)	1.66 (0.75-3.66)
<20	>5	2.15 (0.93-4.94)	2.33 (1.00-5.40)
>20	>5	4.38 (1.72-11.17)	4.90 (1.90-12.62)

a: data presented as number of occasions per 14 days

14. Although the risk associated with the use of both alcohol and tobacco is greater than the risk associated with the individual factors, for these cancers the interaction between alcohol and tobacco was not statistically significant

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15. The studies presented indicate that the combined use of alcohol and tobacco interact to induce cancers of the mouth, neck and squamous cell carcinoma of the oesophagus in a greater than additive manner. However the synergism is not apparent for oesophageal adenocarcinoma and cancers of the gastric cardia.

Induction of CYP enzymes by ethanol

16. Alcohol itself is not considered to be a carcinogen in experimental animals (IARC 1988). The mechanism by which alcohol produces enhanced carcinogenicity is not well understood although there are several hypotheses which have received attention. Although alcohol is metabolized principally by alcohol dehydrogenase, CYP2E1 is thought to be responsible for metabolizing 20% of ethanol at low blood concentrations (Schoedel and Tyndale 2003). Furthermore, ethanol is also known to induce CYP2E1, via protein stabilization and prevention of the degradation of the 26S proteasome (Anderson et al 1995). The significance of this induction to ethanol's apparent synergy with tobacco in the carcinogenic process is apparent when it is considered that most carcinogenic nitrosamines present in tobacco smoke are metabolically activated by the CYP family of enzymes.

17. There are a number of studies in which the relevance of the various CYP enzymes on the metabolism of different nitrosamines is considered which are relevant to understanding the association of alcohol and tobacco intake on cancer incidences. A discussion of the organ distribution of the CYP isoforms or which of these is capable of metabolically activating which specific nitrosamines is outside the scope of the current assessment. However a number of papers are included to provide an indication of how ethanol induced enzyme induction may impact on the carcinogenicity of nitrosamines in tobacco. Of particular note is the induction of CYP2E1 by ethanol.

18. Mori et al (2002) assessed CYP1A1/2, 2B1/2, 2E1 and 3A2 levels using western blotting in rats given N-nitrosomethylbenzylamine (NMBA; 0.5mg/kg) with or without ethanol in the drinking water (10%) and also used S9 prepared from these livers in an Ames test with a variety of N-nitrosamines and heterocyclic amines. NMBA did not increase any of the CYPs measured and ethanol induced 2E1 only. This in turn was shown to increase the mutagenic activity of dimethylnitrosamine (DMN) and diethylnitrosamine (DEN) in the Ames test, but not NMBA mutagenicity.

19. Another approach indicating that induction of CYP2E1 may play a role in ethanol's synergistic effect with nitrosamines in oesophageal carcinogenesis utilized evaluation of the formation of oesophageal polyps, considered an early tumour marker (Tsutsumi et al 2006). In this study rats were given daily doses of N-nitrosomethylbenzylamine (NMBA) for 10 weeks together with ethanol in a liquid diet for 30 weeks (from 8 weeks before NMBA administration until 12 weeks after). An increase in polyps was recorded in treated animals when compared to control animals, and increased CYP2E1 expression was also apparent in the oesophageal mucosa. However there are some earlier studies which concluded that 2E1 was not expressed in rat oesophagus although microsomes from this tissue were shown to metabolise N-nitrosodiethylamine (Ribeiro Pinto et al 2001). However this study did not investigate the potential of ethanol to induce this isoform.

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20. There are a number of studies which investigate CYP2E1 induction by ethanol in man, the expression of oesophageal CYP enzymes and the role of various isoforms in the metabolic activation of nitrosamines associated with tobacco smoke.

21. Confirming the similarities of man and rat with regards to induction, Takahashi et al (1993) demonstrated that CYP2E1 expression is elevated in the centrilobular region of the livers of alcoholics. More specific to the current review, CYP expression in human oesophageal mucosa was investigated by Lechevrel et al (1999) using immunohistochemical techniques. CYP1A, 2E1, 3A and 4A isozymes were detected. CYP2E1 was consistently identified (17/19 samples) although there was up to 43-fold variation in expression. The effects of alcohol and tobacco exposure were monitored although no significant correlation was reported for any of the isoforms evaluated. Similarly, expression of CYP1A1, 1A2, 2A6/2A7, 2E1 and 3A4 mRNAs in human mucosal specimens taken from patients undergoing oesophagectomy was performed with a view to establishing a role in the etiology of oesophageal cancer (Godoy et al 2002). It was shown that 96% and 61% of patients expressed oesophageal CYP2E1 and 2A6/2A7 respectively although the 2A6/7 expression was somewhat variable between samples. It was tentatively suggested that this co-localised to areas susceptible to tumours. In another report, human oesophageal microsomes were shown to metabolise tobacco nitrosamines and that these microsomes were immunoreactive for CYP2E1 (Smith et al 1998).

22. These observations provide a plausible hypothesis for the clear synergism of alcohol and tobacco in the induction of some cancers in that the induction of CYP enzymes by ethanol, particularly 2E1 increases the metabolic activation of some carcinogenic nitrosamines present in tobacco smoke. However, this is evidently not straightforward for a number of reasons. Induction of CYP in the liver would usually be associated with increased clearance of substrates and thus decreasing their availability to distant organs. However, Swann (1984) highlighted the fact that ethanol in fact significantly reduces nitrosamine metabolism in the liver thus blocking first pass clearance. This is likely a consequence of competitive inhibition, as ethanol itself will be concomitantly metabolised by the CYP enzymes it induces. Therefore it is more likely that the induction of the enzymes at the tumour site itself would be a more relevant mechanism of activation. Although CYP2E1 has not been shown to be consistently expressed in the oesophagus for example it is possible that induction by alcohol increases local concentrations of the metabolically activated nitrosamines present in tobacco. Anderson et al (1995) also supports the postulate that hepatic clearance of nitrosamines is reduced resulting in elevated levels at post-hepatic target tissues.

23. There are reports which have investigated polymorphisms of other CYP enzymes, such as CYP 2B1 and 1B1, which also have the capacity to metabolise nitrosamines, and polyaromatic hydrocarbons and their relevance to the induction of cancer (Schoedel and Tyndale 2003, Thier et al 2002). Alcohol and aldehyde dehydrogenase genes (ALD1B and ALDH2) and glutathione-s-transferase (GSTM1, GSTT1, GSTP1) polymorphisms have also been examined with regards to their impact on alcohol and tobacco carcinogenesis. Peters et al (2006) showed combined effects of alcohol and tobacco induced head and neck squamous cell carcinoma and deletion of GSTM1 enhanced this interaction. A Taiwanese study demonstrated an interaction between ADH1B and ALDH2 polymorphisms, smoking and alcohol consumption on the risk squamous cell carcinoma of the oesophagus (Lee et al 2008). Together it is clear that the presence and induction of enzymes responsible for metabolizing and detoxifying both

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carcinogenic nitrosamines contained in tobacco smoke and ethanol are likely to contribute to the synergism observed in the induction of tumours.

Increased permeability of epithelial cells

24. Another postulate which remains plausible is that alcohol increases the permeability of the oral mucosa to carcinogens. It is known that ethanol potentiates the diffusion of co-administered chemicals across the skin (ref). With a view to investigating a potential mechanism for the synergistic effects of alcohol and tobacco on oral cancers, Du et al (2000) investigated the effects of ethanol on the penetration of nitrosonornicotine (NNN) in an *in vitro* model using porcine oral mucosal cells. A threshold concentration of 25% alcohol, nicotine at 0.2% and the combination of alcohol and nicotine enhanced the penetration of NNN across the oral mucosal cells in culture. The authors suggest that local permeabilising effects may be one mechanism which explains the synergy. However the concentrations used seem high and extrapolation is difficult with a lack of understanding of the relevance of the model used.

25. An *in vitro* system was also employed to assess the impact of ethanol on the rates of absorption of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and benzo[a]pyrene (BaP) (Azzi et al 2005). The mucosal layer of whole porcine esophagus was established in Franz diffusion cells, concentrations of ethanol and menthol were selected in an attempt to mimic *in vivo* exposure (5% and 0.08% respectively) and the extent which chemicals permeated the reception chamber was measured. Alcohol and menthol did not effect the transit of the relatively water soluble NNK but did significantly enhance the penetration of the more lipophilic BaP. Confocal microscopy showed that BaP was more concentrated in the basement membrane adjacent to the smooth muscle tissue. Ethanol and menthol together showed synergism. It is concluded that the results support the supposition that the use of mentholated cigarettes and consumption of alcohol may have a marked effect on the absorption and fate of carcinogenic tobacco chemicals.

Conclusion

26. These data suggest that enhancing permeability of the oral mucosa by ethanol could increase local concentrations of carcinogenic nitrosamines present in tobacco. Two plausible mechanisms by which alcohol and tobacco act synergistically on the induction of a number of cancers, specifically those of head and neck, larynx, and oesophagus are presented. It is likely that there are other factors that contribute to this synergism, for example, other enzymes capable of metabolizing both ethanol and tobacco related nitrosamines. Furthermore the relative distribution, inducibility and substrate specificity of the different CYP family of enzymes may impact on other cancers at other sites. However, this provides an example of how chemicals may interact to enhance tumour formation.

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