UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
FACULDADE DE MEDICINA
CAMPUS DE BOTUCATU

INFLUÊNCIA DA EXPOSIÇÃO À FUMAÇA DO CIGARRO E AO ETANOL SOBRE AS ALTERAÇÕES DA MUCOSA DO ESÔFAGO INDUZIDAS POR DIETA MODIFICADA EM CAMUNDONGOS C57BL/6

JOYCE REGINA ZAPATERINI

Dissertação apresentada ao Programa de Pós-Graduação em Patologia da Faculdade de Medicina de Botucatu, Universidade Estadual Paulista – UNESP para obtenção do título de Mestre em Patologia.

BOTUCATU – SP
2010

UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
FACULDADE DE MEDICINA
CAMPUS DE BOTUCATU

INFLUÊNCIA DA EXPOSIÇÃO À FUMAÇA DO CIGARRO E AO ETANOL SOBRE AS ATERAÇÕES DA MUCOSA DO ESÔFAGO INDUZIDAS POR DIETA MODIFICADA EM CAMUNDONGOS C57BL/6

MESTRANDA: JOYCE REGINA ZAPATERINI

ORIENTADOR: DR. LUIS FERNANDO BARBISAN
CO-ORIENTADORA: DRA. MARIA APARECIDA MARCHESAN RODRIGUES

Dissertação apresentada ao Programa de Pós-Graduação em Patologia da Faculdade de Medicina de Botucatu, Universidade Estadual Paulista – UNESP para obtenção do título de Mestre em Patologia.

BOTUCATU – SP
2010

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. E TRAT. DA INFORMAÇÃO
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CAMPUS DE BOTUCATU - UNESP
BIBLIOTECÁRIA RESPONSÁVEL: ***ROSEMEIRE APARECIDA VICENTE***

Zapaterini, Joyce Regina.
Influência da exposição à fumaça do cigarro e ao etanol sobre as alterações da mucosa do esôfago induzidas por dieta modificada em camundongos C57BL/6 / Joyce Regina Zapaterini. – Botucatu, 2010.

Dissertação (mestrado) – Faculdade de Medicina de Botucatu, Universidade Estadual Paulista, 2010.
Orientador: Prof. Dr. Luis Fernando Barbisan
Co-orientador: Profª. Drª. Maria Aparecida Marchesan Rodrigues
Assunto CAPES: 4010006.

1. Patologia. 2. Câncer. 3. Álcool. 4. Cigarro.

Palavras chave: Ácido deoxicólico; Álcool; Câncer do trato digestivo; Cigarro; Deficiência do zinco.

SUMÁRIO

Capítulo I

Revisão da literatura

Capítulo II

Article

Dedicatória

Aos meus pais José Roberto e Fátima

Durante a nossa vida:
Conhecemos pessoas que vem e que ficam,
Outras que, vem e passam.
Existem aquelas que,
Vem, ficam e depois de algum tempo se vão.
Mas existem aquelas que vem e se vão com uma enorme vontade de ficar... (Charles Chaplin)

Agradecimentos

Agradeço primeiramente a Deus, pela vida que me foi concedida, pela luz nos momentos em que pensei estar sozinha, pela família e pessoas que tenho ao meu lado.

Ao homem, exemplo de honestidade e luta, por ter-me ensinado a importância do caráter e perseverança, pelo enorme esforço desprendido para que eu pudesse prosseguir meus estudos, AO MEU PAI, que agora agradeço pela proteção espiritual que me foi dada durante toda essa jornada e, que de uma forma ou de outra, caminha constantemente ao meu lado.

À minha mãe, fonte inesgotável de força, pela infinita dedicação, pelo aconchego nas horas difíceis e por todos os gestos de afeto, carinho e amor que sempre teve para comigo. Obrigada por fazer de mim o que sou e por sempre estar ao meu lado.

Aos meus irmãos, Tatiana e Diego, pelo companheirismo, amor e amizade incondicionais que sempre tiveram para comigo.

Ao meu namorado André, pelos gestos de amor e carinho nos momentos alegres e especialmente nos difíceis, pela sinceridade, paciência e pela grande ajuda principalmente nos momentos que mais precisei. Obrigada por ter caminhado junto comigo durante toda esta jornada.

À minha avó Josefa,minha segunda mãe, por sempre estar de braços abertos oferecendo carinho e afeto e sempre pronta para uma palavra amiga.

Às minhas primas, eternas amigas, pelos altos momentos de descontração, apoio e confidencialismo.

A todos os meus familiares, meu porto seguro.

Ao meu orientador Dr.Luis Fernando Barbisan, pela dedicação durante as etapas deste trabalho, pela compreensão quando mais precisei e por acreditar em minha capacidade. Obrigada pela enorme contribuição para com minha formação.

À minha co-orientadora Dra. Maria Aparecida Marchesan Rodrigues, por todo ensinamento concedido durante esta jornada tornando possível a concretização deste trabalho.

À Prof. Dra. Daniela C. dos Santos, exemplo de amizade e profissionalismo, sempre pronta para ajudar e contribuir para com minha formação..

À minha grande amiga Nelci, sempre pronta para me ajudar quando precisei e sempre colocando um sorriso em meu rosto nos momentos angustiantes, pela enorme ajuda, especialmente, durante a árdua etapa experimental. Obrigada por ter caminhado comigo durante todas as etapas deste trabalho. E, não poderia deixar de agradecer, pelas altas risadas, palhaçadas e foras que demos e vivemos, isto será realmente inesquecível! "É debaixo do pé de Inguá DAÍ!!!!!".

Ao companheirismo dos amigos do Departamento de Morfologia e Patologia: Marcos, Lívia, Lucas, Kelly, Flávia, Gisele, Justulin, Tony, Jossimara, Luciano etc, etc, etc…..

A todos os funcionários do Departamento de Morfologia – IB e do Departamento de Patologia – FMB pela constante dedicação, visando proporcionar a cada dia, o melhor para nós alunos.

Ao bioterista do Departamento de Patologia – FMB, Paulo César (PC), pelo tratamento adequado e dedicação dado aos animais no biotério.

Ao técnico de laboratório de imunohistoquímica, Marquinhos, pela enorme ajuda e disposição em me ajudar quando mais precisei.

À Fundação de Amparo á Pesquisa (FAPESP) pela bolsa mestrado e auxílio científico (processo n° 07-57544-1 e 08/ 53366-4, respectivamente).

À todos os animais utilizados neste experimento, pois sem eles nada teria sido possível.

É difícil mencionar todas as pessoas que me ajudaram, por isso agradeço a todos que diretamente ou indiretamente contribuíram para a realização deste trabalho.

Bom mesmo é ir à luta com determinação, abraçar a vida com paixão, perder com classe e vencer com ousadia, porque o mundo pertence a quem se atreve e a vida é muito para ser insignificante.
(Charles Chaplin)

A vida é uma peça de teatro que não permite ensaios.
Por isso, cante, chore, dance, ria e viva intensamente, antes que a cortina se feche
e a peça termine sem aplausos.
(Charles Chaplin)

"É melhor tentar e falhar, que preocupar-se e ver a vida passar;
É melhor tentar, ainda que em vão, que sentar-se fazendo nada até o final.
Eu prefiro na chuva caminhar, que em dias tristes em casa me esconder.
Prefiro ser feliz, embora louco, que em conformidade viver ..."
Martin Luther King

Para ser grande, ser inteiro; nada teu exagera ou exclui; ser todo em cada coisa; põe quanto és no mínimo
que fazes; assim em cada lago, a lua toda brilha porque alta vive.
Fernando Pessoa

Capítulo I

Revisão da literatura

1. Câncer de esôfago e de língua: epidemiologia e subtipos.

As neoplasias malignas têm sido apontadas como uma das principais causas de mortalidade no mundo ocidental moderno (WCRF, 1997; WHO, 1998). São doenças crônico-degenerativas, multifatoriais que envolvem alterações genéticas progressivas, expressas por alterações celulares metabólicas e morfológicas. Cada tipo de neoplasia possui características clínicas e biológicas peculiares que devem ser investigadas para aprimorar seu diagnóstico, tratamento e prognóstico (Robbins, 2005).

O câncer de esôfago destaca-se pela incidência crescente nas últimas décadas, apresentando-se como a terceira neoplasia maligna do trato gastrointestinal mais prevalente do mundo (Stoner et al., 2001; Pack et al, 1999; Engel et al., 2003). No Brasil, o câncer de esôfago está entre os dez de maior incidência e o sexto em mortalidade de acordo com dados obtidos dos Registros de Base Populacional de 2008 (INCA, 2008). Além disso, apresenta desenvolvimento rápido e, na maioria dos casos, o prognóstico é ruim com sobrevida média abaixo de cinco anos (Enzinger & Mayer, 2003;.Kollarova et al, 2007).

A incidência do carcinoma epidermóide do esôfago em humanos é influenciada por fatores ambientais locais, predisposição genética e estilo de vida, sendo mais freqüente em algumas áreas da França, China, Irã, África do Sul e América do Sul (Craddock, 1992; Day & Varghese, 1994). As áreas de alto risco estão localizadas ao longo do chamado “cinturão do câncer do esôfago” que vai do Irã às repúblicas do sudoeste da antiga URSS, oeste e noroeste da China. Alguns países da América do Sul, sudoeste da África e da Europa também apresentam alta incidência deste tipo de câncer (Tomatis et al., 1990; Craddock, 1992; Day & Varghese, 1994). Predomina nos homens e sua incidência aumenta com a idade, sendo o pico de freqüência entre os 50-70 anos (Kollarova et al, 2007). No Rio

Grande do Sul a incidência de câncer do esôfago está em torno de 14,3/100.000 casos para os homens e 4,2/100.000 e para as mulheres (Barros et al., 2000).

Cerca de 90% das neoplasias do esôfago inclui os carcinomas epidermóides que provém de seu epitélio de revestimento e cerca de 7% dos casos inclui os carcinomas que podem originar-se de glândulas da submucosa do esôfago e, mais comumente, do epitélio cilíndrico do chamado esôfago de Barrett (Pera et al., 1993; Cameron et al., 1995). Entretanto nos EUA, ambos possuem taxas semelhantes de incidência, a qual é mais freqüente em homens sendo que os negros possuem um risco maior do que os brancos de até quatro vezes (Blot et al.,1991).

Estudos epidemiológicos têm mostrado que os principais fatores de risco para o desenvolvimento do câncer de esôfago são o uso de álcool, o tabagismo, deficiência de micronutrientes, consumo de bebidas quentes (em especial de chás) e o baixo nível socioeconômico, sendo a predisposição genética pouco definida (Victora et al., 1987; Craddok, 1992; Blot et al., 1999).

O papilomavírus humano (HPV) é freqüentemente encontrado nos carcinomas epidermóides de regiões com alta incidência, enquanto o consumo de vegetais verdes e de frutas parece exercer papel protetor (Li et al., 1993; Tampi et al., 2005; Farhadi et al., 2005). Supõe-se que os fatores ambientais e nutricionais possam aumentar o risco desta neoplasia, enquanto as deficiências nutricionais possam atuar como promotoras ou potencializadoras de cancerígenos ambientais. Por exemplo, compostos *N*-nitrosos presentes na dieta e no tabaco podem ser os responsáveis pelo amplo espectro de mutações de ponto do gene supressor de tumores TP53, que estão presentes em mais de 50% dos cânceres de esôfago (Putz et al, 2002). As mutações no p16 e a perda de alelos (perda da

heterogeneidade) envolvendo outros cromossomos também são prevalentes nessas neoplasias, de acordo com o conceito de que a aquisição escalonada e acúmulo de alterações genéticas levam a formação do câncer (Putz et al, 2002).

No processo de inflamação crônica relacionada ao uso do mate em temperaturas elevadas, ocorre a formação de nitrosaminas e radicais livres endógenos que podem ser responsáveis pela alta freqüência de mutações de transição G>A e C>T no gene TP53 observado em carcinomas epidermóides de pacientes do sul do Brasil. A prevalência de transição G>A destes casos foi mais alta do que as relatadas nos bancos de dados mundiais (IARC, Lion-França) (Putz et al, 2002).

Acredita-se que a chave precursora para o surgimento dos adenocarcinomas de esôfago é o desenvolvimento do esôfago de Barrett (EB), caracterizado pelo crescimento anormal de células do tipo colunar no esôfago (Murphy et al., 2005). A condição para a ocorrência desta metaplasia glandular é a doença do refluxo gastroesofágico (DRGE). Esta é uma afecção crônica bastante comum na população mundial (Farhadi et al., 2002), caracterizada pelo refluxo do conteúdo gastroduodenal para o esôfago, o que leva à formação de esofagite que, se não tratadas, evolui para o esôfago de Barret. Apesar do EB ser determinante no desenvolvimento do adenocarcinoma de esôfago, a esofagite, desempenha papel importante no processo de carcinogênese (Farhadi et al., 2002). Estudos experimentais e epidemiológicos evidenciam que o influxo de neutrófilos nas vias aéreas, no intestino e no canal urinário, pode levar ao aparecimento de neoplasias de pulmão, cólon e bexiga, respectivamente. Os neutrófilos são conhecidos como a maior fonte de espécies reativas de oxigênio (ROS) (Knaapen et al., 2006). As ROS induzem danos no DNA, através de reações de oxidação, depurinação, metilação, desaminação (Knaapen et al.,

2006) e quebras no DNA (Baik et al., 1996). Além disso, o excesso de ROS é prejudicial à integridade dos tecidos afetados, mantendo a inflamação (Farhadi et al., 2002).

Para as esofagites, cita-se a reação de oxidação, como processo de dano ao DNA provocado pelas ROS, em conseqüência ao RGE. De acordo com Farhadi et al. (2002), o processo de formação de bases oxidadas no DNA resulta em alterações no funcionamento e divisão das células. Estudos experimentais em animais evidenciam que o processo de oxidação do DNA evolui com o aumento do grau de esofagite, sendo severo no EB e no duodeno, de acordo com o aumento dos níveis de ROS nestes estágios (Olyaee et al., 1995 e Farhadi et al., 2002).

O câncer oral é o câncer que mais freqüentemente afeta a região cervicofacial sendo comum em todo mundo, principalmente, em países em desenvolvimento, como Índia, Vietnan e Brasil, representando 25% de todos os tipos de câncer (Magrath and Litivak, 1993). No Brasil, o câncer oral ocupa a sétima posição entre os de maior incidência, estando em torno de 19,72/100.00 casos para os homens e 6,26/100.00 para as mulheres no estado de São Paulo de acordo com dados obtidos dos Registros de Base Populacional de 2008 (INCA, 2008).

O câncer de língua representa 30% dos tipos de câncer oral, sendo o carcinoma de células escamosas (SCC) o tipo histológico mais freqüente da neoplasia da língua (Davidson et al., 1987; Funk et al., 2002), afetando principalmente homens na sexta década de vida (Liewellyn et al., 2001). O prognóstico de pacientes com SCC da lingua é ruim com sobrevida de 5 anos inferior a 50% (Ferrari et al., 2009). Este prognóstico pode ser atribuído a vários fatores, como por exemplo, por ser uma doença freqüentemente assintomática até atingir estágio avançado e por afetar principalmente idosos (Zhen et al., 2004).

O desenvolvimento do câncer de língua envolve a soma de vários fatores ambientais, genéticos e estilo de vida, responsáveis pela progressiva transformação maligna e disseminação metastática (Vogelstein, 1990).

Estudos demonstram que a deficiência em zinco, má higiene bucal e o consumo de álcool e tabaco, são os principais fatores de risco para o desenvolvimento do câncer de língua (Boffetta et al., 1992; Mashberg et al., 1993; Friedlander et al., 1998; Lopes et al., 2002; Fong et al., 2005).

Assim como no esôfago, a proteína P53 apresenta-se mutada em aproximadamente 50-60% dos casos de câncer de língua (Linden et al., 1994; Forastiere et al., 2001), sendo os compostos N-nitrosos presentes no cigarro e no álcool, os responsáveis pela alta taxa de mutações no gene supressor de tumor TP53.

A infecção secundária pelo papiloma vírus humano (HPV) e pelo vírus da Herpes Simplex (HSV-1) são também considerados fatores de risco para o SSC da língua, porém, há controvérsias quanto ao HPV (Friedlander et al., 1998; Lopes et al., 2002). Herrero et al., 2003 e Sugerman & Shilliote, 1997, relatam que o HPV é capaz de aumentar o risco para o desenvolvimento do câncer de língua de 3 a 5 vezes, porém, Dahlgren et al., 2004, afirmam que a presença do HPV influenciou positivamente a sobrevida dos pacientes.

As alterações histológicas observadas no epitélio da língua e do esôfago durante o processo da carcinogênese, inclui alterações não proliferativas, como hiperceratose e inflamação e, alterações proliferativas, como hiperplasia, displasia, metaplasia, papiloma e, finalmente, o carcinoma (Bertran et al., 1996; Whiteley et al., 1996).

O aumento de hiperceratose no tecido epitelial da língua e do esôfago está relacionado com um nível de proteção quando o epitélio está exposto a agressões de curto prazo, sendo ainda considerada, juntamente com a hiperplasia, a alteração histológica mais comumente

observada no início do processo da carcinogênese (Ribeiro et al, 2005, Carrard et al., 2009; Al-Damouk, 1993).

2. Fatores de risco para o desenvolvimento do câncer de esôfago e de língua.

*2.1- Álcool e cig*arro

O consumo crônico de etanol está relacionado diretamente com o desenvolvimento de tumores no trato digestivo superior, tais como, cavidade oral, faringe, laringe, esôfago e próstata, sendo que, cerca de 25% a 80% dessas neoplasias podem ser atribuídas ao consumo crônico de etanol (Brown, 2005).

Além desses órgãos, o consumo excessivo de álcool leva ao desenvolvimento de neoplasias do fígado, intestino grosso, mama e pâncreas (Brown, 2005; Boffeta and Hashibe, 2006). Vários estudos epidemiológicos, revisados em Poschl and Seitz, 2004, mostram forte correlação entre a ocorrência dessas neoplasias e a ingestão de álcool, sugerindo que o etanol é um fator de risco importante para o desenvolvimento dessas doenças. Estudos realizados na França demonstraram aumento de 4.6 e 10.9 na incidência de câncer de esôfago em pacientes que fumam 35 ou mais cigarros ou que bebem 3 ou mais doses de álcool por dia (aproximadamente 25 gr), respectivamente. Esses fatores, quando associados, tiveram seus efeitos potencializados, apresentando risco final multiplicado (Kollarova et al, 2007).

A maioria das neoplasias do esôfago e da língua são freqüentemente atribuídas ao uso do álcool e do fumo, visto que, apresentam quantidades significativas de substâncias mutagênicas e/ou cancerígenas, em especial, de nitrosaninas (Lu et al., 1991; Yang et al., 1992; Boffeta et al., 1992; Mashberg et al., 1993; Straif et al., 2000; Du et al., 2000).

A ingestão de álcool leva a deficiências nutricionais, tais como, alterações no metabolismo lipídico, protéico, de carboidratos, de vitaminas, principalmente vitamina A e do complexo B, e micronutrientes, como o zinco (Bunout, 1999). Essas deficiências contribuem para a carcinogênese, uma vez que o consumo excessivo de etanol promove hipometilação do DNA, inclusive de genes supressores de tumor, aumentando a necessidade de ingestão de doadores de metil, cuja absorção fica comprometida após o consumo de álcool (Stickel et al., 2002; Pöschl and Seitz, 2004). Não obstante, o aldeído causa destruição do folato na célula, o que inibe a transmetilação de genes envolvidos na carcinogênese (Pöschl and Seitz, 2004).

O etanol, ainda pode apresentar efeito co-carcinogênico, pois seu consumo abusivo pode reduzir os níveis de substâncias antioxidantes, como as glutationas (redutases e transferases), α-tocoferol e β-caroteno, seja pela perda de estoques teciduais, por inibição de síntese ou por destruição através da geração de radicais livres (Kock et al., 2004; Valko et al., 2006), além de alterar a função de enzimas hepáticas antioxidantes, como a glutationa-S-transferase em decorrência da formação de adutos acetaldeído-enzima (Sultana et al., 2005). Seu consumo também aumenta a expressão da enzima CYP2E1 em vários órgãos, em especial no fígado (Kushida et al., 2005; Tsutsumi et al., 1993; Stickel et al., 2002; Pöschl and Seitz, 2004; McKillop and Schrum, 2005). Essa enzima, pertence à família do citocromo P450, que é responsável pela biotransformação do etanol a acetaldeído e de vários outros pró-cancerigenos, tais como, as aflatoxinas, nitrosaminas, hidrocarbonetos policíclicos, em suas formas reativas (Tsutsumi et al., 1993; Stickel et al., 2002; Pöschl and Seitz, 2004; Yu and Yuan, 2004; Brooks and Theruvathu, 2005). Estas substâncias estão presentes no ar, são produzidas no preparo de carnes e no cozimento dos

alimentos, no uso de cigarros e consumo de bebidas alcoólicas (Tsutsumi et al., 1993; Stickel et al., 2002; Karim et al., 2003; Pöschl and Seitz, 2004; McKillop and Schrum, 2005; Marrero, 2005; Kushida et al., 2005).

Como o etanol altera o metabolismo das nitrosaminas em animais experimentais e no homem (Pinto, 2000), aumentando suas concentrações no esôfago e diminuindo no fígado, verifica-se aumento no número de tumores esofágicos frente administração concomitante de etanol e nitrosaminas. Muft et al. (1989) observou que a administração simultânea de etanol e do cancerígeno N-nitrosomethylbenzylamine (NMBZA) em ratos Sprague-Dawley inibiu a ocorrência de tumores esofágicos durante a fase de iniciação, mas promoveu o desenvolvimento destes tumores quando administrado na pós-iniciação. Entretanto, ratos F344 machos que receberam etanol na água de beber durante a fase de iniciação, apresentaram maior índice de tumores esofágicos induzidos pela DEN (Aze et al., 1993), não observando diferenças estatísticas na incidência e multiplicidades destes tumores quando o etanol era administrado no pós-iniciação do cancerígeno NMBA (Morimura et al, 2001).

O efeito da ingestão crônica do etanol também tem sido alvo de estudo para o câncer de língua. Ratos Wistar machos que receberam etanol 36% na água de beber apresentaram aumento no tamanho da camada e dos núcleos das células basais (Born et al, 1996) e Al-Damouk (1993) observou aumento na espessura de hiperceratose na parte dorsal da língua de hamsters Sirios machos após tratamento com etanol 10% *ad libitum*. Sofritti et al. (2002) verificaram que a administração de etanol (10%) na água de beber induziu o desenvolvimento de tumores na língua e na boca de ratos Spraque–Dawley machos/fêmeas. Outros estudos demonstram que a ingestão de etanol 40% *ad libitum* aumentou a proliferação celular na camada epitelial intermediária da língua de camundongos CF1

fêmeas, assim como aumentou a keratinização do epitélio (Maito et al, 2003; Carrard et al., 2004), sendo que, a exposição tópica ao etanol não ocasionou diferenças significativas na proliferação celular do epitélio da língua (Maito et al., 2003).

Entre os vários cancerígenos presentes na fumaça do tabaco, os hidrocarbonetos aromáticos policíclicos, as aminas aromáticas e as nitrosaminas apresentam-se como os componentes mais importantes para o desenvolvimento de neoplasias em humanos, dentre eles, o câncer do trato digestivo superior (Hoffmann et al.,1990; Denissenko et al., 1996; Hecht, 2002). A exposição as nitrosaminas pode se dar através da dieta, do tabaco e da síntese endógena que ocorre no estômago pela reação do nitrito com aminas secundárias (Magee, 1989). As nitrosaminas, tais como a 4(metilnitrosamina)-1-(3-piridil)-1-butanona (NNK) e a N´-nitrosonornicotina (NNN) tem sido associadas ao desenvolvimento do câncer oral, de esôfago, pulmão e pâncreas em fumantes (Hoffman et al., 1990; Hecht et al., 1990; Hecht, 1998, 1999).

O consumo de cigarros destaca-se como um dos principais fatores de risco associados ao desenvolvimento de neoplasias do esôfago (Vaughan et al., 1995; Gammon et al., 1997). Entretanto, há poucos estudos que avalia a importância da inalação da fumaça (fumantes passivos) e do tipo de tabaco.

Launoy et al. (2000) demonstraram que a melhor relação entre o risco para o câncer de esôfago e o cigarro, depende do tipo de tabaco e do padrão morfológico da neoplasia, sendo consistentes com estudos que revelam que o cigarro escuro apresenta maior risco para o desenvolvimento dessa doença (De Stephani et al., 1993). O nível de exposição ao cigarro e o risco para o desenvolvimento de carcinoma de células escamosas depende da intensidade e duração do contato com a fumaça do cigarro (Doll et al., 1994; Castellsague et al., 1999; Lagergren et al., 2000). Lagergren et al. (2000) observou que pessoas que

fumam mais de 20 cigarros ao dia por mais de 35 anos desenvolvem mais carcinomas de células escamosas do que adenocarcinoma de esôfago, concluindo, portanto, que o fumo está mais associado com o desenvolvimento do SCC.

O rato é a espécie mais suscetível ao desenvolvimento de neoplasias de esôfago induzido por nitrosaminas que, induz tumores preferencialmente ou exclusivamente neste órgão, independente do meio de administração (Lijinsk, 1992). A ativação metabólica das nitrosaminas é catalisada pelo citocromo P450 (CYP), e o produto formado é extremamente instável podendo reagir com o DNA, induzindo mutações (Magee, 1989), sendo que no fígado, a CYP2E1 é a enzima mais importante envolvida nesta ativação (Yang et al., 1991). O esôfago de ratos não expressa a enzima CYP2E1, mas em contrapartida, expressa a CYP2A3 responsável em grande parte pelo metabolismo das nitrosaminas (Pinto et al., 2001, 2003).

O consumo do cigarro é considerado um dos principais fatores envolvidos da tumorigênese da cavidade oral (Chen et al., 2002), entretanto, são poucos os estudos experimentais que avaliam os efeitos da exposição á fumaça do cigarro sobre a carcinogênese do esôfago e da língua.

Izzotti et al, (1998) expôs fêmeas BD6 ao etanol 5% e à fumaça do cigarro (1hora por dia por oito meses), verificando que a exposição ao tabaco induziu a formação de adutos de DNA no pulmão e no coração mas não no esôfago. Porém, a combinação do etanol, resultou na formação destes adutos no esôfago.

Estudo realizado por Ribeiro et al (2008) demonstraram que ratos Wistar machos expostos á fumaça do cigarro durante 75 dias, apresentaram na mucosa da língua, focos da enzima Glutationa S-transferase (GSTs), enzima esta, detectada nos tecidos quando

expostos a agentes carcinogênicos, assim como superexpressão das proteínas bcl-2 e bax envolvidas no processo apoptótico (Assis et al 2005).

2.2- Sal biliar e Deficiência de zinco.

Estudos epidemiológicos e experimentais indicam que a deficiência de zinco está relacionada com o aumento no risco de desenvolvimento do câncer de esôfago, língua e cólon (Prassad et al., 2002; Fong et al., 1998,1999,2006; Doer et al., 1997) e que a obesidade e a irritação crônica pelos ácidos biliares são considerados os fatores de maior risco para o desenvolvimento do câncer de cólon, do trato digestivo superior e do chamado Esôfago de Barret (EB) (Ozata et al., 2002; Oh et al., 2006)

O zinco é um importante componente de várias proteínas, como por exemplo, a proteina p53, envolvida no reparo de danos do DNA, a qual também se apresenta mutada na metade dos tumores humanos (HO et al., 2002). A concentração de zinco no corpo humano é de 2g a 4g, entretanto, muitas vezes, uma suplementação regular faz-se necessária. Uma ingestão insuficiente de zinco pode prejudicar a defesa antioxidante e comprometer os mecanismos de reparo do DNA, além de que, sua deficiência pode levar à hipometilação do DNA por reduzir a absorção de grupos metil.

Pacientes com câncer oral, geralmente apresentam deficiência em zinco (Doerr et al., 1997; Kleier et al., 1998) e baixa função do gene supressor de tumor p53 (Greenblatt et al., 1994). A deficiência de zinco por si só causa uma condição pré-cancerosa na língua, no esôfago e no estômago de ratos por causar aumento na taxa de proliferação celular, superexpressão da enzima ciclooxigenase-2 (Cox-2) e hiperplasia, além de hiperceratose e paraceratose (Follis 1996; Swenerton and Hurley 1968; Fong et al. 1996, 2005).

Estudos experimentais demonstram que o tratamento com dieta deficiente em zinco aumentou a taxa de proliferação celular e a incidência de tumores esofágicos em camundongos C57BL/6 tratados com N-nitrosomethylbenzylamine (NMBA) (Fong et al. 1999) e acelerou a carcinogenese de esôfago e de estomago de ratos Sprague-Dawley machos e de camundongos machos $p53^{-/-}$ causada por uma única dose de (NMBA) (Fong et al 1997; 2003).

Ratos machos Sprague-Dawley alimentados com dieta contendo 3ppm de zinco apresentaram aumento de proliferação celular na mucosa esofágica após 5 semanas de tratamento (Liu et al, 2005) e 20% (vinte por cento) dos camundongos $p53^{-/-}$ desenvolveram tumores e metaplasia glandular semelhantes ao EB após 3 semanas de tratamento com dieta deficiente em zinco e com uma única dose de NMBA (Fong et al 2003).

Estudo conduzido por Fong et al (2005) demonstrou que, a dieta deficiente em zinco e a administração da 4-nitroquinoline 1-oxide (NQO) em ratos Sprague-Dawley machos, ocasionou hiperplasia, aumento de proliferação celular e da expressão Cox-2 no epitélio da língua, assim como, alta incidência de SCC na língua e esôfago de camundongos C57BL/6 $p53^{+/-}$ (Fong et al 2006).

O aumento de hiperceratose e paraceratose no epitélio do esôfago, língua e de outras partes da cavidade oral de roedores é a alteração histológica mais comumente observada em modelos experimentais de deficiência de zinco. Joseph et al. (1981) observou aumento de paraceratose no epitélio lingual de coelhos machos New Zealand, assim como, ratos Sprague-Dawley machos apresentaram aumento de hipercertose na face dorsal da língua, sendo mais proeminente entre as papilas (Orback et al., 2007). A dieta deficiente em zinco também aumentou a taxa de hiperceratose e/ou paraceratose no esôfago de camundongos

machos C57BL/6 (Guy et al., 2007) e no esôfago de ratos machos Sprague- Dawley iniciados com a NMBA (Fong et al., 2001).

Por outro lado, a reposição do zinco reverte rapidamente a proliferação celular, estimula apoptose e reduz a expressão da COX-2 no epitélio esofágico e da língua de ratos, inibindo, portanto, a tumorigênese (Fong et al. 2001, 2005; Liu et al., 2005).

A bile é um componente do refluxo gastroduodenal (Kauer et al., 1997) e os ácidos biliares primários, como o ácido glicocólico, taurocólico, quenodeoxicólico e glicodeoxicólico, estão predominantemente presentes no esôfago de pacientes que apresentam doença do refluxo gastroesofágico (GERD) (Theisen et al., 2000). A forma mais tóxica dos ácidos biliares (AB) são os ácidos não-conjugados, como por exemplo, o ácido deoxicólico (DOC), presente no refluxo de pacientes com EB (Nehra et al., 1999).

Os AB são foco de diversos estudos *in vivo* e *in vitro* que demonstram sua ação sobre células epiteliais de órgãos, tais como cólon e esôfago (Weisburg et al., 1983; Suterland et al., 1994; Stamp, 2002)

O desenvolvimento de tumores, pode ser ocasionado pelo fato dos sais biliares selecionar as células resistentes a apoptose e por estimular a atividade da cicloxigenase 2 (COX-2), a qual por sua vez, estimula a invasão de células malignas (Glinghammar et al., 2002; Jurek et al., 2005; Zhang et al. 1998). Os AB ainda são capazes de promover aumento de proliferação celular pela indução da expressão de proto-oncogenes, como o c-fos e c-jun (Velazquez et al., 1996), sendo o ácido deoxicólico (DOC) capaz de reduzir a transcrição do fator NF-kB e da enzima ADP ribose envolvidas no reparo oxidativo do DNA (Payne et al., 1998).

Estudo realizado por Rosignoli et al. (2008) demonstrou que o tratamento de colonócitos humanos com DOC e CDCA (acido quenodeoxicólico), podem agir como

promotor e iniciador da carcinogênese. Os AB secundários possuem propriedades detergentes que lesam a membrana plasmática das células epiteliais do cólon levando à morte celular (Narisawa et al., 1974; Reddy et al., 1976; Deschner et al., 1981). A conseqüente proliferação celular compensatória pode aumentar a susceptibilidade das células a mutagênicos e, conseqüentemente, ao câncer (De Kok et al., 2000).

Guy et al.(2007), desenvolveram um modelo experimental animal para o estudo de esofagite e EB através de dieta deficiente em zinco e suplementada com DOC em camundongos C57BL/6. Neste estudo, dos animais que receberam a dieta DOC+Zinco^{-}, 63% apresentaram modificações gastroesofágicas semelhantes ao EB, e 100% apresentaram hiperproliferação de células basais, hiperqueratinização, hipertrofia papilar, aumento de infiltração de células inflamatórias e de angiogênese, assim como, metaplasia entre 69 e 152 dias de tratamento. Segundo Guy et al. (2007), este modelo mimetiza a ingestão de uma dieta deficiente em micronutrientes, deficiência esta, presente nas dietas hipercalóricas a qual contribui para o desenvolvimento do refluxo gastroesofágico. Em conseqüência, este, pode levar ao desenvolvimento de lesões tipo EB, podendo ser potencializadas com a suplementação do ácido biliar deoxicólico.

Referências

3- Referências

Al-Damouk JD. Oral epithelial response to experimental chronic alcohol ingestion in hamsters. Oral Surg Oral Med Oral Pathol 1993;76(6):736-41.

Assis GF, Ceolin DS, Marques MAEA, Salvadori DM, Ribeiro DA. Cigarette smoke affects apoptosis in rat tongue mucosa: role of bcl-2 gene family. J Mol Histol 2005; 36:483-89.

Aze Y, Toyoda K, Furukawa F, Mitsumori K, Takahashi M. Enhancing effect of ethanol on esophageal tumor development in rats by initiation of diethylnitrosamine. Carcinogenesis 1993;14(1):37-40.

Baik SC, Youn HS, Chung MH, Lee WK, Cho MJ, Ko GH et al. Increased oxidative DNA damage in infected human gastric mucosa. Cancer Res. 1996;56:1279-82.

Barros SGS, Ghisolf ES, Luz LP. Mate (chimarrão) é consumido em alta temperatura por população sob risco para o carcinoma epidermóide de esôfago. Arq Gastroenterol 2000;37:25-30.

Bertram TA, Markovits JE, Juliana MM. Non-proliferative lesions of the alimentary canal in rats. Guides for Toxicol Pathol 1996; 1-16.

Blot WJ, Devesa SS, Kneller RW, Fraumeni JFJr et al. Rising incidence of adenocarcinoma of the esophagus and gastric cardia. J Am Med Assoc. 1991;265:1287-89.

Blot WJ, McLaughlin JK. The Changing epidemiology of esophageal cancer. Semin Oncol 1999;26:2-8.

Bofetta P, Hashibe M. Alcohol and cancer. Lancet Oncol 2006;7:149-56.

Boffetta P, Mashberg A, Winkelmann R, Garfinkel L. Carcinogenic effect of tobacco smoking and alcohol drinking on anatomic sites of the oral cavity and oropharynx. Int J Cancer 1992;52(4):530–33.

Brooks TJ, Theruvathu JA. DNA adducts from acetaldehyde: implications for alcohol-related carcinogenesis. Alcohol 2005;35:187-93.

Brown LM. Epidemiology of alcohol-associated cancers. Alcohol 2005;35:161-68.

Bunout D. Nutritional and metabolic effects of alcoholism: their relationship with alcoholic liver disease. Nutr1999;15:583-89.

Cameron AJ, Lomboy CT, Pera M, Carpenter HA. Adenocarcinoma of the esophagogastric junction and Barret´s esophagus. Gastroenterol. 1995;109:1541-46.

Carrard VC, Filho MS, Radosb PV, Chaves AC, Lauxen Ida S. Quantification of silver-staining nucleolar organizer region in epithelial cells of tongue of mice after exposure to, or intake of, alcohol.Alcohol 2004;34:233-38.

Carrard VC, Pires AS, Mendez M, Mattos F, Moreira JCF, Sant'Ana Filho M. Effects of acute alcohol consumption and vitamin E co-treatment on oxidative stress parameters in rats tongue. Food Chem Toxicol 2009;47:1058–63.

Castellsague X, Munoz N, De Stefani E, Victora CG, Castelletto R, Rolón PA et al. Independent and join effects of tobacco smoking and alcohol drinking on the risk of esophageal cancer in mem and women. Int J Cancer 1999;82:657-64.

Chen Y, Si JM, Liu W, Cai JT, Du Q, Wang LJ et al. Induction of experimental acute ulcerative colitis in rats by administration of dextran sulfate sodium at low concentration followed by intracolonic administration of 30% ethanol. J Zhejiang Univ Sci 2007; 8(9):632-37.

Craddock VM. Etiology of esophageal cancer: some operative factors. Eur J Cancer Prev. 1992;1:89-103.

Dahlgren L, Dahlstrand H, Lindquist D, et al., Human papillomavirus is more common in base of tongue than in mobile tongue cancer and is a favorable prognostic factor in base of tongue cancer patients. Inter J Cancer 2004;112(6):1015–19.

Davidson TM. Squamous cell carcinoma of the base of the tongue. Head Neck Surg 1987;9:312–13.

Day NE, Varghese C. Esophageal cancer. Cancer Surv. 1994;19-20:43-54.

De Kok TM, Van Maanen JMS. Evaluation of fecal mutagenicity an colorectal cancer risk. Mutat Res 2000;463:53–101.

De Stephani E, Barrios E, Ferrio L. Black (air- cured) and blond (fluecured) tobacco and cancer risk III:oesophageal cancer. Eur J Cancer. 1993;5:763-66.

Denissenko MF, Pao A, Tang MS, Pfeifer GP. Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in p53. Sci 1996,274:43032.

Deschner EE, Cohen BI, Raicht RF. Acute and chronic effect of dietary cholic acid on colonic epithelial cell proliferation. Digestion 1981;21:290–96.

Doerr TD, Prasad AS, Marks SC, Beck FW, Shamsa FH, Penny HS et al. Zinc deficiency in head and neck cancer patients. J Am Coll Nutr 1997;16:418–22.

Doll R, Peto R, Wheatley K, Gray R, Sutherland I. Mortality in relation to smoking. 40 years´ observations on male Britsh doctors. BMJ 1994;309:901-11.

Du X, Squier CA, Kremer MJ, Wertz PW. Penetration of N-nitrosonornicotine (NNN) across oral mucosa in the presence of ethanol and nicotine. J Oral Pathol Med 2000;29:80-5.

Engel LS, Chow WH, Vaughan TL, Gammon MD, Risch HA, Stanford JL et al. Population attributable risks of esophageal and gastric cancers. J Natl Cancer Inst 2003;95:1404-13.

Enzinger PC, Mayer RJ. Esophageal cancer. N Engl J Med. 2003;349(23):2241-52.

Farhadi A, Fields J, Banan A, Keshavarzian A. Reactive oxygen species: Are they involved in the pathogenesis of GERD, Barrett's esophagus and the latter's progression toward esophageal cancer? Am J Gastroenterol. 2002;97(1):22-26.

Farhadi M, Tahmasebi Z, Merat S, Kamangar F, Nasrollahzadeh D, Malekzadeh R. Human papillomavirus in squamous cell carcinoma of esophagus in a high-risk population. World J Gastroenterol 2005;11(8):1200-3.

Ferrari D, Codec`a C, Fiore J, Moneghini L, Bosari S, Foa P. Biomolecular Markers in Cancer of the Tongue. J Oncol 2009:1-11.

Follis RHJr. The pathology of zinc deficiency. In: Prasad, AD. (ed.). Zinc Metabolism. C.Thomas 1966;129-41.

Fong LY, Ishii H, Nguyen VT, Vecchione A, Farber JL, Croce CM et al. P53 deficiency accelerates induction and progression of esophageal and forestomach tumors in zinc-deficient mice. Cancer Res 2003;63:186–95.

Fong LY, Jiang Y, Farber JL. Zinc deficiency potentiates induction and progression of lingual and esophageal tumors in p53-deficient mice. Carcinogenesis 2006;27(7):1489–96.

Fong LY, Lau KM, Huebner K. Induction of esophageal tumors in zinc-deficient rats by single low doses of N-nitrosomethylbenzylamine (NMBA): analysis of cell proliferation, and mutations in H-ras and p53 genes. Carcinogenesis 1997;18:1477–84.

Fong LY, Li JX, Farber JL, Magee PN. Cell proliferation and esophageal carcinogenesis in the zinc-deficient rat. Carcinogenesis 1996;17:1841–8

Fong LY, Magee PN. Dietary zinc deficiency enhances esophageal cell proliferation and N-nitrosomethylbenzylamine (NMBA)-induced esophageal tumor incidence in C57BL/6 mouse. Cancer Lett 1999;143:63–9.

Fong LY, Nguyen VT, Farber JL. Esophageal cancer prevention in zinc-deficient rats: rapid induction of apoptosis by replenishing zinc**.** J. Natl. Cancer Inst 2001; 93(20):1525–33.

Fong LY, Pegg AE, Magee PN. Difluoromethylornithine inhibits Nnitrosomethylbenzylamine induced esophageal carcinogenesis in zinc deficient rats: effects on esophageal cell proliferation and apoptosis. Cancer Res 1998;58:5380–8.

Fong LY, Zhang L, Jiang Y, farber JL. Dietary zinc modulation of COX-2 expression and lingual and esophageal carcinogenesis in rats. J. Natl Cancer Inst 2005;97:40–50.

Forastiere A, Koch W, Trotti A, Sidransky D. Head and neck cancer. N Engl J Med. 2001;345:1890-900.

Friedlander PL, Schantz SP, Shaha AR, Yu G, Shah JP. Squamous cell carcinoma of the tongue in young patients: a matched-pair analysis. Head & Neck. 1998;20:363-8.

Funk GF, Karnell LH, Hoffman HT, Zhen W, Menck HR. Presentation, treatment and outcome of oral cavity cancer: a National Cancer Data Base report. Head Neck 2002; 24:165–180

Gammon MD, Schoenberg JB, Ahsan H .Tobacco, alcohol,and socioeconomic status and adenocarcinomas of the esophagus and gastric cardia. J Natl Cancer Inst 1997;89(17): 1277–84.

Guy NC, Garewal H, Holubec H, Bernstein H, Payne CM, Bernstein C et al. A novel dietary-related model of esophagites and Barrett´s esophagus, a premalignant lesion. Nutr Câncer 2007;59(2):217-27.

Glinghammar B, Inoue H, Rafter JJ. Deoxycholic acid causes DNA damage in colonic cells with subsequent induction of capsases, COX-2 promoter activity and the transcription factors NF.kB and AP-1. Carcinogenesis 2002;23:839–45.

Greenblatt MS, Bennett WP, Hollstein M. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res 1994;54:4855–78.

Hecht SS. Biochemistry, biology, and carcinogenicity of tobacco-specific N-niyrosamines. Chem Res Toxicol. 1998;11(6):559-603.

Hecht SS. Cigarette smoking and lung cancer: chemical mechanisms and approaches to prevention. Lancet Oncol 2002;3:461-69.

Hecht SS. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst. 1999;91(14):1194-1210.

Hecht SS., Hoffman D. The relevance of tobacco-specific nitrosamines to human cancer. Cancer Surv 1990;8:273-95.

Herrero R, Castellsagu´e X, Pawlita M, Lissowska J, Kee F, Balaram P et al. Human papillomavirus and oral cancer: the international agency for research on cancer multicenter study. J Natl Cancer Inst 2003;95(23):1772–83.

Ho E, Ames BN. Low intracellular zinc induces oxidative DNA damage, disrupts p53, NFkappa B, and AP1 DNA binding, and affects DNA repair in a rat glioma cell line. Proc Natl Acad Sci U S A. 2002;99(26):16770-5.

Hoffman D, Hecht SS. Advances in tobacco carcinogenesis. In:Cooper CS, Grower PL, editors. Handbook of experimental pharmacology. Heidelberg: Spinger-Verlag; 1990;.94:63-102.

Instituto Nacional do Cancer – INCA. Câncer de esôfago, 2008. Disponível em: http://www.inca.gov.br/estimativa/2008.

Izzotti A, Balanski RM, Blagoeva PM, Mircheva ZI, Tulimiero L, Cartiglia C et al. DNA alterations in rat organs after chronic exposure to cigarette smoke and/or ethanol ingestion. J Faseb 1998;12:753-58.

Joseph CE, Ashrafi SH, Waterhouse JP. Estructural Changes in Rabbit Oral Epithelium Caused by Zinc Deficiency. J Nutr 1981; 111:53-7.

Jurek D, Fleckl E, Marian B. Bile acid induced gene expression in LT97 colonic adenoma cells. Food Chem Toxicol 2005;43:87–93.

Karim MR, Wanibuchi H, Wei M, Morimura K, Salim EI, Fukushima S. Enhancing risk of ethanol on MeIQx-induced rat hepatocarcinogenesis is accompanied with increase levels of cellular proliferation and oxidative stress. Cancer Lett 2003;192:37-47.

Kauer WK, Peters JH, DeMeester TR, Feussner H, Ireland AP, Stein HJ et al.Composition and concentration of bile acid reflux into the esophagus of patients with gastroesophageal reflux disease. Surg 1997;122:874–78.

Kleier C, Werkmeister R, Joos U. Zinc and vitamin A deficiency in diseases of the mouth mucosa. Mund Kiefer Gesichtschir 1998;2:320–25.

Knaapen AM, Güngor N, Schins RP, Borm PJ, Van Schooten FJ. Neutrophils and respiratory tract DNA damage and mutagenesis: A rewiew. Mutagen 2006;21(4):225-36.

Kock OR, Pani G, Borrello S, Colavitti R, Cravero A, Farrè S et al. Oxidative stress and antioxidant defenses in ethanol-induced cell injury. Mol Aspects Med 2004;25(1-2):191-98.

Kollarova H, Machova L, Horakova D, Janoutova G, Janout V. Epidemiology of esophageal câncer – An Overview article. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2007; 151(1):17-28.

Kushida M, Wanibuchi H, Morimura K, Kinoshita A, Kang JS, Puatanachokchai R et al. Dose-dependence of promotion of 2-amino-dimethylimidazo[4,5-f]quinoxaline-induced rat hepatocarcinogenesis by ethanol: evidende for a threshold. Cancer Sci 2005;96:747-57.

Lagergren J, Bergstrom R, Lindgren A, Nyren O. The role of tobacco snuff and alcohol use in the aetiology of cancer of the oesophagus and gastric cardia. Int J Cancer 2000;85:340-6.

Launoy G, Milan C, Faivre G, Pienkowski P, Gignoux M Tobacco type and risk of squamous cell cancer of the oesophagus im males: a French multicentre caso-control study. Int J Epidemiol. 2000;29:36-42.

Li JY, Taylor PR, Li B, Dawsey S, Wang GQ, Ershow AG, Dawsey S, Wang GQ, Ershow AG et al. Nutrition intervention trials in Linxian, China: multiple vitamin/mineral supplementation, cancer incidence and disease-specifc mortality among adults with esophageal dysplasia. J Natl Cancer Inst. 1993;85:1492-8.

Li YT, He B, Wang YZ. Exposure to cigarette smoke upregulates AP-1 activity and induces TNF-alpha overexpression in mouse lungs. Inhal Toxicol 2009, 21(7):641-7.

Lijinsky W. Chemistry and biology of N-Nitroso compounds.Cambr. Univ. Press, UK, 1992.

Linden MD, Nathanson SD, Zarbo RJ. Evalution of anti-p53 antibody staining quality control and technical considerations. Appl Immunohistochem 1994;2:218-24.

Liu CG, Zhang L, Jiang Y, Chatterjee D, Croce CM, Huebner K, et al. Modulation of gene expression in precancerous rat esophagus by dietary zinc deficit and replenishment. Cancer Res 2005;65:7790–99.

Llewellyn CD, Johnson NW, Warnakulasuriya KA. “Risk factors for squamous cell carcinoma of the oral cavity in young people—a comprehensive literature review”. Oral Oncology 2001;37(5):401–18.

Lopes FF, Cutrim MCFN, Casal CP, Fagundes DM, Montoro LA. Aspectos epidemiológicos e terapêuticos do câncer bucal. Rev Bras Odontol 2002;59(2):98-9.

Lu SH, Chui SX, Yang WX, Hu XN, Guo LP, Li FM. Relevance of N-nitrosamines to esophageal cancer in china. In O´Neill,I.K., Chein,J. and Bartsch,H. (eds) Relevance to Human Cancer of N-nitroso Compounds, Tobaco Smoke and Mycotoxin, IARC Sci Public no. 105 IARC, Lyon 1991;105:11-7.

Magee PN. The experimental basis for the role of nitrosos compounds in human cancer. Cancer Surv. 1989;8:207-39.

Magrath I & Litvak J. Cancer in developing countries: opportunity and challenge. J Natl Cancer Inst 1993; 85: 862–874

Maito FLDM, Rados PV, Filho MS, Barbachan JJ, Quadros O. Proliferating cell nuclear antigen expression on tongue of mice after intake of, or topical exposure to, alcohol. Alcohol 2003;31:25–30.

Marrero JA. Hepatocelular carcinoma. Curr. Op. in Gastroenterol 2005;21:308-12.

Mashberg A, Boffetta P, Winkelman RL. Garfinkel L. Tobacco smoking, alcohol drinking, and cancer of the oral cavity and oropharynx among U.S. Veterans. Cancer 1993;72(4):1369–75.

McKillop IH, Schrum LW. Alcohol and liver cancer. Alcohol. 2005;35:195-203.

Morimura K, Hori T, Kaneko M, Nishikawa T, Nishikawa A, Wanibuchi H et al. Promotion of chemically induced rat esophageal tumorigenesis with post-initation ethanol modification. Teratog Carcinog Mutagen. 2001;21(4):295-301.

Mufti SI, Becker G, Sipes IG. Effect of chronic dietary ethanol consumption on the initiation and promotion of chemically-induced esophageal carcinogenesis in experimental rats. Carcinogenesis 1989;10(2):303-9.

Murphy SJ, Anderson LA, Johnston BT, Fitzpatrick DA, Watson PR, Monaghan P et al. Have patients with esophagitis got an increased risk of AC? Results from a population-based study. World J Gastroenterol. 2005;11(46): 7290-95.

Narisawa T, Magadia NE, Weisburger JH, Bryan GT. Promoting effects of bile acids on colon carcinogenesis after intrarectal instillation of N N-methyl-N'-nitro-N-nitrosoguanidine in rats. J Natl Cancer Inst 1974;53(4):1093-7.

Nehra D, Howell P, Williams CP, Pye JK, Beynon J. Toxic bile acids in gastro-oesophageal reflux disease: influence of gastric acidity. Gut 1999;44:598–602.

Oh DS, Hagen JA, Fein M, Bremner CG, Dunst CM, Demeester SR, et al. The impact of reflux composition on mucosal injury and esophageal function. J Gastrointest Surg 2006;10:787–96.

Olyaee M, Sontag S, Salman W, Schnell T, Mobarhan S, Eiznhamer D et al. Mucosal reactive oxygen species production in oesophagitis and Barrett's esophagus. Gut. 1995;37:168-73.

Orbak R, Kara C, Ozbek E, Tezel A, Demir T. Effects of zinc deficiency on oral and periodontal diseases in rats. J Periodont Res 2007;42:138–143.

Ozata M, Mergen M, Oktenli C, Aydin A, Sanisoglu SY, Bolu E et al. Increased oxidative stress and hypozincemia in male obesity. Clin Biochem 2002;35:627–31.

Pack SD, Karkera JD, Zhuang Z, Pak ED, Balan KV, Hwu P et al. Molecular cytogenetic finger printing of esophageal squamous cell carcinoma by comparative genomic hybridization reveals a consisted pattern of chromosomal alterations. Genes Chromossomes Cancer. 1999;25(2):160-68.

Payne CM, Crowley C, Washostultz D, Briehl M, Bernstein H, Bernstein C, et al. The stress-response proteins poly(-ADP)ribose polymerase and NF-kB protect against bile salt-induced apoptosis. Cell Death Differ 1998;5(7):623-36.

Pera M, Trastek VF, Carpenter HA, Fernandez PL, Cardesa A, Mohr U et al. Influence of pancreatic and biliary reflux on the development of esophageal carcinoma. Ann Thorac Surg. 1993;55:1386- 93.

Pinto LF, Moraes E, Albano RM, Silva MC, Godoy W, Glisovic T, et al. Rat oesophageal cytocrome P450 (CYP) monooxygenase system: comparison to the liver and relevance in N-nitrosodiethylamine carcinogenesis. Carcinogenesis. 2001;22:1877-83.

Pinto LFR, Rossini AMT, Albano RM, Felzenszwalb I, de Moura Gallo CV, Nunes RA et al. Mechanisms of esophageal câncer development in Brazlians. Mutat Res. 2003;544:365-73.

Pinto LFR. Differences between isoamyl alcohol and ethanol on the metabolism and DNA ethylation of N-nitrosodiethylamine in the rat. Toxicology. 2000;11:73-9.

Pöschl G, Seitz HK. Alcohol and cancer. Alcohol. 2004;39:155-65.

Prasad AS, Kucuk O. Zinc in cancer prevention. Review Cancer Metastasis Rev 2002;21:291–5.

Pütz A, Hartmann AA, Fontes PR, Alexandre CO, Silveira DA, Klug SJ et al. TP53 mutation pattern of esophageal squamous cell carcinomas in a high risk area (Southern Brazil): role of life style factors. Int J Cancer. 2002;98:99-105.

Reddy BS, Narisawa T, Weisburger JH, Wynder EL. Promoting effects of sodium deoxycholate on colon adenocarcinomas in germfree rats. J. Natl Cancer Inst 1976;56:441–2.

Ribeiro DA, Assis GF. Expression of placental glutathione S-transferase in rat tongue mucosa exposed to cigarette smoke. J Mol Histol 2008;39(1):115-9.

Ribeiro DA, MF. Salvadori DMF, Marques MEA. Abnormal expression of bcl-2 and bax in rat tongue mucosa during the development of squamous cell carcinoma induced by 4-nitroquinoline 1-oxide. Int J Exp Path 2005;86:375–81.

Robbins SL, Cotran RS. Bases Patológicas das Doenças. Ed. Elservier; 7th ed., p.1592, 2005.

Rosignoli P, Fabiani R, Bartolomeo A, Fuccelli R, Pelli MA, Morozzi G. Genotoxic effect of bile acids on human normal and tumour colon cells and protection by dietary antioxidants and butyrate. Eur J Nutr 2008;47:301–09.

Soffritti M, Belpoggi F, Cevolani D, Guarino M, Padovani M, Maltoni C. Results of long-term experimental studies on the carcinogenicity of methyl alcohol and ethyl alcohol in rats. Ann N Y Acad Sci 2002;982:46-69.

Stamp DHT. Three hypotheses linking bile to carcinogenesis in the gastrointestinal tract: certain bile salts have properties that may be used to complement chemotherapy. Med. Hypotheses 2002;59:398-405.

Stickel F, Schuppan D, Hahn EG, Seitz HK. Cocarcinogenic effects of alcohol in hepatocarcinogenesis. Gut 2002;51:132-39.

Stoner GD, Gupta A. Etiology and chemoprevention of esophageal squamous cell carcinoma. Carcinogenesis 2001;22(11):1737-46.

Straif K, Weiland SK, Bungers M, Holthenrich D, Taeger D, Yi S et al. Exposure to high concentrations of nitrosamines and cancer mortality among a cohort of rubber workers. Occup Environ Med 2000;57:180-7.

Sugerman PB, Shillitoe EJ. The high risk human papillomaviruses and oral cancer: evidence for and against a causal relationship. Oral Dis 1997;3(3):130–47.

Sultana R, Raju BS, Sharma V, Reddanna P, Babu PP. Formation of acetaldehyde adducts of glutathione S-transcenase A3 in the liver of rats administered alcohol chronically. Alcohol 2005; 35(1):57-66.

Sutherland LA, Bird RP. The effect of chenodeoxycholic acid on the development of aberrant crypt foci in the rat colon. Cancer Lett 1994;76:101–07.

Swenerton H and Hurley LS. Severe zinc deficiency in male and female rats. J. Nutr 1968;95:8-18.

Tampi C, Pai S, Doctor VM, Plumber S, Jagannath P. HPV-associated carcinoma of esophagus in the young: a case report and review of literature. Int J Gastrointest Cancer. 2005;35(2):135-42.

Theisen J, Nehra D, Citron D, Johansson J, Hagen JA, Crookes PF et al. Suppression of gastric acid secretion in patients with gastroesophageal reflux disease results in gastric bacterial overgrowth and deconjugation of bile acids. J Gastrointest Surg 2000;4:50–4.

Tomatis L, Aitio A, Day NE, Heseltine J, Kaldor J, Miller AB et al. Cancer: cause, occurrence and control. IARC Sci. Publ no 100. Lyon: IARC, 1990.

Tsutsumi M, Lasker JM, Takahashi T, Lieber SC. In vivo induction of hepatic P4502E1 by ethanol: role of increased enzyme synthesis. Arch. Biochem. Biophys 1993;304:209-18.

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced câncer. Chem Biol Interact 2006;160:1-40.

Vaughan TL, Davis S, Kristal A, Thomas DB. Obesity, alcohol, and tobacco as risk factors for cancers of the esophagus and gastric cardia: adenocarcinoma versus squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev. 1995;4(2):85–92.

Velazquez OC, Zhou D, Seto RW, Jabbar A, Choi J, Lederer HM et al. In vivo crypt surface hyperproliferation is decreased by butyrate and increased by deoxycholate in normal rat colon: associated in vivo effects on c-fos and c-jun expression. J Parenter Enteral Nutr 1996;20:243–50.

Victora CG, Muñoz N, Day NE, Barcelos LB, Peccin DA, Braga NM. Hot beverages and esophageal cancer in southern Brazil: a case-control study. Int J Cancer. 1987;39:710-16.

Vogelstein B. “A deadly inheritance”. Nature 1990;348(6303):681–82.

Weisburger JH, Reddy BS, Barnes WS, Wynder EL. Bile acids, but not neutral sterols, are tumor promoters in the colon in man and in rodents. Environ Health Perspect 1983;50:101–07.

Witeley LO, Anver MR, Botts S, Joknen MP. Proliferative lesions of the intestine, salivary glands, oral cavity and Esophagus in rats. Guides for Toxycol Pathol 1996; 1-18.

World Cancer Research Fund. Food, nutrition and prevention of cancer: A global perspective. Washington. Am Inst Cancer Res 1997;35-71:508-40.

World Health Organization. The World Health Report 1998: Life in the 21st century a vision for all. Geneva. WHO 1998:61-111.

Yang CS, Smith Y, Ishizak H, Hong JY. Enzyme mechanisms in the metabolism of nitrosamines. In: O´Neill I.K., Chen J., Barysch H. Relevance to human cancer of nitroso compounds, tobacco smoke and mycotoxins. Int Ag Res Cancer**,** Lyon, 1991;265-74.

Yang WX, Pu J, Lu SH. Studies on the exposure level of nitrosamines in the gastric juice and its inhibition in high risk areas of esophageal cancer. Chin J Oncol. 1992; 14:407-10.

Yu M, Yuan JM. Environmental factors and risk for hepatocellular carcinoma. Gastroenterol 2004;127:72-8.

Zhang F, Subbaramaiah K, Altorki N, Dannenberg AJ. Dihydroxy bile acids activate the transcription of cyclooxygenase-2. J Biol Chem 1998;273:2424–8.

Zhen W, Karnell LH, Hoffman HT, Funk GF, Buatti JM, Menck HR.“The national cancer data base report on squamous cell carcinoma of the base of tongue”. Head and Neck, 2004;26(8):660–74.

Objetivos

4. Objetivos

No presente estudo, utilizamos uma dieta que mimetiza a ingestão inadequada de zinco, suplementando-a com um componente associado ao refluxo gastroesofágico e à dieta hipercalórica, o ácido biliar deoxicólico (DCA), que pode criar uma condição pré-cancerígena no trato digestivo. Desta forma, o objetivo deste trabalho foi verificar a influência da exposição à fumaça do cigarro e ao etanol sobre as alterações epiteliais do esôfago e da língua induzidas pela dieta modificada.

Capítulo II

Article

Effects of cigarette smoke and ethanol intake on tongue and esophagus of male C57BL/6 mice fed a zinc-deficient diet supplemented with deoxycholic acid.

Joyce Regina Zapaterini[1,2], Nelci Antunes de Moura[1], Maria Aparecida Marchesan Rodrigues[2], Luis F. Barbisan[1,2]*

[1]UNESP São Paulo State University, Institute of Biosciences, Department of Morphology, 18618-000 Botucatu, SP, Brazil.

[2]UNESP São Paulo State University, School of Medicine, Department of Pathology, 18618-000 Botucatu, SP, Brazil.

Running title: Esophagus, tongue, cigarette smoking, ethanol, zinc deficiency, deoxycholic acid.

Address correspondence to:

Luis Fernando Barbisan, Ph.D.

Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista (UNESP), 18618-000 Botucatu, SP, Brazil.

Telephone/Fax-simile: 55-14-38116264/ e-mail: barbisan@ibb.unesp.br

Abstract

Upper aero digestive tract (UADT) cancer is an important cause of morbidity and mortality worldwide. Chronic alcohol intake and dietary deficiency zinc, tobacco smoking, and gastroesophageal reflux disease are the major risk factors to the UADT cancer. In the present study, we utilized a diet that mimic inadequate zinc intake (Zn^-) diet and supplemented it with unconjugated bile deoxycholic acid (DCA), a component associated with gastroesophageal reflux disease and high fat diets. The aim of this study was to assess the additional influence of exposure to cigarette smoke and ethanol intake on the epithelial alterations of the esophagus and tongue induced by Zn^-+DCA diet. Male C57BL/6 mice animals were allocated into six groups: Groups 1 to 3 were fed modified diet (Zn^-+ 0.2% DCA) and groups 4 to 6 were fed control diet. After 5-weeks, groups 2 and 5 intake ethanol 10% *ad libitum* and groups 3 and 6 were exposed to cigarette smoke for 15 weeks. All animals were euthanized at the end of week 20 and tongue and esophagus were collected for histological analysis and immunohistochemical analysis of cell proliferation using Ki-67 marker and cyclooxygenase 2 expression (COX-2). The Zn^-+DCA diet treatment trend to increased cell proliferation indexes and the incidence of hyperkeratosis in the tongue and esophagus but not in COX-2 expression. No additional effect of 15-week treatment with ethanol or cigarette smoke was observed. These findings indicate that dietary zinc deficiency supplemented with deoxycolic acid appears to be an important factor of epithelial aggression and that deleterious effect of ethanol and cigarette smoke could be detected in a long-term exposure.

Key words: Deoxycholic acid, zinc deficiency, tobacco exposure, ethanol intake, cell proliferation and mice tongue and esophagus

Introduction

Upper aero-digestive tract (UADT) cancer, including esophageal and tongue tumors, is an important cause of morbidity and mortality worldwide (Parkin, 2000). Chronic alcohol intake and dietary deficiency zinc, tobacco smoking, and gastroesophageal reflux disease are the major risk factors to the esophageal ant tongue cancer (Morimura et al, 2001; Straiff et al, 2000; Fong et al, 2006; Prassad et al, 2002; Stamp et al, 2002; Guy et al, 2007; Farhadi et al, 2002; Brown 2005) .

Zinc is an essential component of over 1,000 proteins, including metalloproteinases, DNA-binding proteins with zinc fingers, copper/zinc superoxide dismutase, and several proteins involved in DNA damage repair such as p53 (Ho et al., 2002). Studies have shown that zinc deficiency creates a precancerous condition in the tongue, esophagus, forestomach and colon by causing unrestrained cell proliferation, inflammation, ulceration, overexpression of the enzyme cyclooxygenase-2, hyperplasia, hyperkeratosis and parakeratosis (Swenerton and Hurley 1968; Follis 1996; Fong et al. 1996, 2005, Dani et al, 2007).

Deoxycholic acid (DCA) is a secondary bile acid present in the gastroduodenal reflux and implicated in various cancer of the digestive tract (Theisen et al, 2000; Nehra et al., 1999; Jenkins et al, 2008). It is a mutagenic and clastogenic agent that can reduce the transcription of the enzyme ADP ribose and activate the procarcinogenic signaling molecule NF-jB (Payne et al., 1998; Jenkins et al., 2004, 2007). Male AKR/J mice receiving supplemented diet with 0.2% DCA, during or after azoxymethane (AOM) administration, developed more aberrant crypt foci (ACF) that respective control group (Flyn et al., 2007). Also, male Nos2 knockout mice fed a diet supplemented with 0.2% DOC for eight months developed colitis, associated with increased oxidative DNA damage,

increased angiogenesis and colonic cell proliferation (Bersnstein et al., 2007). Guy et al. (2007) showed that dietary zinc deficiency diet supplemented with 0.2% DCA led to inflammation, basal cell proliferation, papillary hypertrophy and hyperkeratosis of esophageal epithelium in male C57BL/6 mice.

It is also well established by epidemiological and experimental studies that tobacco smoking and consumption of alcoholic beverages contribute to the development of tongue and esophagus cancer (Blot et al., 1992; Straiff et al, 2000; Sofritti et al, 2002; Carrard et al., 2004; Brown, 2005). Both tobacco smoking and alcoholic beverages contain significant amounts of mutagenic and/or carcinogenic substances, especially nitrosaminas (Lu et al, 1991; Hecht et al, 1998; StraifF et al, 2000). The tobacco smoking and alcohol intake may cause DNA hypomethylation, changes in the expression of c-myc, c-jun and c-fos, and reduction in the levels of antioxidants among others (Muller and Gebel 1994; Muller 1995; Stickel et al., 2002; Pöschl and Seitz, 2004; Kock, et al., 2004; Valko et al, 2006).

In the present study, we utilized a diet that mimic inadequate zinc intake (Zn^-) diet and supplemented it with a component associated with gastroesophageal reflux disease and high fat diets, unconjugated bile deoxycholic acid (DCA), that could creates an aggressive condition to the tongue and esophagus mucosa from male C57BL/6 mice. The aim of this study was to assess the additional influence of exposure to cigarette smoke and ethanol intake on the epithelial alterations of the esophagus and tongue induced by Zn^-+DCA diet.

Material and Methods

Animals and treatments

The protocols used were consistent with Ethical Principles for Animal Research adopted by the Brazilian College of Animal Experimentation (COBEA) and approved by the Institute of Biosciences/UNESP Ethical Committee for Animal Research (CEEA). Seventy-five 3 weeks-old male C57BL/6 mice were obtained from CEMIB-UNICAMP (Campinas, SP, Brazil). The animals were kept in polypropylene cages covered with metallic grids in a room maintained at 22 ± 2 ^{0}C, 55 ± 10% humidity under a 12-hr light-dark cycle with free access to food and tap water for a 2-week acclimation period.

During experimental period, the mice were fed control diet or zinc-deficient diet supplemented with 0.2% deoxycholic acid (DCA, Sigma-Aldrich Co., St. Louis Mo, USA) (Guy et al., 2007). The Zn^{-}+DCA diet was nutritionally complete and identical to control diet except for the concentration of elemental zinc (Table 1). Mice were randomly allocated to into six groups: Groups 1 to 3 were fed Zn^{-}+DCA diet and groups 4 to 6 were fed control. After 5-weeks, groups 2 and 5 intake ethanol 10% *ad libitum* and groups 3 and 6 were exposed to cigarette smoke for 15 weeks. Ethanol 10% was offered to the mice into bottles as the sole source of drinking fluid (Morimura et al., 2001). All animals were euthanized at the end of week 20 (Figure 1).

Clinical examinations were performed daily, and detailed physical examinations were accomplished weekly. Food, water and ethanol consumption were recorded twice per week and the animals were weighed individually once a week throughout the experimental period. The mice were fasted overnight and sacrificed after a single i.p. dose of 30 mg/kg body weight of sodium pentobarbital anesthesia.

Exposure to Cigarette Smoke

The mice were exposed to cigarette smoke in a chamber (100×80×75 cm) connected to a smoking device based on a model described by Wang et al. (1999). Smoke was drawn from filtered commercial cigarettes (composition per unit: 0.7 mg nicotine, 10 mg tar, and 10 mg carbon monoxide) with a vacuum pump and was exhausted into the smoking chamber. During the first week, the mice were acclimated to the smoke as follows: At 2 different times each afternoon, smoke was administered, first from 5 cigarettes over a 30-minute period and then, via a gradual increase, from 10 cigarettes over a 30-minute period. Subsequently, 10 cigarettes were used in each of 4 smoking trials administered daily for 30 minutes, (2 trials in the morning and 2 in the afternoon). Mice were exposed whole body to the sidestream cigarette smoke five days a week for 15 weeks.

Tissue processing, histology and immunohistochemical procedures

At sacrifice, tongue and esophagus was excised and fixed in 10% phosphate-buffered formalin during 24 h for paraffin embedding. Samples of tongue and esophagus proximal and distal were routinely processed and paraffin blocks were cut into 5-µm-thick sections and stained with hematoxylin-eosin (HE) for histological analysis and for immunohistochemical analysis of cell proliferation using Ki-67 marker (Imunotech Marceille France) or cyclooxigenase 2 (COX-2, Biogen, USA) expression .

Ki-67 and COX-2 antigens were detected using avidin-biotin peroxidase complex (ABC) method (Hsu et al, 1981). Briefly, deparaffinized 5-µm-thick tissue sections on poly-L-lysine coated slides were treated with 3% H_2O_2 in phosphate-buffered saline for 15 min and nonfat milk for 60 min. Tongue and esophagus sections were overnight incubated at 4^0C with polyclonal rabbit anti-Ki-67 or COX-2 at a 1:200 dilution. Then, the slides were

successively incubated with biotinylated secondary antibody horse anti-rabbit (Vector Laboratories, Inc., Burlingame, CA, USA) at a 1:200 dilution for 60 min and avidin-biotin-horseradish peroxidase solution (Vector Laboratories, Inc., Burlingame, CA, USA) at dilution 1:1:50 dilution for 45 min at room temperature. Chromogen color development was accomplished with 3,3´-diaminobenzidine tetrahydrochroride (DAB, Sigma-Aldrich Co., St. Louis Mo, USA) as the substrate to demonstrate the sites of peroxidase binding. The slides were counterstained with Harris´s hematoxylin.

Histological and immunohistochemical analysis

Non-proliferative or proliferative lesions in the tongue and esophagus were classified according to previously criteria published (Bertran et al., 1996; Whiteley et al., 1996). A histological grading score was established by evaluating degrees of hyperkeratosis, hyperplasia, dysplasia and inflammation on a scale of 0 to 3 for presence or absence each of these categories (with 0 being no significant change, 1 being a small detectable change, 2 being a moderate change and 3 a very change) (Guy et al., 2007).

The KI-67-labeling indexes, expressed as a percentage, were calculated by dividing the number of basal epithelial Ki-67-positive cells (S phase) by the total number of basal cells scored in the each tissue sample (500 cells for distal and proximal esophagus and dorsal tongue mucosa).

Statistical analysis.

The body weight, body weight-gain, food, water and ethanol intake, relative liver weight, cell proliferation, histological grading score and were analyzed by ANOVA test or the Kruskal-Wallis test. The incidence of different types of esophageal and tongue lesions

were examined using $\chi 2$ test or the Fischer exact test. Significance was set at $P< 0.05$. Statistical analysis was performed using the Jandel Sigma Stat Software (Jandel Corporation, San Rafael, CA, USA).

Results

General findings

In general, all experimental procedures used in this study, like treatment with ethanol 10%, cigarette smoke and Zn^{-}+DCA diet was well accepted by the mice. Three animals were found dead during the course of the experiment: one mouse from G2 group (Zn^{-} +DCA diet plus ethanol 10%), one mouse from G3 group (Zn^{-}+DCA diet plus cigarette smoke) and one mouse from G4 group (control diet). A complete necropsy was not performed due to advanced postmortem changes.

The values of mean body weight for each experimental group throughout the experiment are shown in Figure 2. The body-weight gain was significantly lower in the G3 group (Zn^{-}+DCA diet plus cigarette smoke-exposed) when compared to the untreated control group (G4) (G3 vs. G4, $P < 0.001$). At week 20, the relative liver weights were higher in the Zn^{-}+DCA diet-treated groups (G1, G2 and G3) when compared with the control diet groups (G4, G5 and G6) ($P< 0.001$) (Table 2). In ethanol-treated groups (G2 and G5), food consumption was significantly reduced ($P < 0.001$, Table 2) in both groups fed Zn^{-}+DCA or control diet. No significant alteration on ethanol intake was observed between the ethanol-treated groups (G2 and G5, 9.40 ml/mice/day and 9.26ml/mice/day respectively).

Histopathology analysis

All organs examined were free of pathological lesions, detectable at macroscopic inspection immediately after the mice were killed. At week 20, hyperkeratosis with nucleolar reactivity areas in esophagus epithelium, but not in tongue, were more

pronounced in Zn^{-}+DCA diet-treated groups (G1, G2 and G3), without additional effect of chronic treatment with ethanol or cigarette smoke (Figure 2 and Figure 3).

The number of cells in the stratum overlying the basal layer in area of hyperkeratosis in esophagus and tongue showed a trend to increase in the treated-groups (G1 to G3 higher than G5 and G6) than in the non-treated group (G4) that usually showed the stratum with 2 or 3 cells in the esophagus and with 4 or 5 cells in the tongue (Figure 2A and Figure 3A). In contrast, the overlying stratum in the treated-groups (G1 to G3 groups > G5 and G6 groups) had averaging 5 to 7 cells in thickness in esophagus and averaging 7 cells in tongue (Figure 2B-F and Figure 3B-F). The Zn^{-}+DCA diet-treated groups (G1 to G3) showed higher incidence and degree of hyperkeratosis in the esophagus (P = 0.064) and tongue (P= 0,145) when compared with fed control diet groups (G4 to G6), but it was not statistically significant (Figure 4 A and B).

Intense and diffuse liver steatosis was observed in the Zn^{-}+DCA diet-treated groups but more pronounced in the G2 group with ethanol intake (data not shown). This finding has been described in the liver from Zinc-deficient rats and mice (Dieck et al., 2003, 2005; Kang et al., 2009).

Cell proliferation analysis and Ciclooxygenase-2 expression

The values of Ki- 67-labeling indexes (Ki-67 LI%) date in the esophageal and tongue mucosa are shown in the Figure 5 A and B . Ki-67-positive basal cells are easily detected in the esophagus and tongue sections (Figure 6 and Figure 7). Cell proliferation indexes showed a trend to increase in the tongue and esophageal epithelium from Zn^{-}+DCA diet-treated groups (G1 to G3 groups) when compared to the control diet groups (G4 to G6 groups). The 15-week ethanol intake or cigarette smoke exposure did not alter cell

proliferation indexes in tongue or esophageal epithelium when compared to respective control groups (G2 and G3 groups *vs.* G1 group and G5 and G6 group *vs.* G4 group).

The COX-2 expression was detected in the tongue and esophagus sections by immunohistochemistry (Figure 8). In general, in the Zn^{-}+DCA diet-treated group (G1) exhibited a moderate Cox-2 staining in the esophageal epithelium, especially in the basal cell layer, when compared with the others groups (G2 to G6 groups) that showed weaker staining this marker. In the tongue, the COX-2 expression was mainly observed in the papillae and it was slightly more intense in the $Zinc^{-}$+DCA group (G1) and $Zinc^{-}$ +DCA/ethanol group (G2) when compared with the respective control group (G4 and G5, respectively). Cigarette smoke exposure did not alter the Cox-2 expression in the tongue and esophageal epithelium when compared to respective control groups (G3 *vs* G1 group and G6 group *vs* G4 group).

Discussion

The present study was undertaken to investigate the additional influence of exposure to cigarette smoke and ethanol 10% intake on the epithelial changes of the esophagus and tongue induced by dietary deficiency zinc and supplemented with 0.2% deoxycolic acid in male C57BL/6 mice. The findings this study suggest that the Zn^{-}+DCA diet for 20-weeks trend to increase cell proliferation indexes, incidence of hyperkeratosis and COX-2 expression mainly in the esophageal epithelium. Also, a 15-week cigarette smoke or ethanol intake period did not exert pronounced additional effects on cell proliferation or histology from both tongue and esophageal mucosa. These findings indicate that dietary zinc deficiency supplemented with deoxycolic acid appears to be an important factor of esophageal aggression and that deleterious effect of ethanol and cigarette smoke could be detected in a long-term exposure such as 12 months (Maito et al., 2003)

Zinc is an important nutrient that is required as a cofactor for the activity of many proteins (Sandstaead 1994). Zinc deficiency creates a precancerous condition in the tongue, esophagus and forestomach by increasing cell proliferation, hyperplasia and hyperkeratosis (Follis et al., 1996; Fong et al, 2000, 2005; Liu, 2005). Uncontrolled cell proliferation is an important event observed during experimental and human carcinogenesis induced by genotoxic and non-genotoxic agents (Cohen and Ellwein, 1990; 1991; Butterworth et al, 1995) and it can be observed at early stages of carcinogenesis in the upper gastrointestinal tract (Eastwood, 1992).

Dietary zinc deficiency increased the cell proliferation in esophagus epithelium of male Spraque-Dawley rats and male C57BL/6 mice treated with N-nitrosomethylbenzylamine (NMBA) (Fong et al, 1999; Liu et al., 2005). Also, zinc deficiency led to hyperplasia and increase in cell proliferation in the tongue epithelium of

male Sprague-Dawley rats and male C57BL/6*p*+/+ mice initiated with 4-nitroquinoline 1-oxide (NQO) (Fong et al., 2005). Beside, COX-2 overexpression accompanies hyperplasia in the tongue and esophagus of zinc-deficient male Sprague-Dawley rats. (Fong et al., 2005)

Bile acids are normal constituents of gastro-intestinal tract and the concentration of this, in particular unconjugated bile acids, may cause oxidative stress and DNA damage, (Bernstein et al., 2005; Guy et al ., 2007). They are implicated in development of gastro-intestinal cancers by modulating cell signaling pathways, increasing cell proliferation and apoptosis resistance and overexpression of the enzyme cyclooxygenase-2 in long-term exposure (Zhang et al. 1998; Glinghammar et al., 2002; Bernstein et al., 2005; Jurek et al., 2005).

Dietary zinc deficiency associated with 0.2% DCA led to increase in cell proliferation of basal cells, hyperkeratosis and Barrett`s esophagus like lesions in male C57BL/6 mice (Guy et al., 2007). Here, in the same conditions, dietary zinc deficiency in association with 0.2% DCA mild increased cell proliferation indexes in the esophagus epithelium in male C57BL/6 mice but without the development of esophagitis and Barrett`s esophagus like lesions as previously described (Guy et al., 2007). In addition, mice fed Zn^{-}+DCA diet showed a trend to increase the incidence and degree of hyperkeratosis in both tongue and esophageal epithelium as well as a thickened stratum overlying the basal layer. Our findings provide that zinc deficiency plus DCA treatment could be associated to a increase in cell proliferation, suggesting that this may lead to Barrett`s esophagus like lesions in a long-term exposure.

COX-2 is overexpressed in a variety of human premalignant and malignant lesions, including oral and esophageal cancers (Chan et al., 1999; Maaser et al., 2003), with

potential to increase cell proliferation, inhibit apoptosis and, consequently, increase metastatic potential (Tsujii et al., 1995, 1997). Fong et al (2005, 2006) have demonstrated an intensive and abundant expression of COX-2 in the tongue and esophagus of male Sprague-Dawley rats or p53+/+ mice fed a zinc deficient diet and treated with NQO, respectively. Recently Taccioli et al (2009) also reported that male Sprague-Dawley rats fed a zinc deficient diet showed an overexpression of COX-2 in the esophagus. In our study, $Zinc^{-}$/DAC-treated group exhibited a slightly stronger COX-2 immunoreativity, contrastingwith a weak and diffuse cytoplasmic staining of Cox-2 in the esophagus mucosa from control groups. Also, COX-2 staining in the $Zinc^{-}$/Doc diet group was observed in basal layer cell displaying spatial patterns. In the tongue, the Cox-2 expression was observed mainly in the papillae areas and it was more intense in the $Zinc^{-}$+DCA group and in the $Zinc^{-}$+DCA group/ethanol when compared with the respective control diet group.

Chronic ethanol intake is associated with increased risk of upper digestive cancer development (Blot, 1992; Brown, 2005; Boffeta and Hashibe, 2006). Experimental studies have shown that ethanol intake increase the incidence of esophageal tumors in male Fischer 344 rats initiated with dietilnitrosamine (DEN) or in male Sprague-Dawley rats in the post-initiation phase induced by N-nitrosomethylbenzylamine (NMBZA) (Muft et al., 1989; Aze et al. 1993). Tsutsumi et al (2006) also observed squamous polyps and invasive squamous cell carcinoma in esophagus of male Fischer 344 rats fed liquid diet containing ethanol and treated with NMBZA. Sofritti et al (2002) reported the development of tongue tumors in female/male Sprague-Dawley rats that received ethanol 20% *ad libitum* for 104 weeks. Homan et al (1997) and Carrard et al (2004) have demonstrated that chronic ethanol intake increased the cell proliferation in the tongue epithelium of male Wistar rats and of female CF-1 mice. However, in our study, the ethanol-treated mice groups showed a trend to

increase of cell proliferation in the tongue and esophageal epithelium when compared to the respective control groups. In contrast, the ethanol intake increased the hyperkeratosis degree in the tongue epithelium. Also, no additional effect of ethanol exposure on cell proliferation in the esophageal and lingual epithelium was observed in mice fed $Zinc^{-}$ +DCA diet. These findings are in agreement with Homan et al. (1997) and Maito et al (2003) that have reported that effect of alcohol on cell proliferation in the digestive tract of rodents are caused by continuous ethanol intake and occur throughout life.

Study in vitro has shown that ethanol exposure induced moderately Cox-2 expression in the rat gastric mucosal cell line cells (Mustonen et al., 2007). Here, we not observed difference of the ciclooxygenase-2 expression between the ethanol treated-groups and the respective control groups. In the tongue, the Cox-2 staining was slightly stronger in the papillae of ethanol treated group than control group, but without significant difference.

Studies have demonstrated that tobacco smoking contribute to the development of epithelial cancer of upper digestive tract (IARC 1986; Denissenko et al., 1996; Hecht, 2002) because it contains significant amounts of mutagenic and/or carcinogenic substances (Lu et al., 1991; Yang et al., 1992; Straiff et al., 2000, Hecht, 1998, 2002). However, there are few experimental studies that evaluating the effect of sub-chronic tobacco smoke on the epithelium of esophagus and tongue (Izzotti et al, 1998; Port et al., 2004; Ribeiro et al, 2008), especially on the epithelial cell proliferation. In our study, the cigarette smoke exposure increased slightly the hyperkeratosis in the tongue and esophageal epithelium without a notable alteration in cell proliferation indexes. The cigarette smoke exposure for 75 days also not induced histological changes in the tongue epithelium of male Wistar rats (Ribeiro et al., 2008). D'Agostini et al. (2001) and Assis et al (2005) also found no effect of cigarette smoke on the proliferative activity of epithelial cells of tongue in male Sprague-

Dawley and Wistar rats, respectively. In addition, in our study, the cigarette exposure did not alter the COX-2 expression in the esophagus and in the tongue.

The tongue and esophageal epithelium from rat and mice have a high degree of keratinization that possibly provides a relative level of protection when the animals are exposed to short-term period to aggressive agents (Ribeiro et al, 2005; Orback et al., 2007; Guy et al., 2007; Carrard et al., 2009). In our study, the cigarette exposure induced a mild hyperkeratosis degree in both esophagus and tongue, such as the ethanol intake increased the hyperkeratosis degree in tongue mucosa in Zn-/DAC-treated mice. Thus, a expressive histological change in tongue and esophagus mucosa will be achieved with a chronic exposure to cigarette smoke or ethanol intake.

In conclusion, the results of the present study indicate that the Zn^{-}+DCA diet, that mimic a inadequate zinc intake and contains a bile acid (DCA) associated with GERD and a high fat diet, is associated with increase in cell proliferation and appears to be a important factor of aggression to the esophagus and tongue epithelium. Our findings have also indicated that a short-term exposure to cigarette smoke or ethanol intake did not result in important adverse effects on the tongue and esophageal mucosa. Thus, we suggest that studies to evaluate the systemic effects of ethanol intake and tobacco smoking should be long term to avoid misleading conclusions.

Acknowledgments

The study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - 08/ 53366-). De Moura, N.A. and Zapaterini, J.R. were recipients of fellowships from the FAPESP (2009/50854-0) and (2007/57544-1), respectively.

References

Assis GF, Ceolin DS, Marques MAEA, Salvadori DM, Ribeiro DA. Cigarette smoke affects apoptosis in rat tongue mucosa: role of bcl-2 gene family. J Mol Histol 2005; 36:483-89.

Aze Y, Toyoda K, Furukawa F, Mitsumori K, Takahashi M. Enhancing effect of ethanol on esophageal tumor development in rats by initiation of diethylnitrosamine. Carcinogenesis 1993;14(1):37-40.

Bernstein H, Holubec H, Bernstein C, Ignatenko NA, Gerner E, Dvorak K et al. Deoxycholate-Induced Colitis is Markedly Attenuated in Nos2 Knockout Mice in Association with Modulation of Gene Expression Profiles. Dig Dis Sci 2007;52:628–42.

Bertram TA, Markovits JE, Juliana MM. Non-proliferative lesions of the alimentary canal in rats. Guides for Toxicol Pathol 1996; 1-16.

Blot WJ. Alcohol and cancer. Cancer Res 1992;52:2119–23.

Bofetta P, Hashibe M. Alcohol and cancer. Lancet Oncol 2006;7:149-56.

Brown LM. Epidemiology of alcohol-associated cancers. Alcohol. 2005;35:161-68.

Butterworth BE, Conolly RB, Morgan KT. A strategy for establishing mode of action of chemical carcinogens as a guide for approaches to risk assessments. Cancer Lett 1995;93: 129–46.

Carrard VC, Filho MS, Radosb PV, Chaves AC, Lauxen Ida S. Quantification of silver-staining nucleolar organizer region in epithelial cells of tongue of mice after exposure to, or intake of, alcohol.Alcohol 2004;34:233-38.

Carrard VC, Pires AS, Mendez M, Mattos F, Moreira JCF, Sant'Ana Filho M. Effects of acute alcohol consumption and vitamin E co-treatment on oxidative stress parameters in rats tongue. Food Chem Toxicol 2009;47:1058–63.

Chan G, Boyle JO, Yang EK, Zhang F, Sacks PG, Shah JP, et al. Cyclooxygenase-2 expression is up-regulated in squamous cell carcinoma of the head and neck. Cancer Res 1999;59:991– 4.

Cohen SM and Ellwein LB. Cell proliferation in carcinogenesis. Sci 1990; 2491: 1007-11.

Cohen SM and EllweinLB. Genetic errors, cell proliferation and carcinogenesis. Cancer Res 1991;51:6493-6505.

D'Agostini F, Balansky RM, Izzotti A, Bennicelli C, De Flora S. Modulation of apoptosis by cigarette smoke and cancer chemopreventive agents in the respiratory tract of rats. Carcinogenesis 2001;22:375-80.

Dani V, Goel A, Vaiphei K, Dhawan DK. Chemopreventive potential of zinc in experimentally induced colon carcinogenesis. Toxicol. Lett 2007;171:10–18.

Denissenko MF, Pao A, Tang MS, Pfeifer GP. Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in p53. Sci 1996,274:43032.

Dieck TH, Döring F, Roth HP, Daniel H. Changes in rat hepatic gene expression in response to zinc deficiency as assessed by DNA arrays. J Nutr 2003; 133(4):1004-10.

Dieck TH, Döring F, Fuchs D, Roth HP, Daniel H. Transcriptome and proteome analysis identifies the pathways that increase hepatic lipid accumulation in zinc-deficient rats. J Nutr 2005; 135(2):199-205.

Eastwood GL. Epithelial renewal in premalignant conditions of the gastrointestinal tract: a review. J Clin Gastroenterol 1992;14(1):29–33.

Farhadi A, Fields J, Banan A, Keshavarzian A. Reactive oxygen species: Are they involved in the pathogenesis of GERD, Barrett's esophagus and the latter's progression toward esophageal cancer? Am J Gastroenterol. 2002;97(1):22-26.

Flynn C, Montrose D, Swank D, Nakanishi M, Ilsley JN, Rosenberg DW. Deoxycholic Acid Promotes the Growth of Colonic Aberrant Crypt Foci. Mol Carcinog 2007;46:60–70.

Follis RHJr. The pathology of zinc deficiency. In: Prasad,A.D. (ed.), Zinc Metabolism. C.Thomas 1966;129-41.

Fong LY, Jiang Y, Farber JL. Zinc deficiency potentiates induction and progression of lingual and esophageal tumors in p53-deficient mice. Carcinogenesis 2006;27(7):1489–96.

Fong LY, Li JX, Farber JL, Magee PN. Cell proliferation and esophageal carcinogenesis in the zinc-deficient rat. Carcinogenesis 1996;17:1841–48

Fong LY, Magee PN. Dietary zinc deficiency enhances esophageal cell proliferation and N-nitrosomethylbenzylamine (NMBA)-induced esophageal tumor incidence in C57BL/6 mouse. Cancer Lett 1999;143:63–9.

Fong LY, Nguyen VT, Farber JL, Huebner K, Magee PN. Early deregulation of the p16ink4a-cyclin D1/cyclin-dependent kinase 4-retinoblastoma pathway in cell proliferation-driven esophageal tumorigenesis in zinc deficient rats. Cancer Res 2000;60:4589 –95.

Fong LY, Zhang L, Jiang Y, farber JL. Dietary zinc modulation of COX-2 expression and lingual and esophageal carcinogenesis in rats. J. Natl Cancer Inst 2005;97:40–50.

Glinghammar B, Inoue H, Rafter JJ. Deoxycholic acid causes DNA damage in colonic cells with subsequent induction of caspaases, COX-2 promoter activity and the transcription factors NF.kB and AP-1. Carcinogenesis 2002;23:839–45.

Guy NC, Garewal H, Holubec H, Bernstein H, Payne CM, Bernstein C et al. A novel dietary-related model of esophagites and Barrett´s esophagus, a premalignant lesion. Nutr Câncer 2007;59(2):217-27.

Hecht SS. Biochemistry, biology, and carcinogenicity of tobacco-specific N-niyrosamines. Chem Res Toxicol. 1998;11(6):559-603.

Hecht SS. Cigarette smoking and lung cancer: chemical mechanisms and approaches to prevention. Lancet Oncol 2002;3:461-69.

Ho E, Ames BN. Low intracellular zinc induces oxidative DNA damage, disrupts p53, NFkappa B, and AP1 DNA binding, and affects DNA repair in a rat glioma cell line. Proc Natl Acad Sci U S A. 2002;99(26):16770-5.

Homann Nils, Paivi Karkkainen, Koivisto Tiina, Nosova T, Jokelainen K, Salaspuro M. Effects of Acetaldehyde on Cell Regeneration and Differentiation of the Upper Gastrointestinal Tract Mucosa. J Nat Cancer Inst 1997;89(22):1692:97.

Hsu S, Raine I, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cyotchem 1981;29:577-80, 1981.

IARC Biological data relevant to the evaluation of carcinogenic risk to humans: 1.Carcinogenicity studies in animals. In IARC Monographs on the evaluation of the carcinogenic risk of chemical to humans. Tobacco smoking. Inter Ag Res Cancer, Lyon 1986;38:127-39.

Izzotti A, Balanski RM, Blagoeva PM, Mircheva ZI, Tulimiero L, Cartiglia C et al. DNA alterations in rat organs after chronic exposure to cigarette smoke and/or ethanol ingestion. J Faseb 1998;12:753-58.

Jenkins GJ, Harries K, Doak SH, Wilmes A, Griffiths AP, Baxter JN et al. The bile acid deoxycholic acid (DCA) at neutral pH activates NF-kappaB and induces IL-8 expression in oesophageal cells in vitro.Carcinogenesis 2004;25:317-23

Jenkins GJS, Cronin J, Alhamdani A, Rawat N, D'Souza F, Thomas T et al. The bile acid deoxycholic acid has a non-linear dose response for DNA damage and possibly NF-{kappa}B activation in oesophageal cells, with a mechanism of action involving ROS. Mutagenesis 2008;23(5):399-405.

Jenkins GJS, Souza FRD, Suzen SH, Eltahir ZS, James SA, Parry JM et al. Deoxycholic acid at neutral and acid pH, is genotoxic to oesophageal cells through the induction of ROS: the potential role of anti-oxidants in Barrett's oesophagus Carcinogenesis 2007;28(1):136–42.

Jurek D, Fleckl E, Marian B. Bile acid induced gene expression in LT97 colonic adenoma cells. Food Chem Toxicol 2005;43:87–93.

Kang X, Zhong W, Liu J, Song Z, McClain CJ, Kang YJ et al. Zinc supplementation reverses alcohol-induced steatosis in mice through reactivating hepatocyte nuclear factor-4alpha and peroxisome proliferator-activated receptor-alpha. Hepatology 2009;50(4):1241-50.

Kock OR, Pani G, Borrello S, Colavitti R, Cravero A, Farrè S et al. Oxidative stress and antioxidant defenses in ethanol-induced cell injury. Mol Aspects Med 2004;25(1-2):191-98.

Liu CG, Zhang L, Jiang Y, Chatterjee D, Croce CM, Huebner K, et al. Modulation of gene expression in precancerous rat esophagus by dietary zinc deficit and replenishment. Cancer Res 2005;65:7790–99.

Lu SH, Chui SX, Yang WX, Hu XN, Guo LP, Li FM. Relevance of N-nitrosamines to esophageal cancer in china. In O´Neill,I.K., Chein,J. and Bartsch,H. (eds) Relevance to Human Cancer of N-nitroso Compounds, Tobaco Smoke and Mycotoxin, IARC Sci Public no. 105 IARC, Lyon 1991;105:11-7.

Maaser K, Daubler P, Barthel B, Heine B, von Lampe B, Stein H, et al. Oesophageal squamous cell neoplasia in head and neck cancer patients: upregulation of COX-2 during carcinogenesis. Br J Cancer 2003; 88:1217–22.

Maito FLDM, Rados PV, Filho MS, Barbachan JJ, Quadros O. Proliferating cell nuclear antigen expression on tongue of mice after intake of, or topical exposure to, alcohol. Alcohol 2003;31:25–30.

Morimura K, Hori T, Kaneko M, Nishikawa T, Nishikawa A, Wanibuchi H et al. Promotion of chemically induced rat esophageal tumorigenesis with post-initation ethanol modification. Teratog Carcinog Mutagen. 2001;21(4):295-301.

Muft SI, Becker G, Sipes IG. Effect of chronic dietary ethanol consumption on the initiation and promotion of chemically-induced esophageal carcinogenesis in experimental rats. Carcinogenesis 1989;10(2):303-9.

Muller T, Gebel S. Heme oxygenase expression in Swiss 3T3 cells following exposure to aqueous cigarette smoke fractions. Carcinogenesis 1994;15(1):67-72.

Muller T. Expression of c-fos in quiescent Swiss 3T3 cells exposed to aqueous cigarette smoke fractions. Cancer Res 1995;55(9):1927-32.

Mustonen H, Hietaranta A, Puolakkainen P, Kemppainen E, Paimela H, Kiviluoto T et al. Ethanol induced NF-KB activation protects against cell injury in cultured rat gastric mucosal epithelium Am J Physiol Gastrointest Liver Physiol 2007;292:1614–21.

Nehra D, Howell P, Williams CP, Pye JK, Beynon J. Toxic bile acids in gastro-oesophageal reflux disease: influence of gastric acidity. Gut 1999;44:598–602.

Orbak R, Kara C, Ozbek E, Tezel A, Demir T. Effects of zinc deficiency on oral and periodontal diseases in rats. J Periodont Res 2007;42:138–143.

Payne CM, Crowley C, Washostultz D, Briehl M, Bernstein H, Bernstein C, et al. The stress-response proteins poly(-ADP)ribose polymerase and NF-kB protect against bile salt-induced apoptosis. Cell Death Differ 1998;5(7):623-36.

Port JL, Yamaguchi K, Du B, De Lorenzo M, Chang M, Heerdt PM, et al. Tobacco smoke induces CYP1B1 in the aerodigestive tract Carcinogenesis 2004;25(11):2275-81.

Pöschl G, Seitz HK. Alcohol and cancer. Alcohol. 2004;39:155-65.

Prasad AS, Kucuk O. Zinc in cancer prevention. Review Cancer Metastasis Rev 2002;21:291–5.

Ribeiro DA, Assis GF. Expression of placental glutathione S-transferase in rat tongue mucosa exposed to cigarette smoke. J Mol Histol 2008;39(1):115-9.

Ribeiro DA, MF. Salvadori DMF, Marques MEA. Abnormal expression of bcl-2 and bax in rat tongue mucosa during the development of squamous cell carcinoma induced by 4-nitroquinoline 1-oxide. Int J Exp Path 2005;86:375–81.

Sandstead HH. Understanding zinc: recent observations and interpretations. J Lab Clin Med 1994;124:322–27.

Soffritti M, Belpoggi F, Cevolani D, Guarino M, Padovani M, Maltoni C. Results of long-term experimental studies on the carcinogenicity of methyl alcohol and ethyl alcohol in rats. Ann N Y Acad Sci 2002;982:46-69.

Stamp DHT. Three hypotheses linking bile to carcinogenesis in the gastrointestinal tract: certain bile salts have properties that may be used to complement chemotherapy. Med. Hypotheses 2002;59:398-405.

Stickel F, Schuppan D, Hahn EG, Seitz HK. Cocarcinogenic effects of alcohol in hepatocarcinogenesis. Gut 2002;51:132-39.

Straiff K, Weiland SK, Bungers M, Holthenrich D, Taeger D, Yi S et al. Exposure to high concentrations of nitrosamines and cancer mortality among a cohort of rubber workers. Occup Environ Med 2000; 57:180-7.

Swenerton H and Hurley LS. Severe zinc deficiency in male and female rats. J. Nutr 1968; 95:8-18.

Taccioli C, Wan SG, Liu CG, Alder H, Volinia S, Farber JL et al. Zinc Replenishment Reverses Overexpression of the Proinflammatory Mediator S100A8 and Esophageal Preneoplasia in the Rat. Gastroenterol 2009;136:953–966

Theisen J, Nehra D, Citron D, Johansson J, Hagen JA, Crookes PF et al. Suppression of gastric acid secretion in patients with gastroesophageal reflux disease results in gastric bacterial overgrowth and deconjugation of bile acids. J Gastrointest Surg 2000;4:50–4.

Tsujii M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell 1995;83:493–501.

Tsujii M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci U S A 1997;94:3336–40.

Tsutsumi M, Lasker JM, Takahashi T, Lieber SC. In vivo induction of hepatic P4502E1 by ethanol: role of increased enzyme synthesis. Arch. Biochem. Biophys 1993;304:209-18.

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced câncer. Chem Biol Interact 2006;160:1-40.

Witeley LO, Anver MR, Botts S, Joknen MP. Proliferative lesions of the intestine, salivary glands, oral cavity and Esophagus in rats. Guides for Toxycol Pathol 1996; 1-18.

Yang WX, Pu J, Lu SH. Studies on the exposure level of nitrosamines in the gastric juice and its inhibition in high risk areas of esophageal cancer. Chin J Oncol. 1992; 14:407-10

Wang XD, Liu C, Bronson RT, Smith DE, Krinsky NI, Russell Ml. Retinoid signaling and activator protein-1 expression in ferrets given beta-carotene supplements and exposed to tobacco smoke. J. Natl. Cancer, v.91, p. 60-66, 1999.

Zhang F, Subbaramaiah K, Altorki N, Dannenberg AJ. Dihydroxy bile acids activate the transcription of cyclooxygenase-2. J Biol Chem 1998;273:2424–8.

Table 1 - Nutritional profile from AIN-76A purified diet in the groups-diets**.**

A –Vitamin mix

Vitamin A, IU/g	4.0
Vitamin D-3 (added), IU/g	1.0
Vitamin K (as menadione), ppm	0.50
Thiamin hydrochloride, ppm	6.0
Riboflavin, ppm	6.0
Niacin, ppm	6.0
Pantothenic Acid, ppm	15
Folic Acid, ppm	2.0
Pyridoxine, ppm	5.8
Biotin, ppm	0.2
Vitamim B-12, mcg/kg	10
Choline Chloride, ppm	1,000
Ascorbic acid, ppm	0.0

B- Composition of the Mouse Diets (% or ppm)

	DOC/Zinc$^-$ diet	**Standard diet**
Sucrose	49.9990	49.9990
Casein – Vitamim Free	20.0000	20.0000
Corn Starch	15.0000	15.0000
Powdered Cellulose	5.0000	5.0000
Corn Oil	5.0000	5.0000
Vitamin mix (see table 1A)	1.000	1.000
Dl-Methionine	0.3000	0.3000
Choline Bitartrate	0.2000	0.2000
Ethoxyquin (a preservative)	0.0010	0.0010
Protein	18.4	18.4
Calcium	0.52	0.52
Phosphorus	0.56	0.56
Potassium	0.6	0.36
Magnesium	0.05	0.05
Sodium	0.10	0.10
Chlorine	0.16	0.16
Sodium deoxycolate	0.2	0.0
Iron, ppm	35	35
Manganese, ppm	59	59
Copper, ppm	6.0	6.0
Iodine, ppm	0.21	0.21
Chromium, ppm	2.0	2.0
Selenium, ppm	0.11	0.11
Zinc, ppm	0.0	36

Table 2 - Body and relative liver weight data and food consumption values for the different experimental groups. [1]

Group/Treatment[2]	Number of mice	Body Weight (g) Initial	Final	Gain	Food Consumption (g/mouse/day)	Relative Liver Weight (%)
(G1) DM	15	23.73±1.83[a]	26.60±2.23[a,b]	2.87±2.20[a,b]	3.61±0.59[a]	5.62±0.52[a]
(G2) DM/ethanol 10%	15	23.27±1.67[a]	26.00±1.04[a]	2.64±1.69[a,b]	3.20±0.67[b]	5.56±0.53[a]
(G3)DM/cigarette smoke	15	24.20±2.04[a]	25.50±1.45[a]	0.79±2.26[b]	3.43±0.87[a,b]	5.05±0.30[b]
(G4) DS	10	24.30±1.83[a]	28.56±1.81[b]	4.22±1.79[a]	3.43±0.59[a,b]	4.43±0.61[c]
(G5) DS/ethanol 10%	10	23.40±1.8[a]	27.60±1.76[a,b]	4.20±1.48[a]	3.03±0.63[c]	4.10±0.40[c]
(G6)DS/cigarette smoke	10	23.80±1.75[a]	27.40±3.06[a,b]	4.15±2.41[a]	3.24±0.71[a,b]	4.05±0.24[c]

[1]Values are mean ± SD; Group 1= Modified Diet (Zn^-/DCA); Group 2= Modified Diet (Zn^-/DCA) plus ethanol 10%; Group 3= Modified Diet (Zn^-/DCA) plus cigarette smoke; Group 4= Control Diet; Group 5= Control Diet plus ethanol 10% and Group 6= Control Diet plus cigarette smoke. Different letters express difference statistic, $P < 0{,}001$.

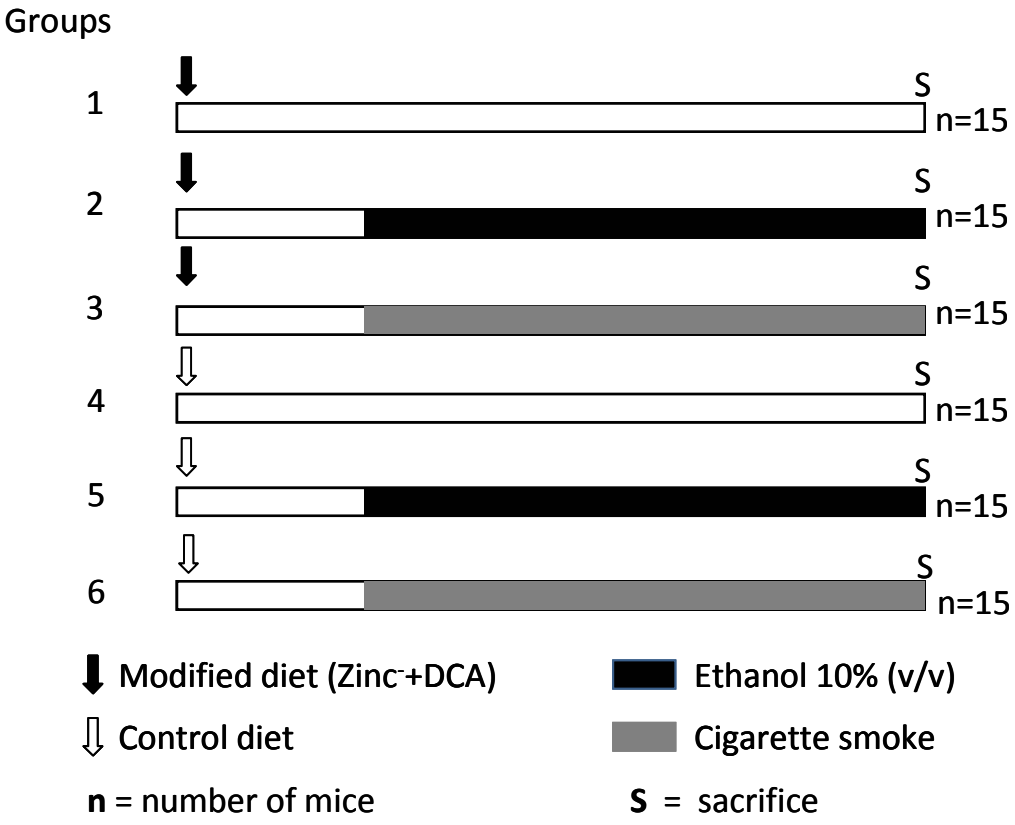


Figure 1 – Experimental Design

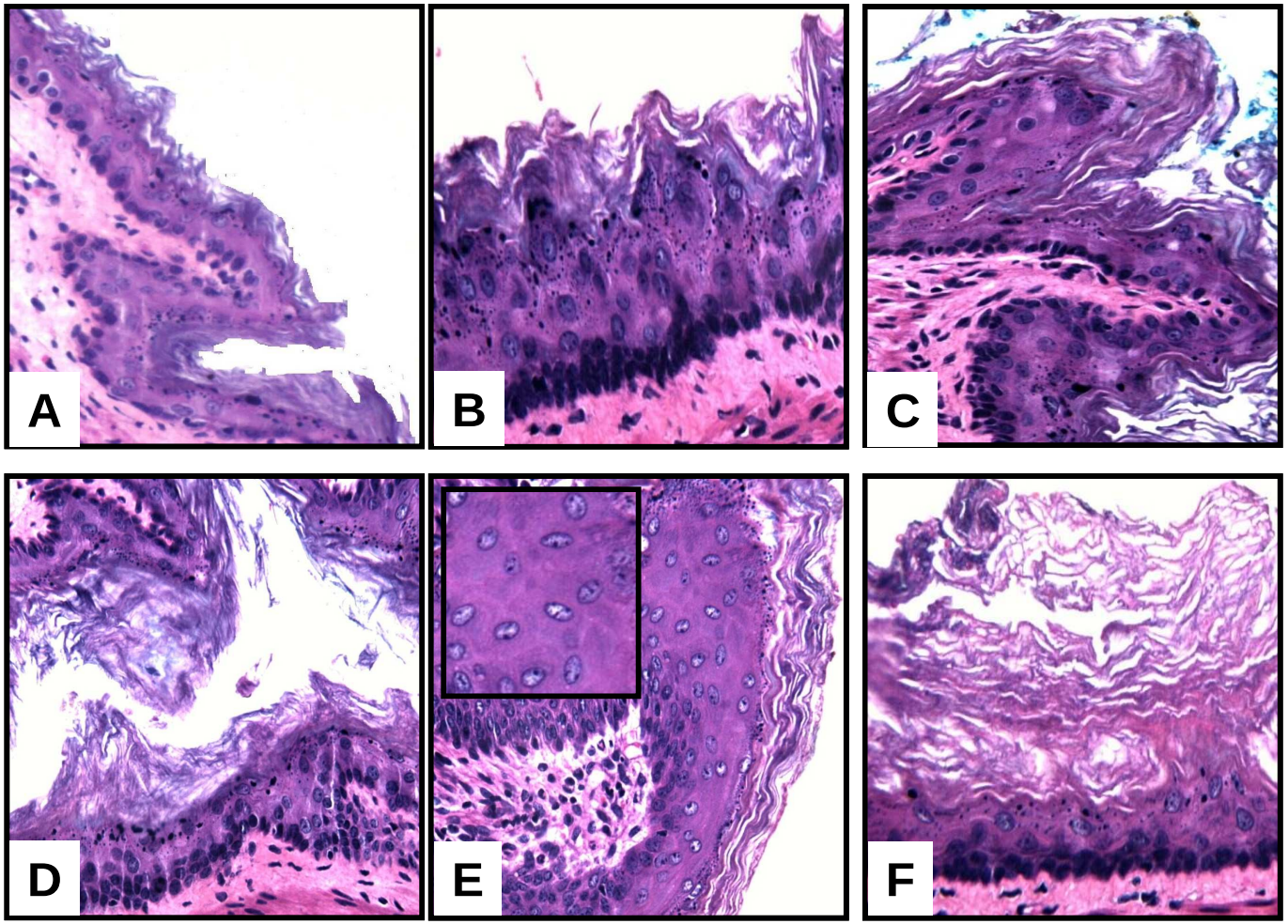


Figure 2-Histological appearance of the esophagus of mice in different experimental groups. A: Control diet. B: Control diet plus ethanol 10%. C: Control diet plus cigarette smoke. D: Zn^{-}+DCA diet. E: Zn^{-}+DCA diet plus ethanol 10%. F: Zn^{-}+DCA diet plus cigarette smoke.

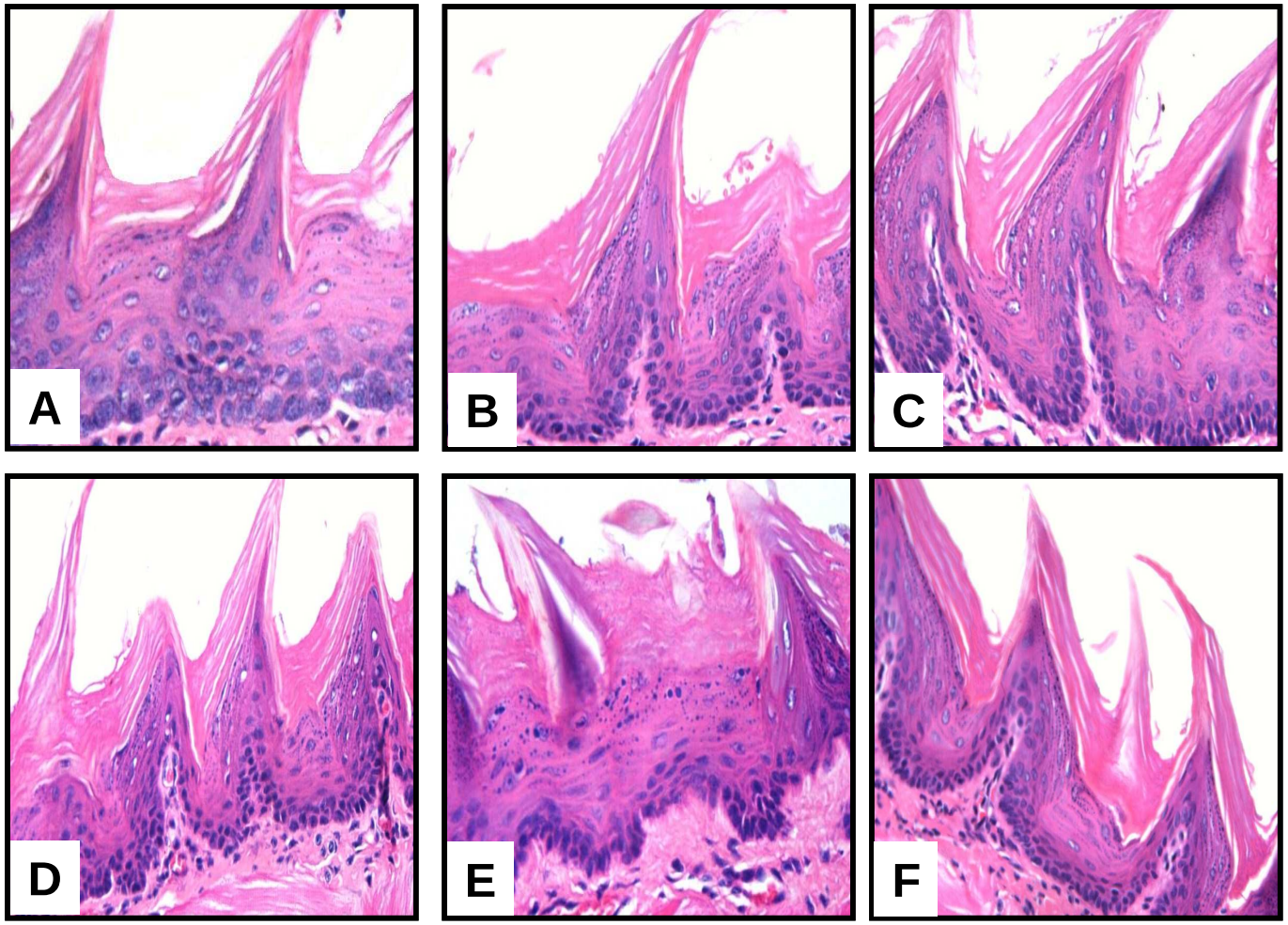
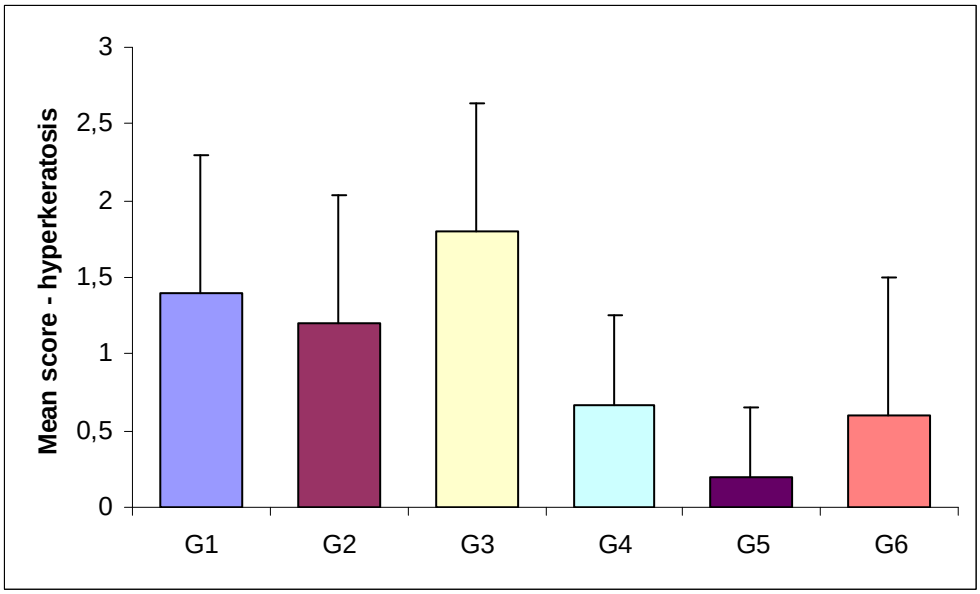


Figure 3 - Histopathology of tongue of mice in different experimental groups. A: Control diet. B: Control diet plus ethanol 10%. C: Control diet plus cigarette smoke. D: Zn^{-}+DCA diet. E: Zn^{-}+DCA diet plus ethanol 10%. F: Zn^{-}+DCA diet plus cigarette smoke.

A



B

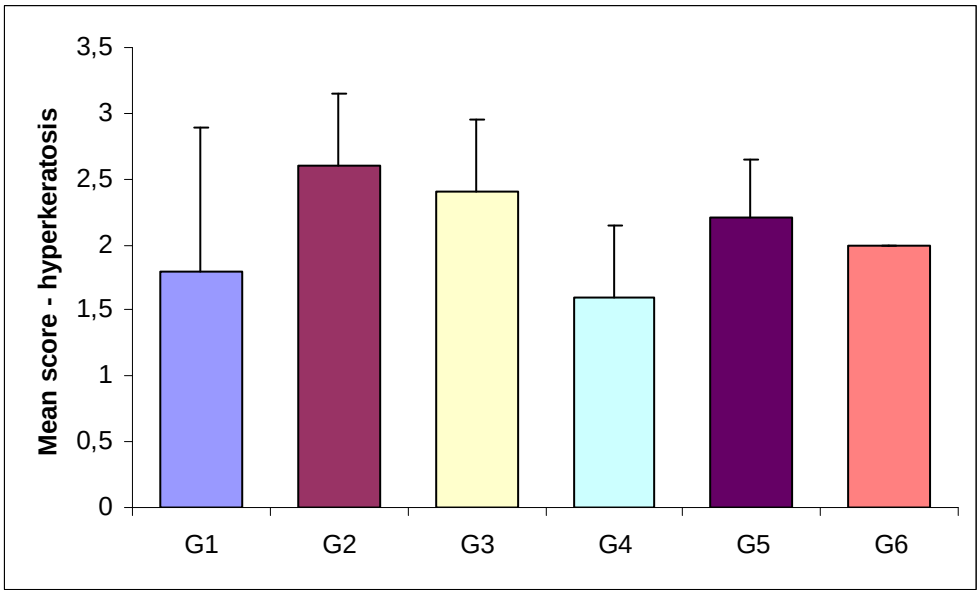
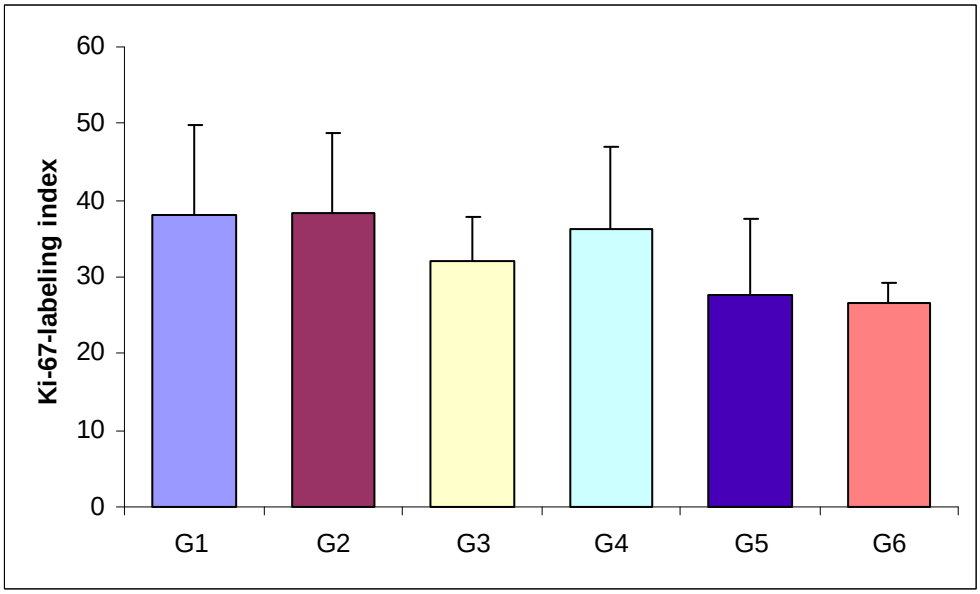


Figure 4 –The mean morphology score based on degree of kyperkeratosis in the esophagus (A) and tongue (B) was determined for each group of mice. G1: Zn^{-} DCA diet. G2: Zn^{-}DCA diet plus ethanol 10%. G3: Zn^{-}DCA plus cigarette smoke. G4: Control diet. G5: Control diet plus ethanol 10%. G6: Control diet plus cigarette smoke.

A



B

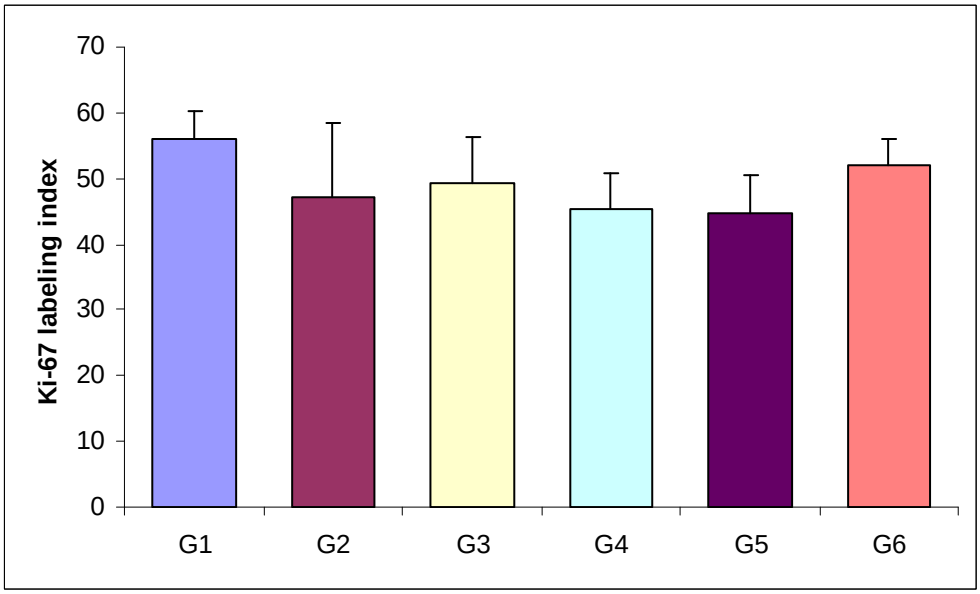


Figure 5- Data of Ki-labeling (Ki LI%) in esophageal and lingual epithelial cells (A and B, respectively) of different experimental groups. G1: Zn^{-}DCA diet. G2: Zn^{-}DCA diet plus ethanol 10%. G3: Zn^{-}DCA plus cigarette smoke. G4: Control diet. G5: Control diet plus ethanol 10%. G6: Control diet plus cigarette smoke.

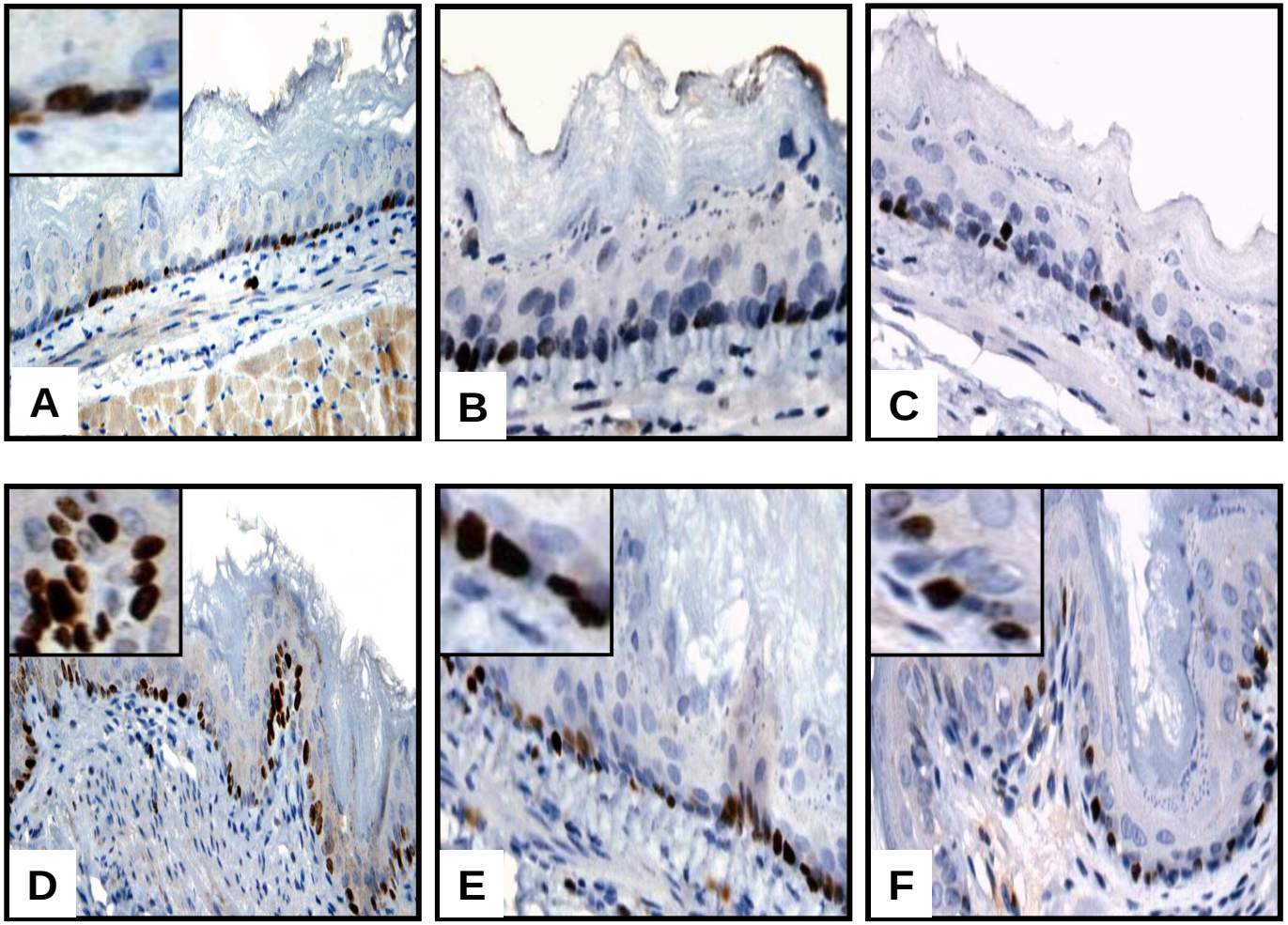


Figure 6- Immunohistochemical analysis of Ki-67 in the esophagus of mice for each group. In Zn^{-}+DCA treated-groups (D-F) the Ki-67 positive cells was more abundant that in control diet groups (A-C). Counterstained by Harris'Hematoxilin. Objective 40x.

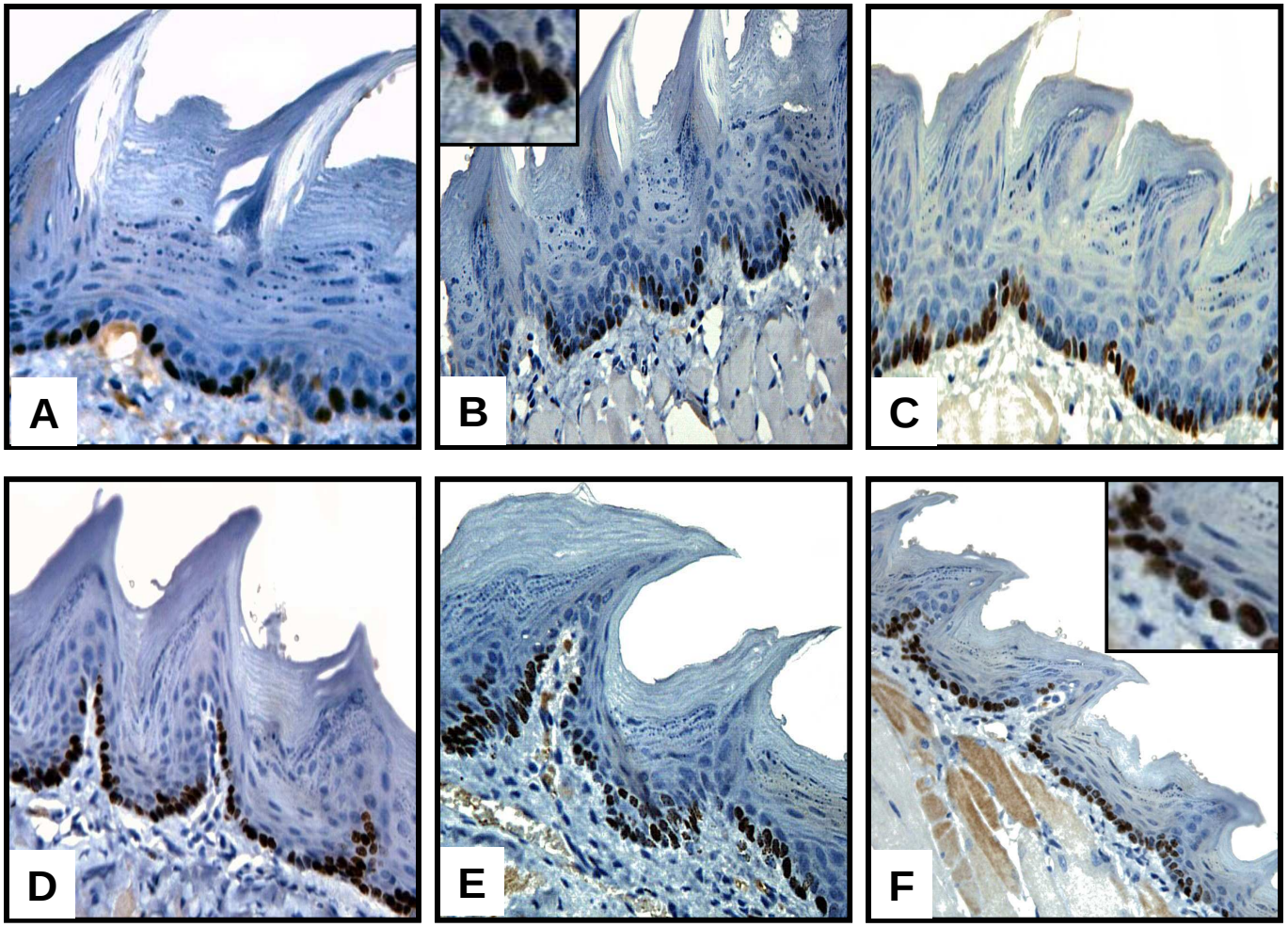


Figure 7- Immunohistochemistry for cell proliferation in the epithelium of tongue from mice of different experimental groups. The non treated-group (A) had a fewer Ki-67 positive cell. Control diet groups (A-C). Zn^{-} DCA treated-groups (D-F). Counterstained by Harris'Hematoxilin. Objective 40x.

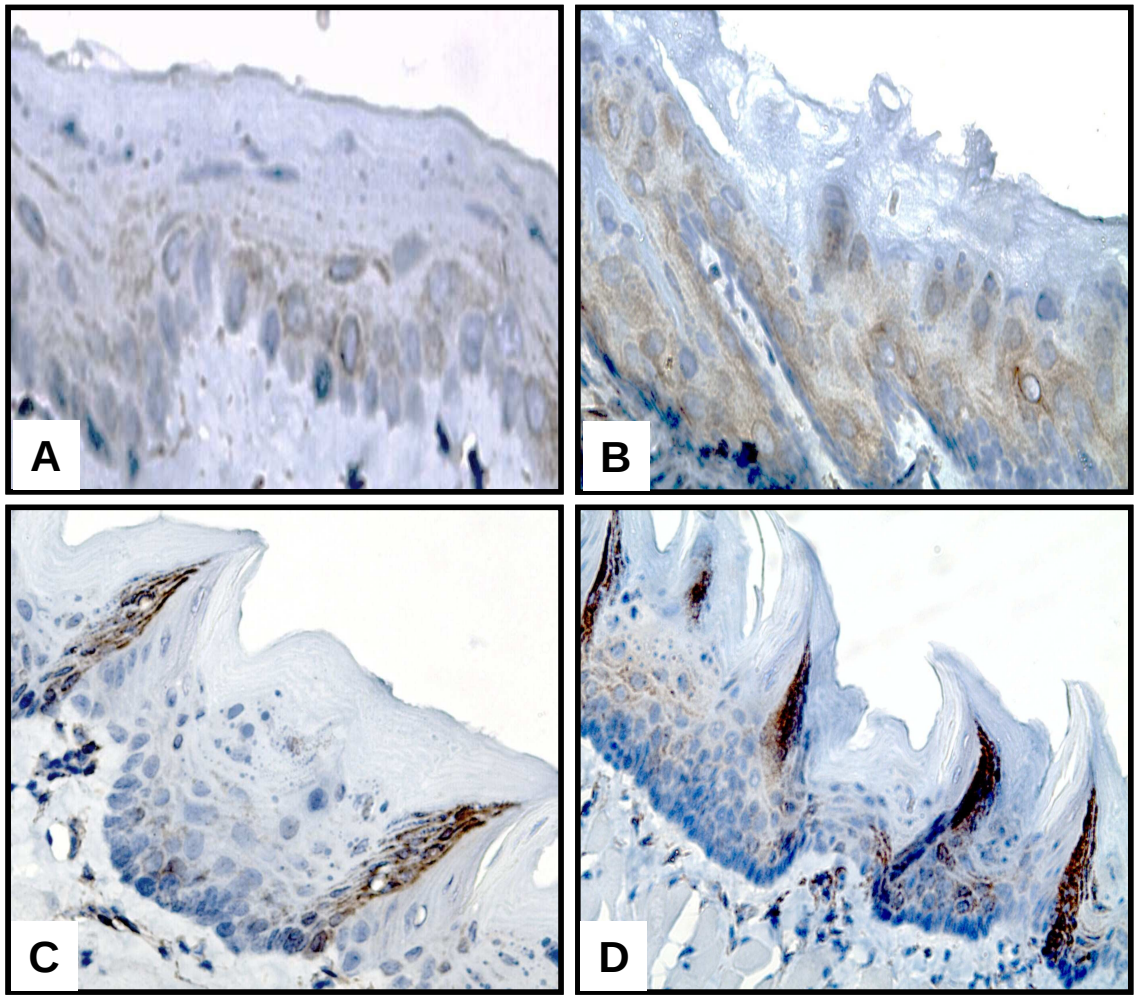


Figura 8- Immunoreactivity for cyclooxygenase-2 (COX-2) in the esophageal (A-B) and tongue (C-D) ephitelium. Non-treated- animals (A and C) and Zn^{-}/DAC-treated animals (B and D). Counterstained by Harris'Hematoxilin. Objective 40x.

Livros Grátis

(http://www.livrosgratis.com.br)

Milhares de Livros para Download:

Baixar livros de Literatura
Baixar livros de Literatura de Cordel
Baixar livros de Literatura Infantil
Baixar livros de Matemática
Baixar livros de Medicina
Baixar livros de Medicina Veterinária
Baixar livros de Meio Ambiente
Baixar livros de Meteorologia
Baixar Monografias e TCC
Baixar livros Multidisciplinar
Baixar livros de Música
Baixar livros de Psicologia
Baixar livros de Química
Baixar livros de Saúde Coletiva
Baixar livros de Serviço Social
Baixar livros de Sociologia
Baixar livros de Teologia
Baixar livros de Trabalho
Baixar livros de Turismo