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4NQO Carcinogenesis: A Model of Oral Squamous Cell Carcinoma

Carcinogenesis Inducida por 4NQO: Un Modelo de Carcinoma Oral de Células Escamosas

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SUMMARY: The experimental oral carcinogenesis induced by the chemical 4-nitroquinoline 1-oxide (4NQO) is one of the most frequent in the study of squamous cell carcinoma of the oral cavity (CCEC). The clear advantage is that the model is very similar to the physiological process of malignancy. The model has clear benefits by and is suitable for applications in therapeutic research.

KEY WORDS: 4NQO; Experimental carcinogenesis; Oral squamous cell carcinoma; Tongue.

INTRODUCTION

Oral cancer, most commonly in tongue, is a major cause of cancer deaths worldwide (Parkin *et al.*, 2001). Oral squamous cell carcinoma (OSCC) represents 90% of oral malignancies (Silverman, 2001). Every year more than 300,000 new cases are being diagnosed with OSCC world wide.

The development of malignant neoplasms -including the oral cancer- is a complex process, and for their understanding animal models of carcinogenesis that accurately represent the cellular and molecular changes associated with initiation and progression of human oral cancer have been deployed (Rivera *et al.*, 2011), from normal tissues to pathological conditions, which are then capable of being analyzed using histopathologic, genetic and biochemical techniques (Herzig *et al.*, 2002).

One of the animal models of carcinogenesis most used is that by chemical induction with 4-nitroquinoline 1-oxide (4NQO) (Dayan *et al.*, 1997; Rivera *et al.*). The 4NQO is a powerful carcinogen in several organs, and it can specifically induce tongue SCC when applied in low concentrations via drinking water (El-Rouby, 2011).

The aim of this review is to present the most used method to induce oral cancer, experimentally.

Carcinogenesis. Carcinogenesis is the process by which normal cells or tissues become transformed into cancer (Levy, 1963; Kumar *et al.*, 2005). This implies a

carcinogen, a substance which will transform normal to malignant cells (Levy *et al.*, 1950). Carcinogens can be essentially divided into DNA-reactive genotoxic and non-genotoxic types on the basis of their mechanisms of action. They can cause initiated cells to develop into preneoplastic and neoplastic lesions by stimulating clonal proliferation (Miranda *et al.*, 2011).

A large number of agents are capable of producing genetic damage and induce malignant transformation. These include chemical and physical carcinogens, oncogenic viruses and some other microorganisms (Kumar *et al.*; Jiang *et al.*, 2007; Choi & Myers, 2008).

In the process of carcinogenesis, cellular alterations at the molecular level are the first to occur, such as alterations in the genetic material which are not detectable by conventional histopathology. The accumulation of these molecular changes over time, causing individual cell changes that subsequently will be massive and may be detected histologically, and then when the number of altered cells is significant, it is possible to observe macroscopic changes in the affected areas.

Chemical carcinogenesis. Many carcinogenic chemicals have been identified that are involved in the development of various tumors. The induction of genetic alterations by chemical carcinogenesis involves 3 stages: initiation, promotion and progression.

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Initiation: Is the response to exposure of cells to sufficient doses of carcinogen (initiator), an initiated cell is altered, making it potentially capable of giving rise to a tumor. However, the initiation alone is not sufficient for tumor formation. The initiation produces permanent damage to the DNA (mutations), therefore it is irreversible and has memory (Kumar *et al.*).

Promotion: This phase is mediated by so-called promoters, agents that can induce tumors in initiated cells, but which by themselves are not tumorigenic and can act on the initiated cells several months after application of the initiator. In contrast to the effects of the initiators, the cellular changes resulting from the application promoters do not directly affect the DNA and are reversible. For example, they can increase proliferation of initiated cells, an effect that may contribute to the development of additional mutations (Kumar *et al.*). The promotion is reversible in the early stages if the promoter agent is eliminated (Stevens & Lowe, 2001).

Progression: If it persists in cell proliferation, initiated cells acquire new genetic alterations in oncogenes, which result in deregulation of growth and, sometimes, in autonomous growth. The end point of this progression is the development of an invasive tumor (Stevens & Lowe).

Although many agents are initiators or promoters, others behave as both, which defines them as a complete carcinogen (Stevens & Lowe).

Experimental Oral Carcinogenesis. The main animal models of carcinogenesis using chemical agents, probably because the chemical constituents of tobacco and alcohol were quickly identified as possibly responsible for most human OSCC (Mognetti *et al.*, 2006).

No animal model is perfectly applicable to any type of human cancer. However, there is consensus to use an animal model is an unavoidable step to understanding the processes and human diseases such as the process of carcinogenesis and the evaluation of therapeutic interventions. Experimental animal models that accurately represent the cellular and molecular changes associated with the initiation and progression of human cancer, are of crucial importance, although some are more suited for certain applications than others (Mognetti *et al.*).

Early attempts to induce malignant oral tumors were largely unsuccessful, as the oral mucosa is considerably more resistant to the actions of chemical carcinogens than skin (Eveson, 1981). This difference in susceptibility is explained by the presence of saliva in the oral cavity, which has a protective effect (Vered *et al.*, 2010).

The first animal model of chemical induced OSCC was used in Syrian hamsters. These animals have a pocket on each cheek, whose anatomical feature allows easy access (MacDonald, 1981). The first successful chemical agent used was 9,10-dimethyl-1,2-benzanthracene (DMBA), either dissolved in acetone or benzene (Salley, 1954).

However, application of DMBA is laborious locally. Also acetone and benzene have local toxic effects. Additionally, Syrian hamster oral mucosa, despite having a stratified squamous epithelium, is very different from the human (MacDonald; Nauta *et al.*, 1995).

Animal models have long been confined to chemically induced oral carcinomas, probably because the chemical components of tobacco and alcohol were quickly identified as responsible for most human OSCC (Mognetti *et al.*).

4NQO oral squamous cell carcinoma model. The medium term tongue carcinogenesis assay is a useful model for studying OSCC phase by phase. The animal model of chemical carcinogenesis induced locally or systemically by 4-nitroquinoline-1-oxide (4NQO) is considered the most representative model to study OSSC (Dayan *et al.*; Rivera *et al.*). This model is based on the multi-step process of carcinogenesis, which is characterized by initiation, promotion, and tumor progression (Fracalossi *et al.*, 2011; Minicucci *et al.* 2011).

The 4NQO (Fig. 1) is a synthetic carcinogen derivative of a quinoline, soluble in water, sensitive to high temperature and light (Wilkey *et al.*, 2009). Is used to study the various stages of oral carcinogenesis, because it is capable of inducing sequentially the phases of carcinogenesis (hyperplasia, dysplasia, severe dysplasia, carcinoma in situ and OSSC) (Wilkey *et al.*). The 4NQO is a powerful chemical carcinogen whose carcinogenic action is initiated by the enzymatic reduction of its nitro group (Nagao & Sugimura, 1976; Kitano, 2000). The four-electron reduction product 4-hydroxyaminoquinoline 1-oxide (4HAQO) is believed to be the proximate carcinogenic metabolite of 4NQO. When metabolized to an electrophilic reactant, selyl-4HAQO, it reacts with DNA to form stable quinoline monoadducts such as 3-(deoxyadenosin-N6-yl)-4AQO and N4-(guanosin-7-yl-4AQO) which are considered responsible for its mutagenicity and genotoxicity (Tada & Tada, 1975; Kohdab *et al.*, 1991). 4-Nitroquinoline 1-oxide (4NQO) elicits its carcinogenicity by producing DNA adducts after being metabolized to 4-hydroxyaminoquinoline 1-oxide, which forms 8-hydroxydeoxyguanosine (8OHdG) which is responsible for its oxidative damage (Arima, 2006).

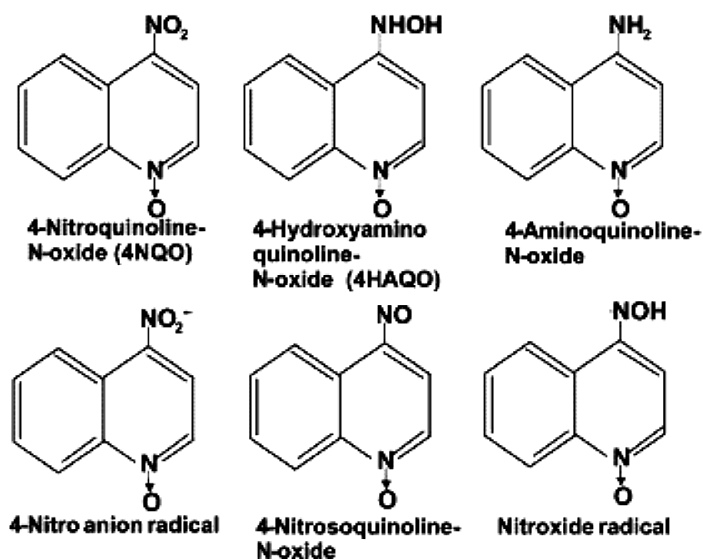


Fig. 1. Structures of 4NQO and its metabolites (Koontongkaew *et al.*, 2000).

Depending upon the dose and duration of treatment, 4NQO induces a spectrum of dysplastic and neoplastic lesions in the oroesophageal epithelium, with morphological and molecular alterations that mimic those occurring in human oral epithelial preneoplastic and neoplastic lesions (Wilkey *et al.*).

The carcinogenic mechanism of action of 4NQO is through the generation of reactive oxygen species (ROS) and nitrogen

(RNS) such as superoxide radicals, hydrogen peroxide and nitric oxide, which induce intracellular oxidative stress (Nunoshiba & Demple, 1993). ROS/RNS damage directly and indirectly to macromolecules, including the DNA, predominantly by binding of guanine residues, leading to adducts (Kanojia & Vaidya, 2006).

The damage described is similar to those caused by other carcinogens present in the tobacco, which is an important risk factor for oral cancer. Also exposure to 4NQO produces molecular and cellular changes that lead to histopathological changes similar to those seen in human OSCC (Kanojia & Vaidya).

Chronic administration of 4NQO in water simulates tongue carcinogenesis in rats, similar to its human homolog (Minicucci *et al.*, 2009). The oral mucosa of tongue dorsum of mice has also been used to induce tumors massively, mainly in strains CBA, C57BL/6, BALB/C and others. There has been a 100% incidence of lesions in animals. Histopathological analysis indicated that these lesions were squamous dysplasias, papillomas and invasives OSSC (Tang *et al.*, 2004). Figure 2 shows a well-differentiated carcinomas induced by 4NQO.

The model of carcinogenesis in rat and mouse is more appropriate to study OSSC,

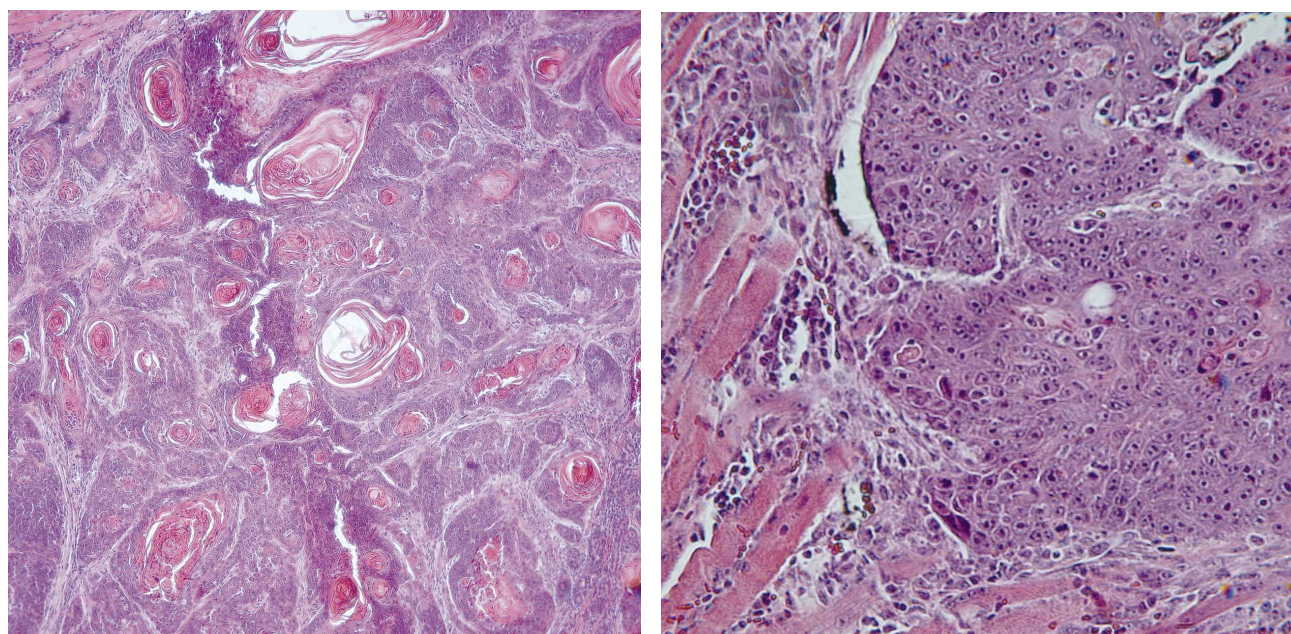


Fig. 2. Well-differentiated (A) and moderately differentiated (B) OSSC induced by 4NQO.

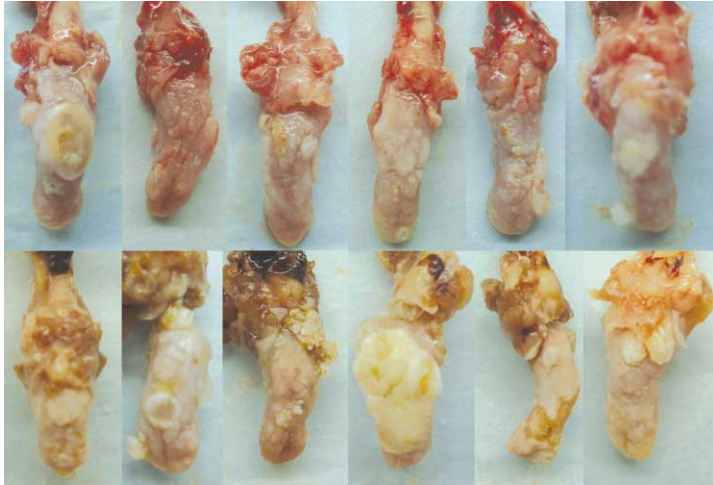


Fig. 3. Mice tongue lesions induced by 4NQO.

because the oral mucosa of these animals is similar to human oral mucosa, especially in palate and tongue (MacDonald; Nauta *et al.*, 1996). Another advantage of this model is the possibility of chemical management in systemic, long-term and lower doses, reducing further adverse effects (Vered *et al.*, 2005).

There is a lag time between the onset of molecular changes (not detectable with conventional light microscopy), the onset of histopathological changes (detectable) and the macroscopic and clinical appearance of lesions (MacDonald; Ribeiro *et al.*, 2004; Vered *et al.*). Figure 3 shows macroscopic tongue lesions.

The histopathological changes in the tongue epithelium during carcinogenesis pass from mild to moderate and severe dysplasia before the onset of OSSC. The initiation, with irreversible cellular changes, occurring within 4 weeks of systemic administration of 4NQO, through drinking water (Steidler & Reade, 1986). The latency period between first histopathology manifestations and macroscopic lesions in this model is 8 weeks (Salley, 1954, Vered *et al.*).

Experimental protocols used vary extensively, however, concentrations of carcinogen used are similar (Steidler & Reade; Nauta *et al.*, 1996; Vered *et al.*).

In order to validate models of chemical carcinogenesis in animals have been developed for comparative studies in humans and animals OSSC, relative to the carcinogen activation markers, oxidative stress, cell proliferation, apoptosis, invasion, and angiogenesis. The results reveal aberrant expression of multiple molecules in the main signaling pathways in both OSSC (Nagini *et al.*, 2009), establishing these models of study as an important tool for monitoring oral oncogenesis.

DISCUSSION

Animal models permit the development and testing of new approaches to disease prevention and treatment, identification of early diagnostic markers and novel therapeutic targets, and an understanding of the *in vivo* biology and genetics of tumor initiation, promotion, progression and metastasis.

The aim of chemical carcinogenesis model is to provide further understanding concerning the multi-step process of carcinogenesis in order to investigate hazard risk caused by environmental agents as well as to develop strategies for early diagnostic and/or cancer prevention (Miranda *et al.*).

The 4NQO can be administered orally in drinking water and therefore is easy to apply (Tang *et al.*). This model is effective because of a close similarity to human oral carcinogenesis at both histological as well as molecular level. Here, controlled conditions can be maintained with limited variations, which allow the reproducible isolation of all the stages of carcinogenesis and hence provide an excellent opportunity to intervene the neoplastic progression, as the premalignant stages are identifiable. Unfortunately, conditions often vary from study to study, such as the doses of 4NQO and species and strains used.

The two most frequent used experimental procedures for investigating therapies are *in vitro* cell lines and the nude mouse. The 4NQO model has several advantages and disadvantages over these laboratory experiments. Compared with the cell lines and the nude mouse model, the close similarity of the 4NQO model to the physiological process is a big benefit. The biggest drawback of the nude mouse model is the lack of an immunocompetent component. The clear disadvantage of the 4NQO model is that both the cell line and the bare mouse are less time consuming and that cell line experiments are less costly and most of the time readily available (Schoop *et al.*, 2009).

Currently it has started to be used simultaneously 4-NQO (200 µg/ mL) more and arecoline (500 µg/mL), in order to increase the incidence of malignant lesions (Chang *et al.*, 2010).

The 4NQO model is still the most used, however, transgenic models are very recent and very promising, considering that the genetics of animals is known, these models seek to standardize the model of oral carcinogenesis, an example of them, the K14-CreERTam/LSL-K-rasG12D/+p53flox/flox two-hit animal model system (Raimondi *et al.*, 2009) that may represent a suitable platform for exploring the underlying molecular mechanism and genetic and

epigenetic event determining the susceptibility to malignant progression of tumoral lesions arising from the distinct stratified epithelia of the oral cavity.

In conclusion, taking advantages and disadvantages in to account the 4NQO oral tumor model has clear benefits and seems suitable for therapeutic research applications, however we must remain alert to emerging models.

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RESUMEN: La carcinogénesis oral experimental inducida por el químico 4-nitroquinolina 1-óxido (4NQO) es uno de los métodos más frecuentes en el estudio del carcinoma de células escamosas de la cavidad oral (CCECO). La clara ventaja del modelo radica en el gran parecido al proceso fisiológico de la neoplasia maligna. El modelo tiene beneficios claros y es adecuado para las aplicaciones de la investigación terapéutica.

PALABRAS CLAVE: 4NQO; Carcinogénesis experimental; Carcinoma oral de células escamosas; Lengua.

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