Brief Review on 4-Nitroquinoline-1-oxide induced oral carcinogenesisin murine test system.

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Abstract: Oral cancer, especially squamous cell carcinoma has been a leading type of cancer in North-east India. The high abundance of this cancer type is due to the indiscriminate use of tobacco and smoking. However, to study the exact mechanism of carcinogenesis and its progression, one needs a large amount of sample to analyze from the subjects. This particularly become problematic as there is scarcity of adequate human sample and the whole genesis cannot be studied at first. To address this problem, scientists are now using a very potent oral cancer forming agent known as 4-Nitroquinoline-1-oxide(4NQO) to produce oral cancer in mice test systems *in vivo* and study the pathway from developing stages to ultimate cancer form and the time needed for complete tumorigenesis. So, it is critical to know the nature of this carcinogen and its usability and hence this review provides a comprehensive knowledge about this water-soluble carcinogen and its mode of action along with some potential utilitarian perspective.

Kewords: *oral cancer, 4NQO, carcinoma, carcinogen, tobacco*

1. INTRODUCTION

Cancer is brought about by changes in a cell's DNA – its hereditary "blueprint"(Chapman, 2017). Substances and exposures that can prompt to cancer are called carcinogens(Hecht, 2003). This can be a chemical substance, an infection, or even the medicines and radiation that are generally administered to treat malignancy(American Cancer Society, 2015). While numerous malignancies are brought about by a carcinogens or blend of numerous carcinogens, the propensity to develop cancer may likewise be acquired as a feature of our genome(Pardo et al., 2009). A few cancer-causing agents don't influence DNA directly, however prompt to malignancy in different ways such as by altering its growth rate and increasing chances of error prone DNA generation (American Cancer Society, 2015). Many national and international agencies have contributed in classifying human carcinogens. 4-Nitroquinoline-1-oxide(4NQO), an artificial water-soluble carcinogen, was found to be a more robust substance for the production of oral carcinogenesis as compared to other similar products in mouse model(Wallenius & Lekholm, 1973). It is used as a common substance in murine models, to study varied stages of oral carcinogenesis(Liu, Athar, Lippai, Waldren, & Hei, 2001; Vered, Allon, Buchner, & Dayan, 2007). The successive stages of carcinogenesis like dysplasia, severe abnormal condition, malignant neoplastic disease and SCC are evoked by 4NQO. 4NQO is a quinoline by-product, which was initially discovered by Nakahara et al., in 1957. They evoked skin carcinogenesis with this chemical and later, intraoral dose of this chemical was given to mice for the induction of malignant neoplastic disease. Researchers had painted0.5% of 4NQO three times a week on the palatal membrane and discovered tumors in surface, base of tongue and in abdomen once seven months(Wallenius & Lekholm, 1973). 4NQO exerts potent intracellular oxidative

stress and therefore the metabolic product of it binds to DNA preponderantly at guanine residues(Kanojia & Vaidya, 2006). These insults seem almost like harm obligatory by alternative carcinogens present in tobacco, which may be a major risk marker for carcinoma. Additionally, 4NQO exhibits similar microscopic anatomy yet as molecular changes as determined in human oral carcinogenesis(Hawkins et al., 1994; Tang et al., 2004; Wallenius & Lekholm, 1973).

2. The Synthetic Carcinogen: 4-Nitroquinoline-1 Oxide

4-Nitroquinoline-1-oxide, a synthetic water soluble carcinogen is widely used as a carcinogen in murine models, to study various stages of oral carcinogenesis(Wallenius & Lekholm, 1973). The sequential stages of carcinogenesis like hyperplasia, dysplasia, severe dysplasia, in situ carcinoma and SCC are induced by 4NQO(Hawkins et al., 1994; Kanojia & Vaidya, 2006). It also produces similar histological as well as molecular changes as seen in human oral carcinogenesis(Nauta et al., 1996; Tang et al., 2004).Initially it was established as a chemotherapeutic agent but later used to develop skin carcinogenesis. In a study done in 1984, researchers applied 4NQO repeatedly to the palates of male CBA mice for 2, 4, 6, 8, 12, or 16 weeks, and the animals were observed for the remainder of the 50-week experimental period. Oral epithelial atypia and squamous cell carcinoma were observed with increasing prevalence as the period of carcinogen exposure was increased. Carcinomas developed by 50 weeks in all animals that received 4NQO for 16 weeks(Steidler& Reade, 1984). Oral administration of 0.001% 4-nitroquinoline l-oxide(4NQO) in drinking water of rats had resulted in a 100% incidence of squamous cell carcinoma in the oral mucosa (tongue, palate, and gingiva), while tumour induction in other organs was rare (Ohne et al., 1985). Changes observed in a subsequent study by the group included carcinoma in situ and invasive carcinoma. Carcinoma in situ showed erosion, leukoplakia, and a gross papillary appearance. Histologically, most carcinomas in situ showed full-thickness alteration of epithelium (Ohne et al., 1985). 4NQO is broken down in vivo by a diaphorase, 4NQO reductase (E.C. 1.6.99.2), to produce an active molecule believed to be responsible for carcinogenesis (Booth, 1990). In liver cytosols, NADH: 4NQO nitroreductase was the predominant enzyme catalyzing the reduction of 4NQO. Rat liver cytosol catalyzed the conversion of 4NQO to either 4HAQO or a glutathione conjugate depending upon coenzyme or co-substrate availability. Whereas NADPH: quinonereductase NADPH : (quinone acceptor) oxidoreductase; DT diaphorase; EC 1.6.99.2) was the predominant 4NQO reductase present in liver cytosol from Sprague-Dawley rats, dicumarol-resistant NADH:4NQO nitroreductase specific activities were comparable with those of mouse liver cytosols(Benson, 1993).Study by Nauta et al.,1995clearly show that repeated applications of 4NQO to the rat palatal mucosa induce different stages of epithelial dysplasia before squamous cell carcinoma can be noted. These induced premalignant changes have both macroscopic and microscopic characteristics comparable to those observed in humans. The study shows that short

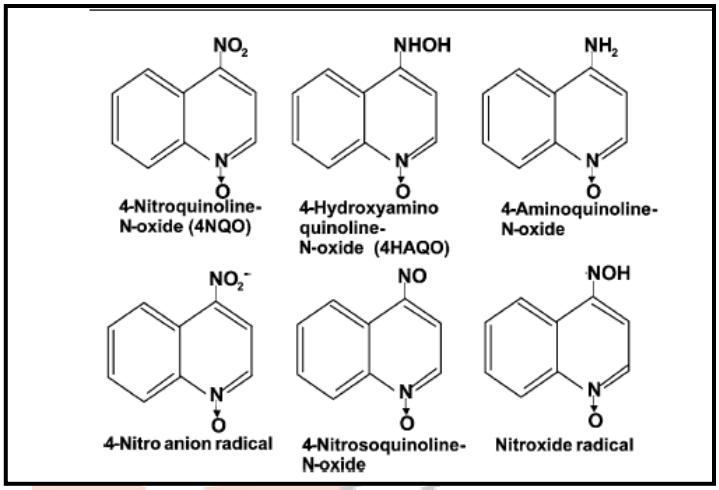

Figure 1: 4NQO structure and its metabolites(Adapted from Koontongkaew et al., 2000)

periods of application of the carcinogen 4NQO results in the induction of squamous cell carcinoma and the development of these tumours can be accelerated by prolonged application periods (20-26 weeks) which ultimately result in oral squamous cell carcinoma (Nauta et al., 1995; Nauta et al., 1996). Another experiment from 1997 examined the effect of the stable transfection of latent TGF-beta-1 cDNA, under the control of a cytomegalovirus promoter in the expression vector pcDNA3, into a 4NQO- induced clonal rat oral keratinocyte cell line that formed undifferentiated spindle cell tumours following subcutaneous transplantation to athymic mice. Test cells containing latent TGF-beta-1 cDNA produced a 2.3-fold increase in TGF-beta-1 protein compared to pcDNA3 controls as demonstrated by ELISA (Davies et al., 1997)(Davies et al., 1997)(Davies et al., 1997)(Davies et al., 1997). Yuan et alin their previous study of 1994, showed that 4-nitroquinoline-1-oxide (4NQO)-induced murine oral squamous cell carcinomas (SCC) have Hras1 mutations. In another study, topical application of a 0.5% 4NQO solution dissolved in glycol to the palate was used for 4 months to induce malignant transformation in a desalivated rat model (Kaplan et al., 2002). Histomorphometric analysis of proliferating cell nuclear antigen (PCNA), a cell cycle regulator and a proliferation marker, was performed. It has been found that manifestation of PCNA significantly increased as the observed histologic changes progressed from hyperkeratosis, to mild or moderate dysplasia, severe dysplasia and squamous cell carcinoma. Differences in manifestation of PCNA among the diagnostic groups were significant. In the desalivated group, PCNA expression was significantly higher than in control and normal groups, in both tongue and palate after 2 and 4 months. This lead the authors to a conclusion thatan unknown component of saliva has a temporary anti-carcinogenic protective effect, which can both delay and decrease the level of proliferation induced by the carcinogen 4NQO (Kaplan et al., 2002; Yang et al., 2013). 4NQO has the potential to initiate apoptosis, but yet no clear idea about the exact path is known. However, researchers found that 4-NQO could induce considerable damage to the mitochondrial membrane (Han et al., 2007). Thus they inferred that 4-NQO might induce apoptosis through the mitochondrial signalling pathway resulting from DNA damage. Further investigation showed that the apoptosis induced by 4-NQO was p53-dependent and expression levels of bax and bcl-2, closely related to mitochondrial signalling pathway, were also up and down-regulated, respectively. Meanwhile, the activity of caspase-9 and -3, lying in downstream of mitochondrial, was also enhanced (Han et al., 2007). In subsequent studies it has been found that a protein known as survivin, an inhibitor of apoptosis plays a crucial role in such malignancies. It is a multifunctional protein that suppresses apoptosis by association with caspases and Smac/DIABLO and regulates mitosis by interacting with other chromosomal passenger proteins(Altieri, 2003).

3. Similarity of 4NQO induced cancer model with tobacco induced cancer:

4NQO isn't found in natural conditions because it is synthesized for analysis purpose solely. In case of humans, tobacco may be a major risk issue for carcinoma, that contains quite sixty totally different carcinogens(Heicht, 2003; Lewin et al., 2014). Tobacco contains polycyclic aromatic hydrocarbons (PAH), nitrosamines, aromatic amines, aldehydes, phenols, volatile hydrocarbons, nitro compounds alternative inorganic and organic compounds as carcinogens. NNK (4-(Methylnitrosamino)-1-(-3-pyridyl)- 1-butanone) found in tobacco is metabolically activated by a hydroxylation, forming methyldiazo hydroxide, that binds specifically to G residues (7 alkyl radical guanine, 06 alkyl radical G in DNA)(Hoffmann & Wynder, 1986). A number of the Nitrosamines like NNN (N-nitrosonornicotine), NNK (4-(methylnitrosamino)-1-(-3-pyridyl)-1-buta- none), PAH etc. are used for the induction

of tumors within the animal models. However, it's been shown by totally different teams that regardless of the route of administration, NNN and NNK preponderantly turn out carcinoma in experimental model and extremely seldom cancers within the oral cavity(Padma et al., 1989; Yuan et al., 1997). The alterations that took place in human oral as well as oesophageal cancer were vastly similar to the 4NQO induced oral cancer in mice test system (Yang et al., 2013). The carcinogenic features resembled to the tobacco induced cancer both pathologically and morphologically. It was also accompanied by similar molecular alterations involving aberrant expression of Rarb2, p-ERK1/2 and Cox2 which contributed significantly to the cancer pathology (Yang et al., 2013). Such evidences made 4NQO a good choice over other chemicals to study oral cancer related pathways in vivo utilizing mice as test animal.

4. Tissue Specific nature and binding of 4NQO:

4NQO has been extensively used for the induction of carcinoma together with dorsal and ventral tongue, roof of the mouth and oesophagus (Tang et al., 2004; Vered et al., 2007; Wallenius & Lekholm, 1973). However, it can even be created to act on lung by injection of 4NQO(Imaida et al., 1989). Oral administration of 4NQO produces growth in varied sites of rimaoris and aerodigestive track. 4NQO enzyme (diaphorase), that activates 4NQO by reduction, has been involved within the condition to 4NQO. Diaphorase is found in great deal in oesophagus and hence exhibits higher susceptibility to 4NQO iatrogenic carcinogenesis as compared to alternative sites of digestive tract. This finding is supported by researchers relating to institution of oesophageal carcinogenesis model in mice via 4NQO in water (Tang et al., 2004). A correlation was conjointly found between concentration of 4NQO enzyme and incidence of SCC in oral mucous membrane. These findings counsel that prime diaphorase activity leads to increase vulnerability to 4NQO induced malignancy. 4NQO undergoes metabolic activation by cellular enzymes, and is changed accordingly. Modified 4NQO has been shown to make polymer adducts at numerous positions hencedisrupts normal configuration (Tada &Tada, 1976).In vivo experiments counsel that this chemical reacts preferentially with guanine residues and this binding affinity for guanine residues has additionally been verified by Nuclear Magnetic resonance (NMR) studies (Hecht, 2003). Acetylated derivative of 4NQO reacts via its third and fourth position with N2 and C8 positions of G residue (Galiegue-Zouitina et al., 1985) The high mutagenic potential of 4NQO has been ascribed to N2 guanine adduct, but also C8 adduct is found to be hepatotoxic and some mutations caused by these adducts ends up in guanine to pyrimidine substitution(Fronza et al., 1992; Galiegue-Zouitina et al., 1989). 4NQO has additionally been said as an ultraviolet radiation mimetic. Light cause pyrimidine variable resistor formation, that area unit large polymer adducts and area unit repaired by ester excision repair machinery. 4NQO is additionally believed to cause large polymer ad- ducts like ultraviolet radiation (Waters et al. 1992). There are also unit variations within the ultraviolet radiation and 4NQO iatrogenic damages which ultimately trigger the repair pathways in response to those genetic insults (Kanojia & Vaidya, 2006; Lewin et al., 2014).

5. Conclusion:

The water-soluble carcinogen 4NQO has been proved to be one of the best chemicals to study carcinogenesis of oral cavity *in vivo.* Its similarities with tobacco induced carcinogenic growth and tissue specific tumour formation makes it a candidate of choice for wide studies in oral squamous cell carcinoma. This chemical facilitates in depth analysis of tumour initiation, its progression and its metastatic growth even. One can study the signalling pathways, the

systemic effects (other than tongue) and the nature of tumour growth and progression to evaluate exact carcinogenesis pathway. It also facilitates various treatment trials to be done on the mouse models created efficiently with this chemical and hence ease the clinical trial work may fold. Overall it is a potent laboratory carcinogen, synthetic and with great specificity and can be utilized widely for oral cancer studies including epidemiology, prophylaxis and clinical drug trials.

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