The Effect of N-nitrosodimethylamine and Metabolic Activation in the Liver on the Formation of Nasopharyngeal Carcinoma

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Abstract: Nasopharyngeal carcinoma (NPC) is a common tumor of the head and neck with originates in the nasopharynx cancer, especially in Asia. Moreover, N-nitrosodimethylamine (NDMA) is a highly toxic, carcinogenic nitrosamine, $C_2H_6N_2O$. It is generally accepted that NDMA is the main cause of NPC. This research aims to investigate the level of NDMA necessary to induce the formation of NPC. To achieve the purpose of this experiment (how much NDMA will cause the formation of NPC) I first injected different amounts of NDMA and pyrazole into the 3 Male Ydd strain mice (inject nothing into the control mice), then carry out a timecourse, lastly use MRI to test the formation of NPC in the Male Ydd strain mice. The result obtained from the experiment is when the level of NDMA is higher than 6.25nmole/200kd of water/mouse and the level of metabolic activation is lowered NPC would be formed (Not found out yet). This study sheds light on the future study about the formation of NPC, also suggests that people should eat less smoked food.

1 INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a common tumor of the head and neck with originates in the nasopharynx (Osong Public Health and Research Perspectives. 2016), especially in southern China. (Cao, Simons and Qian. 2011) There is less than one case for every 100,000 people in most parts of the world (including the United States) per year. (American Cancer Society. 2018) Whereas in part of southern China 141.7/100,000 people would be diagnosed with NPC per year. (Cao, Simons and Qian. 2011) A previous study (Smoked cooked meat as a risk factor for Nasopharyngeal Carcinoma: A case-control study among Saudi populations (Saudi Journal of Oto-Rhino-Laryngology Head and Neck Surgery. 2018) has reported that smoked food is one of the biggest causes of NPC. And in the newest study, it's suggested that NDMA in smoked food is the main cause of NPC. (Khalid Hakami, N Prepageran, Talal Al Thubaity, Eidah Al Juaid, Nawaf Al Solami, 2018) In the work, I investigated whether the level of NDMA in the body tissues and metabolic activation in the liver (IARC Sci Publ. 1980) causes the rate of getting Nasopharyngeal carcinoma (NPC) to increase.

1.1 Purpose

Nasopharyngeal carcinoma (NPC) is a common tumor of the head and neck with originates in the nasopharynx (Osong Public Health and Research Perspectives. 2016), especially in southern China. (Su-Mei Cao, Malcolm J. Simons and Chao-Nan Qian. 2011) There is an increasing amount of people diagnosed with NPC in Guangdong in the last 29 years (1970-1999). (Karen Michell Othaya Kumar and Rabiatul Basria S.M.N. Mydin. 2019) A previous study has reported that NPC is mainly caused by N-nitrosodimethylamine (NDMA). This study will investigate whether the level of NDMA in the body tissues and metabolic activation in the liver (IARC Sci Publ. 1980) causes the rate of getting Nasopharyngeal carcinoma (NPC) to increase.

1.2 Methods

The experiments will use Male Ydd strain mice, which will be injected with increasing amounts of NDMA as the experiment measures liver function through assay. An increasing amount of pyrazole will also be injected, for speeding up the reduction of metabolic activation in the liver. pyrazole (used to replace part of the NDMA → speeding up the reduction of metabolic. By doing so, it is less time

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consuming and can save money) (Metabolic activation means the chemical conversion of a relatively benign substance into a more hazardous one by normal biochemical processes in cells and tissues.)

A timecourse experiment is then performed, for measuring the amount of NDMA in the liver and body tissues. Also, the time takes for NPC to form under different dosages of pyrazole. The amount of NPC formed is measured using an MRI.

1.3 Possible Results

There are three most possible results: (1) Mice pretreated with a larger amount of pyrazole would have a higher recovery of NDMA administration; (2) pH value would decrease administration of pyrazole; (3) NPC is to form in pretreated mice quicker than in control mice.

1.4 Methods

Materials

This experiment will use

- male ddY strain mice (20-25g)
- pyrazole (100-300 mg/5 ml of saline/kg): Speeding up the reduction of metabolic activation in the liver of mice.
- Saline(5ml/kg): Clean the mice, clear possible cancerogen that may affect the result.
- NDMA (6.25-100 nmole/200kd of water/mouse): Main source of causing NPC.
- 14-C Aminopyrine (0.063-1.0 kdmole/100kd of water/mouse)：ABT rate measures the severity level of liver tissue.
- MRI: Detect NPC in the mice.

Animals:

Male ddY strain mice (20-25g).

ABT rate and metabolic activation of liver tissues:

[Control mice]: First, clear out all the possible cancergen inside the control mice by injecting it with Saline(51/kg), 60 minutes before the administration. Then use 14-C aminopyrine (0.063-1.0xmole/100~tlof water/mouse) for measuring the ABT rate (severity level of liver tissue) and the metabolic activation of the liver tissues in the control mice.

[Mice-1]: First, clear out all the possible cancergen inside mice-1 by injecting it with Saline(51/kg). Second, inject mice-1 with NDMA (6.25-100nmole/200kd of water/mouse), which provides mice-1 with small amount of cancergen of NPC and source for reduction of metabolic activation in liver. Third, inject mice-1 with pyrazole(100mg/kg), for speeding up the reduction of metabolic activation in mice-1's liver. (60 minutes before the administration). Last, use 14-C aminopyrine (0.063-1.0xmole/100~tlof water/mouse) for measuring the ABT rate (severity level of liver tissue) and the metabolic activation of the liver tissues in mice-1.

[Mice-2]: First, clear out all the possible cancergen inside mice-2 by injecting it with Saline(51/kg). Second, inject mice-2 with NDMA (6.25-100nmole/200kd of water/mouse), which provides mice-2 with medium amount of cancergen of NPC and source for reduction of metabolic activation in liver. Third, inject mice-2 with pyrazole(200mg/kg), for speeding up the reduction of metabolic activation in mice-2's liver. (60 minutes before the administration). Last, use 14-C aminopyrine (0.063-1.0xmole/100~tlof water/mouse) for measuring the ABT rate (severity level of liver tissue) and the metabolic activation of the liver tissues in mice-2.

[Mice-3]: First, clear out all the possible cancergen inside mice-3 by injecting it with Saline(51/kg). Second, inject mice-3 with NDMA (6.25-100nmole/200kd of water/mouse), which provides mice-3 with large amount of cancergen of NPC and source for reduction of metabolic activation in liver. Third, inject mice-3 with pyrazole(300mg/kg), for speeding up the reduction of metabolic activation in mice-3's liver. (60 minutes before the administration). Last, use 14-C aminopyrine (0.063-1.0xmole/100~tlof water/mouse) for measuring the ABT rate (severity level of liver tissue) and the metabolic activation of the liver tissues in mice-3.

Amount of NDMA remaining in the body tissues:

Measure the amount of NDMA in the body by testing the blood/urine.

Time takes for an NPC to form based on the amount of pyrazole used:

[Control mice]: First, Inject the control mice with no pyrazole, measure the time it takes to form an NPC in control mice by using timecourse. Then, detect the formation of NPC with an MRI. Check the amount of NPC formed in the control mice 7days per once.

[Mice-1]: First, inject mice-1 with NDMA (6.25-100nmole/200kd of water/mouse) second, inject mice-1 with pyrazole(100mg/kg). （Pyrazole is used to speed up the reduction of metabolic activation of all the body tissue without using a large amount of NDMA which is a way of saving time and money.）Measure the time it takes to form NPC in mice-1 by using timecourse. Last, detect the formation of NPC with an MRI. Check the amount of NPC formed in mice-1 7days per once.

[Mice-2]: First, inject mice-2 with NDMA (6.25-100nmole/200kd of water/mouse) second, inject mice-2 with pyrazole (200mg/kg). Measure the time it takes to form a NPC in mice-2 by using timecourse. Last detect the formation of NPC with an MRI. Check the amount of NPC formed in mice-2 7days per once.

[Mice-3]: First, inject mice-3 with NDMA (6.25-100nmole/200kd of water/mouse) second, inject mice-3 with pyrazole (300mg/kg). Measure the time it takes to form a NPC in mice-3 by using timecourse. Last detect the formation of NPC with an MRI. Check the amount of NPC formed in mice-3 7days per once.

1.5 Result

Table 1 Percentage of NDMA recovery 60 minutes after the NDMA administration based on amount of pyrazole injected to the mice. With amounts of pyrazole injected to the mice listed.

Male ddY strain mice (20-25g)	Amount of pyrazole injected to the mice (mg/kg)	Percentage of NDMA recovery 60 minutes after the NDMA administration (%)
Control mice	0mg/kg	0
Mice-1	100mg/kg	50
Mice-2	200mg/kg	71
Mice-3	300mg/kg	84

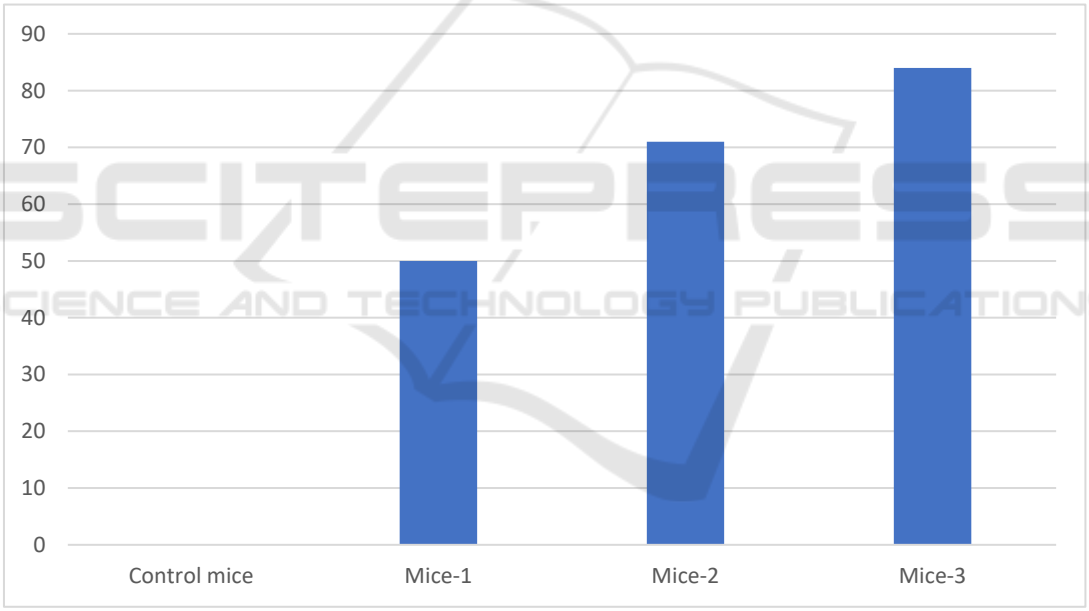

Figure 1 Percentage of NDMA recovery 60 minutes after the NDMA administration based on amount of pyrazole injected to the mice.

Table 2 Comparison of positive or negative result of NDMA presence between control mice, Mice-1, Mice-2, Mice-3.

	Control mice	Mice-1	Mice-2	Mice-3
Formation of NPC	-	+	+	+
NDMA present in urine	-	+	+	+
NDMA present in blood	-	+	+	+

Table 3 time takes for an NPC to form based on the amount of pyrazole used

	7 days	14days	21days	28days	35days	43days	50days	57days
Control mice	-	-	-	-	-	-	-	-
Mice-1	-	-	-	-	-	-	+	+
Mice-2	-	-	-	-	+	+	+	+
Mice-3	-	-	+	+	+	+	+	+

Possible result 1: No recovery of NDMA in control mice.

Possible result 2: Mice pretreated with 100mg/kg of pyrazole, NDMA recovery is below 50% 60 minutes after the NDMA administration.

Possible result 3: Mice pretreated with 200mg/kg of pyrazole, NDMA recovery is about 71.2% 60 minutes after the NDMA administration.

Possible result4: Mice pretreated with 300mg/kg of pyrazole, NDMA recovery is about 83.7% 60 minutes after the NDMA administration. Also, more than 80% of NDMA was not metabolized.

Possible result 5: NPC is to formed in pretreated mice quicker than in control mice.

1.6 Discussion

Pass research shows that NDMA is the main factor to increase the possibility of NPC (Hakami, Prepageran, Thubaity, Juaid, Solami, 2018) forming in the human body. In this research, I found that the rate of diagnosed with NPC is directly related to the amount of NDMA in the tissue body and metabolic activation of the liver. Possible result 1as shown in table 2, neither NPC nor recovery of NDMA is found in control mice as it wasn't injected with any NDMA, therefore would not cause a reduction of metabolic activation in the liver.

On the other hand, possible result 2, 3, 4 and 5 as shown in table 2, are injected with both NDMA and pyrazole, show appearance of NPC, which prove that NDMA causes NPC.

The percentage of recovery of NDMA based on the amount of pyrazole injected, as shown in figure 1, proves that once the metabolic activation of the liver is wakened the NDMA is uneasy to be destroyed by other organ tissues.

The appearance of NDMA in blood, urine and formation of NPC proves that if the liver is unable to metalize the NDMA other body tissue would take the job. The body tissue is unable to clear NDMA out of the body completely which then cause the rate of diagnosed with NPC to increase.

Both table 1 and table 3 show that the higher amount of pyrazole was injected into the mice, the faster NPC is formed, which proves that NDMA is the main cause of NPC.

One of the problems in this experiment that may cause unreliable results is that I haven't repeat the experiment and number of mice (4) used are not enough to make the experiment result accurate. Because their may be human and environmental errors (injected too much or too less amount of pyrazole or NDMA into the mice, timecourse carried out inaccurately, NPC in mice that's too small to detect by MRI, one, two, three or four of the four mice's gene that has higher possibility to allow NPC forming in their body (e.g. heredity)). Moreover, data used for experiment prediction have a time gap for 41 years long (1980- 2021), which makes the prediction inaccurate to the result (The effect of N-nitrosodimethylamine and metabolic activation in the liver on the formation of Nasopharyngeal carcinoma). On the other hand, if the experiment was repeated I could have collected the result as an average, which would make the experiment more reliable. Also, if data are collected from literature written around the same time prediction to the result (The effect of N-nitrosodimethylamine and metabolic activation in the liver on the formation of Nasopharyngeal carcinoma) would have been more specific. However, colleting literature from 1980- 2021 about the topic NPC shows that the scientist are aware of the problem and the research on NPC has been improving continuously.

2 CONCLUSION

The result of this study provides doctors with a larger reference of the cause of NPC in future medical diagnoses. Future studies will focus on decreasing amount of NPC taken in by human body through food by medicines/ health care products.

On average, about 1/100,000 people are diagnosed with NPC per year all around the world [less than 1% of children (0-10) are diagnosed with NPC (Journal of Cancer Research and Practice. 2016). There are two peaks for males to be diagnosed with NPC: between 30 to 39 and 50 to 59 years old. Whereas the peak for females is 20-39 years old. (Beyene, Ketema, Alebachew, Saleh and Gebremariam. 2021) About 8.8/100,000 people are diagnosed with NPC per year in southeast China

(Hamid. 2020); About 32/100,000 people are diagnosed with NPC per year in Guangzhou and 13.9/100,000 people are diagnosed with NPC per year in Hong Kong. (Cao, Simons and Qian. (2011); (Dr. Anthony C.H. YING. 2008) NPC also has a high risk of causing death. Around 0.84/100,000 people died per year, globally. And 31413/100,000 people died per year in China. (Xu, Zheng, Zhang, Zou and Chen. 2013) Furthermore, the annual cost of cancer treatment in China (2015) is 221.4 billion RMB (Cai, Xue, Chen, Hu, Miao, Lan, Zheng and Meng. 2017). And the population in parts of China being diagnose with NPC is 3085 in 2009. Therefore, NPC is a financial burden towards China. Research in the past has found that salted fish and Siu Mei contain a large amount of NDMA, (Food and Cosmetics Toxicology. 1981) People from Guangzhou and Hong Kong love eating these two kinds of food, which explains why people around these areas have such a high rate of being diagnosed with NPC comparing to the world's probability. Inside the human body, once NDMA is being detected, the liver would metabolize it out within a short period, which causes the other organs not to have enough interactions with NDMA. Causing the level of NDMA found in peripheral blood lower than expected (based on total dietary exposure). The reduction of the metabolic activation in the liver (IARC Sci Publ. 1980) causes other tissues to be used to metabolize NDMA. NPC then starts to form between the head and neck of the human body (Osong Public Health and Research Perspectives. 2016), as other tissues can't metabolize NDMA fully and take too long. Therefore, I prediced the reduction of liver ability to clear NDMA will cause NDMA induced NPC in the human body. This paper talks about the tumor Nasopharyngeal carcinoma, which is a common tumor in Asia of the head of the neck with originates in the nasopharynx (Osong Public Health and Research Perspectives. 2016); (Cao, Simons and Qian. 2011). In this work, I investigated: 'The effect of different levels of N-nitrosodimethylamine in the body tissues and metabolic activation in liver on the rate of formation of Nasopharyngeal carcinoma to increase.'. (IARC Sci Publ. 1980) The experiments will use Male Ydd strain mice, which will be injected with increasing amounts of NDMA as the experiment measures liver function through assay. An increasing amount of pyrazole will also be injected, for speeding up the reduction of metabolic activation in the liver. (Metabolic activation means the chemical conversion of a relatively benign substance into a more hazardous one by normal biochemical processes in cells and tissues.)

A timecourse experiment is then performed, for measuring the amount of NDMA in the liver and body tissues. Also, the time takes for NPC to form under different dosages of pyrazole. The amount of NPC formed is measured using an MRI. There are three most possible results: (1) Mice pretreated with a larger amount of pyrazole would have a higher recovery of NDMA administration; (2) pH value would decrease administration of pyrazole; (3) NPC is to form in pretreated mice quicker than in control mice.

Generally, this study explores the effect of NDMA on the formation of NPC. The result of my study will indicate how the rate of formation of NPC is effected by amount of NDMA in the human body. The possible results on NPC shows that the larger amount NDMA intaking the worse the metabolic activation is being damaged. FEHD as an example, should stop business selling food that contain high amount of NDMA, as Hong Kong is one of the place that has high rate of population diagnosed with NPC. And in the future study, scientists can focus on the prevalence of NPC in Asia, and investigate the connection between the dietary habit of Asia and the formation of NPC.

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