

## Anti-tumor agent celecoxib activity toward SP-C1 tongue cancer cells invasion (in vitro)

Harun Achmad<sup>\*)</sup>, Mieke H. Satari<sup>\*\*</sup>, Roosje Oewen<sup>\*</sup>, Supriatno<sup>\*\*\*</sup>

<sup>\*)</sup>Department of Pedodontics Faculty of Dentistry Universitas Padjadjaran

<sup>\*\*</sup>Department of Oral Biology Faculty of Dentistry Universitas Padjadjaran

<sup>\*\*\*</sup>Department of Oral Medicine Faculty of Dentistry Universitas Gadjah Mada

### ABSTRACT

Invasion is a characteristic of the occurrence of cancer and indicates the cancer cells' capability to destroy and degrade the border between the epithel and basal membrane to further spread into the surrounding extra-cellular matrix. The purpose of this research was to find the existence of impediment at the SP-C1 tongue cancer cell using celecoxib chemopreventive medication. The SP-C1 tongue cancer cells were treated in vitro using celecoxib medication as research subject at the following concentrations 5, 10, 25, 50, 75, 100, 125%; and 0 as control group (only DMEM growth medium treatment). Pure experimental testing was carried out for 24 and 48 hours, with observation and calculation of average number of SP-C1 tongue cancer cells. The data collected were analyzed using the ANAVA test with Newman Keuls paired range test or t test. Research results indicated that the average number of SP-C1 tongue cancer cells invasion after administration of celecoxib medication based on administration concentration and time statistically yielded significant results. The ANAVA test results was statistically significant, that is, average occurrence of the number of SP-C1 tongue cancer cells due to the use of celecoxib at certain concentrations compared to that without celecoxib was different. At celecoxib of zero (control) concentration was 24.4 with celecoxib concentration starting at 5 up to 125% experienced decline from its average 11 to become 2.3. The conclusion of the research was that the greater the celecoxib concentration administered, the greater the effect on the impediment of SP-C1 tongue cancer cell invasion.

**Key words:** Invasion, SP-C1 tongue cancer cell, celecoxib

### ABSTRAK

Invasi merupakan karakteristik terjadinya kanker dan menunjukkan kemampuan sel kanker merusak dan mendegradasi batas antara jaringan epitel dan basal membran untuk selanjutnya menyebar ke dalam matriks ekstraseluler sekitarnya. Tujuan penelitian ini untuk mengetahui adanya hambatan pada invasi sel kanker lidah SP-C1 dengan menggunakan obat kemopreventif celecoxib. Sel kanker lidah SP-C1 pada uji invitro diberi perlakuan dengan menggunakan obat celecoxib sebagai subyek penelitian pada konsentrasi 5, 10, 25, 50, 75, 100, 125, serta 0 sebagai kelompok kontrol (hanya pemberian

---

<sup>\*)</sup>Correspondence author: Harun Ahmad, Department of Pedodontic Faculty of Dentistry Universitas Padjadjaran Jl. Sekeloa Selatan No. 1 Bandung, West Java-Indonesia, Tel./Fax: +6222-2504985/2532805

media pertumbuhan DMEM). Pengujian eksperimental murni dilakukan selama 24 dan 48 jam dengan pengamatan dan perhitungan terhadap rerata jumlah invasi sel kanker lidah SP-C1 setelah pemberian beberapa konsentrasi celecoxib. Data yang diperoleh dianalisis menggunakan uji ANAVA dengan uji berpasangan rentang Newman Keuls atau *t* test. Hasil penelitian menunjukkan bahwa rerata jumlah invasi sel kanker lidah SP-C1 setelah pemberian obat celecoxib berdasarkan konsentrasi dan waktu pemberian secara statistik memberikan hasil yang signifikan. Hasil pengujian dengan ANAVA memberikan *F* hitung = 60,46 yang bersifat bermakna secara statistik, artinya rata-rata terjadinya jumlah invasi sel kanker lidah SP-C1 karena pemakaian celecoxib dengan konsentrasi tertentu dibandingkan tanpa celecoxib adalah berbeda. Pada Celecoxib konsentrasi nol (kontrol) adalah 24,4 dengan konsentrasi celecoxib mulai dari 5 sampai dengan 125 mengalami penurunan dari rata-ratanya 11 hingga menjadi 2,3. Kesimpulan penelitian adalah semakin besar konsentrasi celecoxib yang diberikan akan memberikan efek yang lebih besar pula terhadap hambatan invasi sel kanker lidah SP-C1.

**Kata kunci:** Invasi, sel kanker lidah SP-C1, celecoxib

## INTRODUCTION

Cancer is an abnormal rapid growth of cells, uncontrolled, and there are no clear borders with sound tissues and also have some traits such as anaplasia, invasion, metastatic and rapid growth acceleration.<sup>1</sup> The disease characterized by disorders or failure of multiplication control mechanism in multicellular organism so that uncontrollable behavioral change occurred. The change caused by genetic transformation, especially in genes that regulate cell growth, such as protooncogenes and tumor suppressor genes. The transformed cells continuously proliferate and suppress the normal cells growth.<sup>2</sup>

Squamous cell carcinoma in tongue is a malignancy that originated from oral epithelial mucosa and mostly epidermoid carcinoma.<sup>3</sup> Tongue squamous cell carcinoma accounted for 25 to 50% of all cancers in oral cavity. Out of 441 tongue squamous cell carcinoma reported by Ash and Millar, 25% occurred in women and 75% founded in men with mean age of 63 years old. According to the NCI's SEER statistic (National Cancer Institute Surveillance Epidemiology and End Results) of the U.S. National Institutes of Health Cancer it was assumed 9,800 men and women (6,930 men and 2,870 women) diagnosed tongue cancer.<sup>4</sup> Tongue squamous cell carcinoma had a poor prognosis, so that early diagnose of this disease is important particularly if it was metastatic to a remote area (such as neck and cervical). Tongue carcinoma frequently observed concomitant with another disease such as syphilis and premalignant lesions

as leukoplakia, erythroplasia while according to Frazell and Lucas study, the tongue cancer cases occurred at tongue dorsum was only 4%, but it was more malignant (Undifferentiated epidermoid carcinoma).<sup>5</sup>

Tongue squamous cell carcinoma occurred because of lost control of cell cycles, which is the control cell survival, and control cell motility.<sup>6</sup> The pathogenesis of squamous cell carcinoma is a gradual process, that occurred because of disturbances in growth control function (protooncogenes and tumor suppressor genes) so an increasing growth factors production occur and the cell surface receptors, accelerate the intercellular signal transduction and increasing the transcription factors production.<sup>6,7</sup> Lethal trait of cancer is the ability to invade the surrounding structures, and metastasize to a distant sites of the body.<sup>8,9</sup>

Tongue cancer cell SP-C1 is a tongue cancer cell that isolated from cancer patient lymphonoid. The SP-C1 tongue cancer cell originated from the squamous cell carcinoma moderately differentiated and had not yet invaded the local muscle tissues. This cancer cell characterized by: (1) rapid invasion and metastatic activity; (2) mostly found in human cells; (3) recurrence rate is high even though a radical mode of therapy had been carried out; (4) survival rate is low. This cancer also accounted as a hard to be cured disease and easily metastasized to cervical lymphonoid with high malignancy level.<sup>10</sup>

Prior to invasion process, the squamous cell carcinoma developed locally in the cancer origin epithelial, and had not yet penetrate the basal

membrane, this condition known as carcinoma in situ. But because of the invasive cancer growth pattern, they went out of their origin tissue, to influence the adjacent organ surface function.<sup>11</sup>

Squamous cell carcinoma will invade the underlying structure, and metastasize based on their malignancy level. Invasion is a characteristic of a cancer. Invasion showed the ability of cancer cell to destroy or degrade the border of epithelial tissues and basal membrane.<sup>11</sup>

The invasion process occurred as it infiltrate into the border tissue, impair the basal membrane, extracellular matrix and destroy the tissue structure even organ function.<sup>8</sup> Cancer cell metastatic activity occurred because of the epithelial cell migration. The epithelial cell migration is a very essential for many physiological and pathological processes. Migration of carcinoma cell involved the molecular mechanism similar to the physiological migration. Cell behavioral change occurred as a result of the impaired molecular signal and different ability of tumor cells in respond to this signal. The tumor cell invasion involving the bonding process of receptor and ligand and interaction between proteins and enzymes of basal membrane.<sup>8</sup>

Two important facts regarding the Non-Steroid Anti Inflammatory Drugs (NSAIDs) usage in radiotherapy of cancer disease treatment. First, on preventive side (cancer preventive) that eventually resulted in long term effect. Second, on curative side of cancer. Celecoxib is a kind of NSAIDs while in use not only act as an analgesic, antipyretic and antiinflammatory therapeutic

agents but also act in cancer prevention measure. Celecoxib (NSAID group) can retard the proliferation process and inhibit the invasion of cancer cells and also causes death to cancer cells (in some percentage). Celecoxib as an anti-cancer agent has an important role in cell cycle intervention.<sup>12</sup>

## METHODS

In this study, an observation toward several concentrations of celecoxib used to obtain data regarding the inhibition of SP-C1 tongue cancer cell *Supri's clone* invasion using the Boyden Chamber Assay measurement device. In period of 24 hours and 48 hours observation and counting the alive cancer cells amount done eventually, in order to obtain the numbers of cancer cells survived from invasion inhibition after celecoxib applied, with each concentration of 5, 10, 25, 50, 75, 100, 125%, and as comparison there also been a control group with growth medium applied (DMEM).

## RESULT

Based on observation data (Table 1 and 2), it can be described as follows: generally the result and the counting of tongue cancer cell SP-C1 in 24 hours group in polycarbonate membran under the light microscope, there was a significant change in SP-C1 cancer cell inhibition, from the concentration 5 to 125. Instead, compared to the control group of 24 hours (mean 95) and 48 hours showed an increasing invasion numbers of SP-C1

Table 1. Analysis of SP-C1 cancer cell invasion amount

| Time (A)   | Concentration (B) |      |      |      |      |      |      |      | Total | Average |
|------------|-------------------|------|------|------|------|------|------|------|-------|---------|
|            | Without           | 5%   | 10%  | 25%  | 50%  | 75%  | 100% | 125% |       |         |
| 24 hour    | 95                | 65   | 40   | 24   | 22   | 18   | 16   | 13   |       |         |
|            | 96                | 52   | 39   | 25   | 19   | 18   | 14   | 12   |       |         |
|            | 94                | 54   | 40   | 23   | 23   | 17   | 13   | 9    |       |         |
| Total I    | 285               | 171  | 119  | 72   | 64   | 53   | 43   | 34   | 841   |         |
| Average    | 95.0              | 57.0 | 39.7 | 24.0 | 21.3 | 17.7 | 14.3 | 11.3 |       | 35.0    |
| 48 hour    | 98                | 30   | 27   | 22   | 19   | 18   | 11   | 7    |       |         |
|            | 99                | 32   | 24   | 20   | 20   | 16   | 15   | 6    |       |         |
|            | 103               | 32   | 26   | 21   | 19   | 17   | 13   | 7    |       |         |
| Total II   | 300               | 94   | 77   | 63   | 58   | 51   | 39   | 20   | 702   |         |
| Average    | 100.0             | 31.3 | 25.7 | 21.0 | 19.3 | 17.0 | 13.0 | 6.7  |       | 29.3    |
| Total I+II | 585               | 265  | 196  | 135  | 122  | 104  | 82   | 54   | 1543  |         |

Table 2. Cell invasion comparison based on period and drug concentrations

| Time (A) | Concentration |         |         |         |       |         |         |         |         | F count  | p value |
|----------|---------------|---------|---------|---------|-------|---------|---------|---------|---------|----------|---------|
|          |               | 0%      | 5%      | 10%     | 25%   | 50%     | 75%     | 100%    | 125%    |          |         |
| 24 hour  | Mean          | 95.0    | 57.0    | 39.67   | 24.0  | 21.33   | 17.67   | 14.33   | 11.33   | 311.920  | < 0.001 |
|          | (SD)          | (1.0)   | (7.0)   | (0.577) | (1.0) | (2.082) | (0.577) | (1.528) | (2.082) |          |         |
|          | Range         | 94-96   | 52-65   | 39-40   | 23-25 | 19-23   | 17-18   | 13-16   | 9-13    |          |         |
| 48 hour  | Mean          | 100     | 31.33   | 25.67   | 21.0  | 19.33   | 17.0    | 13.0    | 6.67    | 1209.220 | < 0.001 |
|          | (SD)          | (2.646) | (1.155) | (1.528) | (1.0) | (0.577) | (1.0)   | (2.0)   | (0.577) |          |         |
|          | Range         | 98-103  | 30-32   | 24-27   | 20-22 | 19-20   | 16-18   | 11-15   | 6-7     |          |         |

tongue cancer cells after recounting of the data in 48 hours period (100). Counting the control group cancer cells, in comparison of control in 24 hours (95), to the control in 48 hours (100) showed an increasing amount for 5%, this pointed out that cancer cells without treatment of celecoxib would return as an increasing number of SP-C1 cancer cells amount (there was an invasion of cancer cells). Observation on cancer cells amount in concentration of celecoxib 5, 10, 25, 50, 75, 100, 125% in 24 hours period (57; 39.6; 24; 21.3; 17.67; 14.3; 11.3) showed a decreasing result and after observation in 48 hours (31.3; 25.7; 21; 19.3; 17; 13; 6.7). This suggested that there was an inhibition toward the SP-C1 tongue cancer cells after application of celecoxib medium.

## DISCUSSION

Basically oral cancer occurred because of abnormal genome, caused by impaired genes that regulate the cell differentiation. The genes that regulate cell growth and differentiation known as protooncogene and tumor suppressor genes, and can be found in every chromosome abundantly. Impaired protooncogenes that undergo changes so that it could eventually be a cancer known as oncogenes.<sup>13</sup>

Understanding of the carcinogenesis process is a strategic development in cancer disease treatment. Cancer therapy approach using the chemopreventive agents is more promising than any other conventional anti-cancer drugs. Chemopreventive agents defined as a compound that inhibit and suppress the carcinogenesis process in human cells so that cancer growth could be prevented.<sup>14</sup>

Chemopreventive agents development based on the cell cycle regulation including the

growth hormone receptors and protein kinase, angiogenesis inhibition, cyclooxygenase-2 (COX-2) enzyme inhibition, and apoptotic induction. Chemopreventive agents specifically targeted to an activity through the molecular mechanisms. The abnormality of cell cycle and apoptotic regulation, COX-2 enzyme increasement, and angiogenesis process only occurred at cells invaded by cancer even though only on several cases angiogenesis observed in the heart.<sup>14</sup>

Celecoxib (NSAID group) could inhibit the cancer cell proliferation and invasion and also killed the cancer cells (at some percentages). Celecoxib as an anti cancer agents played a role in intervention toward the cell cycle. Cyclooxygenase enzyme (COX) that is a target of Non-Steroid Anti-Inflammatory Drugs (NSAID) could be found in two isoform, which is COX-1 and COX-2. Both enzyme catalized the reaction and resulted the same product, prostaglandin, but with different biological functions.<sup>15</sup>

Statistical analysis using the ANAVA showed a significant value, which means that the cancer cells invasion event toward the celecoxib in several concentration is different compared to the group without celecoxib treatment. The control celecoxib group was 97.50 and the concentration with celecoxib concentration of 5  $\mu$ M to 125  $\mu$ M had a mean value change from 44.17 to 9.00.

Analysis of period of time using ANAVA also resulted a significant value, this showed that time factor influenced majorly in celecoxib as an anti-invasion drugs effectivity: the longer time the lower or there would be a decreased cancer cells invasion based on the mean of cancer cells invasion (35.042 in 24 hours while in the 48 hours it mean value was 29.250).

The result of this study correspond with Lucille et al.<sup>16</sup> study which suggest that celecoxib

10  $\mu$ M inhibits the cells invasion or migration through matrix collagen type 1 about 40% in 24 hours. The zymography result concluded that in existence of celecoxib with concentration of 10  $\mu$ M the activity of MMP-2 and MMP-8 enzyme decreased about 30-40%. This in vitro study also showed that there is inhibition in proliferation and invasion of squamous cell carcinoma by the specific inhibitor of COX-2, where the celecoxib resulted an anti-cancer effect through various mechanism of cellular and molecular. This study also tested 10 groups of NSAID available toward oral cancer cells and resulted that celecoxib and sulindac sulfide (Clinoril sulfide) were very effective in killing and inhibiting the cancer cells growth. Study regarding the two drugs, celecoxib and sulindac sulfide suggested that celecoxib proved as more effective, inhibited up to 60 percent of oral cancer cells.

## CONCLUSION

The celecoxib drug inhibits the SP-C1 tongue cancer cells invasion in several concentrations, this showed in decreased numbers of tongue cancer cells after the drug with several concentrations applied from the lowest (5) to the highest (125). While in the control group (without celecoxib application) still showed an increased numbers of tongue cancer cells invasion.

## REFERENCES

1. Warshawsky S, Landolph JR. Molecular carcinogenesis and the molecular biology of human cancer. Boca Raton USA: Taylor & Francis Group; 2006. p. 6.
2. King RJ, Robins MW. Cancer biology. 3<sup>rd</sup> ed. England: Pearson Education Ltd.; 2006. p. 209-29.
3. Wood NK, Sawyer DR. Oral cancer. In: Wood NK, Goaz PW (editors). Differential diagnosis of oral and maxillofacial lesion. St. Louis: Mosby Inc.; 1997. p. 587-95.
4. Hasibuan S. Prosedur deteksi dini dan diagnosis kanker rongga mulut. Medan: USU Digital Library; 2004. p. 1-7.
5. Shah JP, Zelefsky MJ. Cancer of oral cavity. In: Harrison. Head and neck cancer, a multidisciplinary approach. 2<sup>nd</sup> ed. Philadelphia: Lippincott Williams & Wilkins; 2004. p. 266-80.
6. Regezi JA, Sciubba JJ. Oral pathology, clinical pathologic correlations. 3<sup>rd</sup> ed. Philadelphia: W.B. Saunders Co.; 1999. p. 76-90.
7. Williams HK. Molecular pathogenesis of oral squamous carcinoma. J Clin Pathol Mol Pathol 2000;53:165-72.
8. Revianti S, Parisihni K. Peran matriks metallo-proteinase (MMP) pada metastasis karsinoma sel skuamosa rongga mulut. J PDGI Edisi khusus 2005;55:232-6.
9. Liotta LA. Principles of molecular cell biology of cancer, cancer metastasis. In: DeVita. Cancer, principles & practice of oncology. 4<sup>th</sup> ed. Philadelphia: Lippincot-Raven; 1993. p. 134-40.
10. Supriatno, Yuletnawati. Aktifitas anti kanker cepharantine pada kanker lidah manusia invitro (Tinjauan proliferasi, invasi, dan metastasis sel). Jogyakarta: Majalah Kedokteran Gigi UGM; 2006. p. 141-5.
11. Field JK. The role of oncogenes and tumour-suppressor genes in the aetiology of oral, head and neck squamous cell carcinoma. J Soc Med 1995;88:35-9.
12. Roy J, Lucille A. Combining NSAID with chemotherapy, radiation may improve cancer treatment. [cited 2009 Jun 25]. Available from: <http://www.uihealthcare.com/news/news/2007/06/04/cancertreatment.html>.
13. Kumar V, Cotran RS, Robbins SL. Robbins basic pathologic. 7<sup>th</sup> ed. Jakarta: Penerbit Buku Kedokteran EGC; 2007. p.185-224.
14. Kudo Y. Invasion and metastasis of oral cancer cells require methylation of e-cadherin and/or degradation of membranous  $\beta$ -Catenin. Clin Res 2004;10:5455-463.
15. Washart ML. Treating and preventing cancer through COX-2 Inhibition. [cited 2009 May 20]. 2002.
16. Kwak YE, Jeon NK, Kim J, Lee EJ. The cyclooxygenase-2 selective inhibitor celcoxib suppresses proliferation and invasiveness in the human oral squamous carcinoma. Ann N Y Acad Sci 2007;1095:99-111.