ANTI-CANCER CELL ACTIVITY OF RECOMBINANT CANINE INTERFERON (rCaIFN) AND ITS COMBINATION WITH *Curcuma zedoaria* PLANT EXTRACTS ON SEVERAL TUMOR-DERIVED CELL LINES

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ABSTRACT

Anti-cancer cell activity of recombinant canine interferon (rCaIFN) which produced using biology molecular techniques through silk worm bioreactor and baculovirus system, ethanol & chloroform extracts of *Curcuma zedoaria* and the combination between rCaIFN-*Curcuma zedoaria* extracts on canine tumor-derived cell lines (MCA-B1, MCM-B2), human (HeLa) and rat (PC-12) was conducted. The dose tested for ethanol extract of *Curcuma zedoaria* was 70 ppm, while the rCaIFN dose was 10^4 IU/mL. Recombinant canine interferon more effective in canine tumors cells compared to non-canine cells. Maximum antiproliferation activity of rCaIFN on each tumor cells was 59% and 52% for MCM-B2 and MCA-B1, 19% for HeLa and 13% for PC-12 cells. When rCaIFN was combined with ethanol extract of *Curcuma zedoaria* plant extract and exposed to canine cells, antiproliferation activity was higher than rCaIFN or ethanol extract of *Curcuma zedoaria* alone. The maximum activities for combination of rCaIFN with *Curcuma zedoaria* ethanol extract on each cells was 76% for MCM-B2, 84% for MCA-B1, 55% for HeLa and 44% for PC-12; while for combination of rCaIFN with choloroform extract of *Curcuma zedoaria* (the dose was 21 ppm) were 79% for MCA-B1, 73% for MCM-B2, 54% for HeLa and 47% for PC-12 cells, respectively. We suggested that combination of rCaIFN with ethanol and chloroform extracts of *Curcuma zedoaria* have a synergistic effect on the growth inhibition activity on tested tumor cells, and this activity seemed to be more effective on canine derived-tumor cells compared to human and rat cells due to the origin of the recombinant interferon source. This phenomenon seems to be a promising way for the cancer treatment. The combination of rCaIFN with other plants extract is in progress.

Key words : growth inhibition, recombinant canine interferon, tumor cells, *Curcuma zedoaria*

INTRODUCTION

Interferon (IFN) is a cytokine that produced by all living cells due to an infection caused by virus or other intracellular pathogens. It is well known that in their discovery era, interferon is used as an antiviral drug. Recently besides their anti-viral activity, it is also known that IFN had an anti-cancer activity. Recombinant canine interferon (rCaIFN) which produced using molecular biology techniques through silk worm bioreactor and baculovirus system (Prijosoejanto *et al,* 1999) is well known had an anti-proliferation activity on several tumor-derived cell lines singly or in combination with some anticancer drugs such as Vincristin, Vinblastine, Mitomycin-C and Doxorubicin (Prijosoerjanto *et al,* 2000; Prijosoerjanto *et al,* 2002).

Curcuma zedoaria known as "Temu Putih" in Indonesian language is one of the 7,000 Indonesia's medicinal plants out of 30,000 plants found in Indonesia (Indonesia Drugs and Food Agency, 2002). Ethanol and chloroform extracts of *Curcuma zedoaria* plant were known had an anti-proliferation activity on several tumor-derived cell lines (Prijosoerjanto *et al,* 2001). Based on our previous research, the anti-proliferation activity of *Curcuma zedoaria* seem to be more effective in myeloma-derived cell line compared to the carcinoma-derived cell line, and they showed a dose response relationship. In general, the anti-proliferation activity of both extracts from *Curcuma zedoaria* gave a significant inhibition of cell growth on myeloma and carcinoma cells.

The purpose of the present study is to know the synergistic effect on anti-proliferation activity of the combination between rCaIFN and ethanol or chloroform extracts of *Curcuma zedoaria,* in order to find the anti-cancer substances for the treatment of tumor disorders both in human and animal.

MATERIALS and METHODS

Recombinant Canine Interferon (rCaIFN).

The rCaIFN was produced by a biology molecular techniques through silk worm bioreactor and baculovirus system in our previous study (Prijosoerjanto *et al,* 1999). The stock solution of rCaIFN was kept on refrigerator until use, and diluted based on the tested dose for working solution.

Extraction of the Plant

Curcuma zedoaria which used in this study was identified and determined by the Research Center for Biology, Indonesian Institute of Science/LIPI-Bogor. The extracts of *Curcuma zedoaria* were prepared using chloroform and ethanol solutions following the method of Anonimous (1985). Briefly, 50 grams of *Curcuma zedoaria* roots powder were macerated using 500 ml of chloroform or ether and kept for 5 days, and were then filtered. The wastes were dissolved into a sufficient amount of chloroform and were filtered until the total volume of extracts was 100 ml. The extracts were evaporated to get the desired concentrated filtrates and were kept until use. Working concentrations of each extracts were made by dilution the extracts until the tested concentration was achieved (Prijosoerjanto *et al,* 2001).

Anti-proliferation Activity Assay

Two canine tumor-derived cell lines MCA-B1 and MCM-B2 (Prijosoerjanto *et al*, 1995a,b), human (HeLa) and rat (PC-12) cells were used in this study. All cell lines were cultured with the density of 10^3 cell/ml on the 24-well dish using a growth medium comprises from DMEM and 10% FCS (Prijosoerjanto *et al*, 1995a,b ; Prijosoerjanto *et al,* 2002). The tested dose of rCaIFN (10^4 IU/mL) and each plant extracts (70 ppm for ethanol extract and 21 ppm for

chloroform extract) were determined after the LC_{50} of each extracts were recognized on our previous studies (Prijosoerjanto *et al,* 2000; Prijosoerjanto *et al,* 2001). rCaIFN, ethanol and chloroform extracts of *Curcuma* zedoaria alone or combination between rCaIFN and the extracts were added to the culture dish of all tested cell lines (3 holes for each treatment). After the confluence growth was attained on the control negative dishes, the cells on all treated-dishes were harvested and the average of the total number of cells on each treated-dishes were counted using a hemacytometer with Trypan Blue dye.

RESULT and DISCUSSION

Antiproliferation activity of rCaIFN or ethanol and chloroform *Curcuma zedoaria* extracts was detected in all cell lines, although it was vary for each cell line. This result was similar to our previous studies (Prijosoerjanto *et al,* 2001; Prijosoerjanto *et al,* 2002). The highest antiproliferation activity of rCaIFN on each tumor cells was 59% and 52% for MCM-B2 and MCA-B1, 19% for HeLa and 13% for PC-12 cells. For ethanol extract of *Curcuma zedoaria* alone, the maximum anti-proliferation activity were 57% for MCA-B1, 62% for MCM-B2, 485 for HeLa and 53% for PC-12 cells, while for chloroform extract of *Curcuma zedoaria* alone were 61%, 65%, 52% and 49% for MCA-B1, MCM-B2, HeLa and PC-12 cells respectively (Figure 1).

Combination of rCaIFN with ethanol or chloroform extracts of *Curcuma zedoaria* were also gave an antiproliferation activity on all tested cell lines. This combination activity was higher compared to the treatment with single substance (Figure 2). The result of this activity indicated that there was a synergistic effect on the anti-proliferation activity between rCaIFN and ethanol or chloroform extracts of *Curcuma zedoaria*. Similar to their activity as a single substance when they exposed to the tested cell line, variation on the degree of antiproliferation on each cell lines was also encountered.

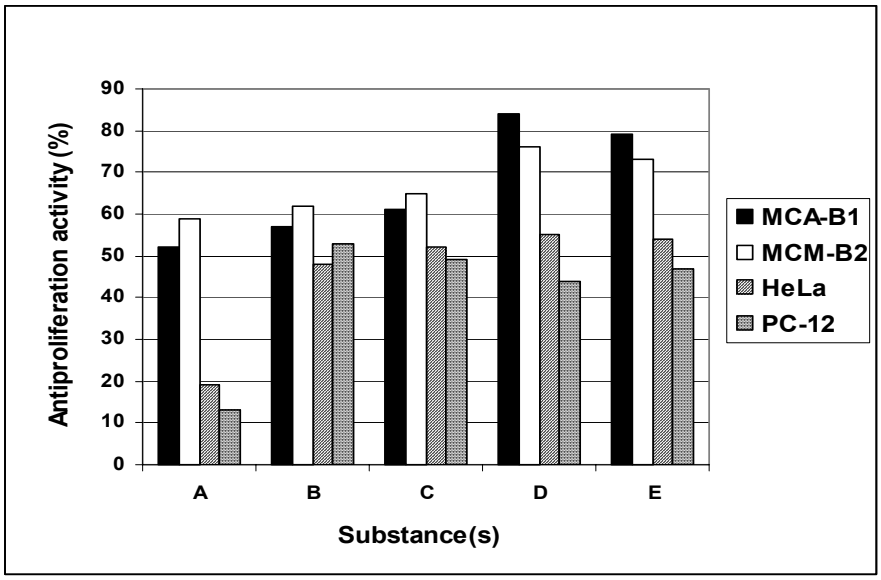

Figure 1. Anti-proliferation activity of each substance(s) on four tumor derived cell lines. A = rCaIFN; B = Ethanol extract of *Curcuma zedoaria*; C = Chloroform extract of *Curcuma zedoaria*; D = Combination of rCaIFN + Ethanol extract of *Curcuma zedoaria* and E = Combination of rCaIFN + Chloroform extract of *Curcuma zedoaria.*

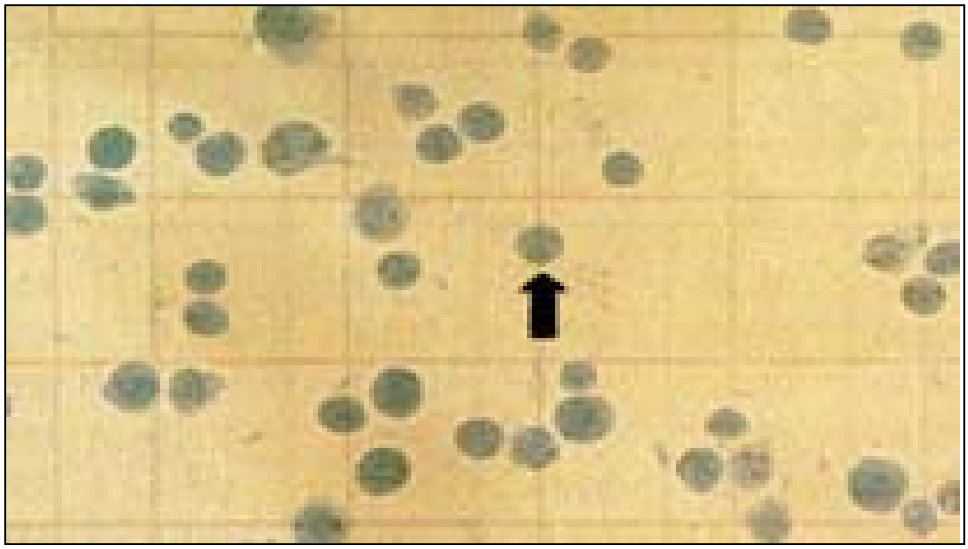

Figure 2. Growth inhibition effect of *Curcuma zedoaria* extracts on MCA-B1 cell. Death cell is marked by blue color (arrow). Hemacytometer, Trypan Blue dye exlusion

The capability of rCaIFN to inhibit the tumor cell proliferation in vitro is seem to be more effective on the canine-derived tumor cells (the same species with the origin of rCaIFN) compared to other cells from different species. This result was in accordance to our previous studies (Prijosoerjanto *et al*, 1995c; Tateyama *et al*, 1995; Prijosoerjanto *et al*, 1997) which use an interferon derived from feline (rFeIFN) and indicated that even interferon (IFN) not always species specific but their activity more effective on the same species cells. The different sensitivity of rCaIFN on the cell lines derived from canine tumor (MCA-B1 and MCM-B2) was also occurred. This phenomenon was seemed due to the different sensitivity on the cell surface including the receptor for rCaIFN (Johnson *et al,* 1994; Prijosoerjanto *et al*, 1995c,d).

Anti-proliferation activity of extracts from *Curcuma zedoaria* was occurred in all cell lines, this result was also same with our previous study even with the different cell lines (Prijosoerjanto *et al,* 2001). Hutapea (1993) reported that *Curcuma zedoaria* contain a volatile oils, saponin, flavonoids and polyphenol. Soedibyo (1998) indicated that extract of this plant has an anti-tumor activity, but how their mechanism work is not fully understood.

The synergistic effect of other recombinant interferon from other animal species (feline interferon/rFeIFN) with some anticancer drugs was also reported on our previous studies (Widyastuti *et al,* 1999a,b). The same result on synergistic anti-proliferation activities of rCaIFN with some anticancer drugs was clearly indicated on our previous studies (Prijosoerjanto *et al,* 2000; Prijosoerjanto *et al,* 2002).

To our knowledge, our present study is the first report of the synergistic effect of the combination of rCaIFN with ethanol or chloroform extracts of *Curcuma zedoaria.* This result indicated that enhancement of the anti-proliferation activity was occurred on these combination of two anti-cancer substances, and this phenomenon is seem to be a promising treatment on the way to produce an anti-tumor drugs for the combating of tumor disorder both in human and animals.

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