

CHAPTER IV

RESULTS

This chapter is composed of four parts, which are demonstrated for the experimental results of curcumin on tumor angiogenesis and on tumor angiogenic biomarkers, COX-2 and VEGF levels.

4.1 The tumor model

2×10^6 cells of Hepatocellular carcinoma cells (HepG2) were inoculated into the dorsal skin-fold chamber, which implanted in nude mice as described previously in Chapter III (Materials and Methods). One day after the inoculation, the growth of HepG2 cell was positively confirmed by H&E examination (as shown in Figure 3.3). Moreover, the growth of tumor cells within the chamber could be easily observed by eye on day 14 as shown by photograph in Figure 4.1. Table 4.1 demonstrated that on day 14 of tumor progression, body weights of all groups, Con, HepG2, Con-cur, and HepG2-cur, were no significant difference ($p > 0.05$).

4.2 Tissue perfusion of tumor area

The Laser Doppler Flowmeter was used to measure the tissue perfusion of tumor area of each animal by using 10 points of reference as described previously in Chapter III (Materials and Methods). The results of changes in tissue perfusion of HepG2, and HepG2-cur groups during any periods as compared to aged-matched control were summarized in Table 4.2 and Figure 4.2. The results showed that the % changes from control in tissue perfusion on day 7 and day 14 in HepG2 groups were significantly increased $43.15 \pm 9.54\%$ and $53.89 \pm 5.59\%$ as compared 3 days HepG2 group, respectively. After 7 days treatment with both low dose (37.79 ± 8.86) and high dose (29.71 ± 5.74) of curcumin, the % changes from control in tissue perfusion within dorsal skin-fold chamber in the tumor bearing mice was reduced but did not significantly different when compared to HepG2. Interestingly, on day 14, the %

changes from control in tissue perfusion of HepG2-cur3,000 were significantly decreased by $28.95 \pm 9.14\%$ as compared to HepG2 group.

It is noted that there was no significant change between groups of control and control treated with curcumin.

4.3 Tumor angiogenesis

By using intravital fluorescent microscopy, the inoculated HepG2 cell caused the changes of microvascular network of the host as follows :

Three days after implantation (3 days HepG2 group), the results showed the appearance of dilatation, tortuosity and hyperpermeability with extravasations of fluorescence tracer from the host existing microvessels (Figure 4.3).

On day 7, at the early stage of tumor angiogenesis onset, the tumor neocapillaries were able to be observed, in which the mother-host vessels underwent endothelial cell sprouting (Figure 4.4A). Some of the proliferating neovessels appeared already to migrate out, approaching to the tumor area (Figure 4.4B).

A number of neovessels in the tumor area on day 14 after the inoculation (14 days HepG2 group) was demonstrated in Figure 4.5. Most of the tumor microvasculature had the converging flow architecture. The results indicated markedly increase in capillary network in the tumor area (Figure 4.5A). In addition, varieties of abnormal neocapillaries such as abrupt changes in the diameter, and from shunts/loops were observed (Figure 4.5B).

The fluorescent videomages of the microvasculature for controls and HepG2 groups at different periods after tumor cells inoculation were demonstrated in Figure 4.6.

Figure 4.7 demonstrated the intravital fluorescent microscopic observation of tumor angiogenesis affected by curcumin treatment. The result showed that the appearance of neocapillaries induced by HepG2 was markedly reduced on 7 and 14

days after treatment of curcumin (3,000 mg/kgBW). In addition, the abnormalities of neocapillary network pattern were attenuated after treatment with high dose of curcumin. It is strongly supported by histological examination. Confocal laser image of an area of tissue in the dorsal skin-fold chamber (stained with hematoxylin-eosin) in 7 and 14 days HepG2 groups with and without treatment was demonstrated in Figure 4.8. The section of 14 days HepG2-cur3,000 showed remarkable decreased in tumor cells deposit.

Based on the recorded videoimage, the tumor neocapillary density and microvasculature were evaluated by using the digital image analysis' as described previously Chapter III (Materials and Methods). The values of neocapillary density (NCD) were calculated using the equation 1 and summarized in Table 4.3 and Figure 4.9. In both Con and Con-cur groups without tumor, the capillary densities remained constant ($28.57 \pm 1.83\%$ to $31.63 \pm 1.00\%$) during any periods. In HepG2 groups, the capillary density increased from $51.67 \pm 4.26\%$ (on day 7) to $69.18 \pm 2.41\%$ (on day 14), which were significantly higher than those of 3 days HepG2 group ($33.57 \pm 1.28\%$) ($p < 0.01$ and $p < 0.001$, respectively) and the aged match control groups ($p < 0.001$).

The mean capillary density in 7 days and 14 days HepG2 groups after treatment with curcumin at the dose of 300 mg/kgBW (HepG2-cur300) ($48.19 \pm 0.78\%$ and $59.86 \pm 2.87\%$, respectively) was decreased but not significantly different compared with untreated group. However, high dose of curcumin (HepG2-cur3,000), could significantly lower the mean capillary densities compared with the levels in HepG2 group and HepG2-cur300 ($59.86 \pm 2.87\%$) group at both periods ($39.88 \pm 1.61\%$ and $42.91 \pm 1.76\%$). But these capillary densities on day 7 and 14 in HepG2-cur300 were still higher than those of control. Therefore, it was meant that the tumor-induced neovascularization still existed. Even though it is worthy to conclude that increases in capillary density with HepG2 were attenuated with treatment of curcumin.

Relationship between capillary density and time courses of vehicle or curcumin treatment in HepG2, HepG2-cur300, and HepG2-cur3,000 groups was presented in Figure 4.10. The results show a significant correlation fitted with a linear relationship was found between capillary density and time courses of vehicle or

curcumin treatment in HepG2, HepG2-cur300, and HepG2-cur3,000 groups ($r^2=0.999$, $p<0.001$; $r^2=0.99$, $p<0.001$; $r^2=0.9202$, $p<0.01$, respectively).

Table 4.4 and Figure 4.11 demonstrated means of capillary diameter of each group at each different time point. The results showed that neocapillary diameters of tumor area were significantly higher than those age-matched control groups. The mean neocapillary diameter of 7 ($10.32\pm 0.41\mu\text{m}$) and 14 ($10.89\pm 0.65\mu\text{m}$) days HepG2 groups were significantly increased as compared to control levels at both periods ($7.33\pm 0.50\mu\text{m}$, and $7.50\pm 0.50\mu\text{m}$). After treatment with high dose of curcumin, the mean capillary diameter in HepG2 group was significantly decreased. But these capillary diameters on day 7 and 14 in HepG2-cur3000 were still higher than those of control.

4.4 Tumor Biomarkers : COX-2 and VEGF

4.4.1 COX-2

Western blotting was used to examine the up-regulation of COX-2 protein expression (Figure 4.12). Since, there was vary small amount of COX-2 protein for each mouse, therefore, we have to pool all 5 specimens together. The results indicated COX-2 protein expression was dramatically detected in HepG2 groups at 3 days period and maintained throughout the experimental period. The up-regulations of COX-2 protein expression in HepG2 groups were suppressed by treatment of curcumin. COX-2 protein was undetectable in the normal skin tissue.

A quantitative comparison using image analysis (Global LabII software) was used to allow for the measurement of the COX-2 protein level. Table 4.5A, 4.5B, and 4.5C demonstrated COX-2 protein levels in 3, 7, and 14 days HepG2 groups, respectively.

Immunoblot analysis indicated a high amount of protein level ($0.1053\pm .0151\mu\text{g}/10\mu\text{g}$ of total protein) in dorsal skin tissue of 3 days HepG2 group. The mean COX-2 protein level of 3 days HepG2 group was significantly decreased after

treatment with both low dose ($0.0638 \pm 0.0147 \mu\text{g}/10 \mu\text{g}$ of total protein) and high dose ($0.0483 \pm 0.0044 \mu\text{g}/10 \mu\text{g}$ of total protein) of curcumin as compared to non-treated group.

Immunoblot analysis indicated a high amount of protein level ($0.1058 \pm .0075 \mu\text{g}/10 \mu\text{g}$ of total protein) in dorsal skin tissue of 7 days HepG2 group. The mean COX-2 protein level of 7 days HepG2 group was decreased but not significantly different after treatment with both low dose of curcumin ($0.0765 \pm 0.0150 \mu\text{g}/10 \mu\text{g}$ of total protein) and significantly suppressed after treatment with high dose of curcumin ($0.0585 \pm 0.0158 \mu\text{g}/10 \mu\text{g}$ of total protein).

Immunoblot analysis indicated a high amount of protein level ($0.1068 \pm .0095 \mu\text{g}/10 \mu\text{g}$ of total protein) in dorsal skin tissue also maintained during 14 days after HepG2 inoculation. The mean COX-2 protein level of 14 days HepG2 group was significantly suppressed after treatment with low dose ($0.0610 \pm 0.0057 \mu\text{g}/10 \mu\text{g}$ of total protein) and high dose ($0.0445 \pm 0.0051 \mu\text{g}/10 \mu\text{g}$ of total protein) of curcumin as compared to non-treated group.

Comparisons of the means COX-2 protein level for control and HepG2 groups at different periods with and without curcumin treatment were demonstrated in Table 4.6 and Figure 4.13.

Relationship between COX-2 protein levels and time courses of vehicle or curcumin treatment in HepG2, HepG2-cur300, and HepG2-cur3,000 groups was presented in Figure 4.14. The results show a significant correlation fitted with a linear relationship was found between COX-2 and the periods of HepG2 groups ($r^2 = 0.9643$, $p < 0.05$). In HepG2-cur300, and HepG2-cur3,000 groups, the relationship between COX-2 protein levels and time courses of curcumin treatment were very low ($r^2 = 0.0287$, $p > 0.05$; $r^2 = 0.055$, $p > 0.05$).

4.4.2 VEGF

The serum VEGF levels of the 7 ($49.52 \pm 5.18 \text{ ng/ml}$) and 14 days HepG2 ($49.96 \pm 5.74 \text{ ng/ml}$) groups were significantly elevated compared with those of the age-matched control levels ($6.38 \pm 0.68 \text{ ng/ml}$ and $6.53 \pm 1.24 \text{ ng/ml}$, respectively) ($p < 0.001$) and than those of 3 days HepG2 group ($16.80 \pm 2.43 \text{ ng/ml}$) (Table 4.7 and Figure 4.15). Elevations of serum VEGF in HepG2 groups were significantly reduced by treatment with both low ($23.43 \pm 4.92 \text{ ng/ml}$) and high doses ($18.56 \pm 3.34 \text{ ng/ml}$) of curcumin at 7 days after treatment ($p < 0.001$). On 14 days the serum VEGF after treatment with high dose ($18.18 \pm 2.76 \text{ ng/ml}$) of curcumin was more significantly reduced than those treatment with low dose ($31.30 \pm 7.84 \text{ ng/ml}$).

Relationship between serum VEGF and time courses of vehicle or curcumin treatment in HepG2, HepG2-cur300, and HepG2-cur3,000 groups was presented in Figure 4.16. The results show a correlation fitted with a linear relationship was found between serum VEGF and the periods of HepG2 groups ($r^2 = 0.7599$, $p > 0.05$). The relationship between COX-2 protein levels and time courses of low dose of curcumin treatment ($r^2 = 0.9826$, $p < 0.01$) was higher than those of high dose of curcumin treatment ($r^2 = 0.7145$, $p > 0.05$).

4.4.3 Relationship between COX-2 expression and serum VEGF and NCD

The relations between the capillary density and COX-2 protein level in HepG2 groups have been carried out. The relationship between the mean values of capillary density (33.57 ± 1.2 , 51.67 ± 4.26 , $69.18 \pm 2.41\%$, respectively) and COX-2 protein levels (0.1053 ± 0.0151 , 0.1058 ± 0.0075 , $0.1068 \pm 0.0095 \mu\text{g}/10 \mu\text{g}$ of total protein, respectively) of 3, 7, and 14 days HepG2 groups was presented in Table 4.8 and Figure 4.17. A significant correlation was found between NCD and COX-2 expression ($r^2 = 0.9607$, $p < 0.01$).

The relationship between the mean values of neocapillary density (33.57 ± 1.2 , 51.67 ± 4.26 , $69.18 \pm 2.41\%$, respectively) and serum VEGF (16.80 ± 2.43 , 49.52 ± 5.18 , 49.96 ± 5.74 ng/ml, respectively) of 3, 7, and 14 days HepG2 groups was presented in Table 4.9 and Figure 4.18. No significant correlation was found between serum VEGF and NCD ($r^2 = 0.7681$, $p < 0.32$).

The relationship between the mean values of COX-2 protein levels (0.1053 ± 0.0151 , 0.1058 ± 0.0075 , 0.1068 ± 0.0095 $\mu\text{g}/10$ μg of total protein, respectively) and serum VEGF (16.80 ± 2.43 , 49.52 ± 5.18 , 49.96 ± 5.74 ng/ml, respectively) of 3, 7, and 14 days HepG2 groups was presented in Table 4.10 and Figure 4.19. No significant correlation was found between COX-2 expression and VEGF production ($r^2 = 0.5829$, $p < 0.447$).



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Figure 4.1. The photograph of the growth of tumor cells could be observed within the chamber on day 14 (as indicated by circular area)

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Table 4.1. Values of mean body weight (g) for each group at different time point with and without treatment of curcumin (mean \pm SEM, all n=5).

Groups	Body weight (g)		
	3 days	7 days	14 days
Con	24.16 \pm 0.77	24.02 \pm 0.50	25.00 \pm 0.85
Con-cur300	25.84 \pm 0.95	24.23 \pm 0.51	23.94 \pm 0.62
Con-cur3,000	24.55 \pm 0.53	24.03 \pm 0.70	24.05 \pm 0.96
HepG2	24.90 \pm 0.74	24.32 \pm 0.64	23.89 \pm 1.33
HepG2-cur300	23.10 \pm 1.74	24.46 \pm 0.28	23.83 \pm 0.89
HepG2-cur3,000	23.29 \pm 0.28	23.27 \pm 0.75	25.94 \pm 1.08

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Table 4.2. Changes in tissue perfusion of HepG2, and HepG2-cur groups during any periods as compared to aged-matched control, manifested as per cents of the control values.

Groups	% Changes from control		
	3 days	7 days	14 days
HepG2	14.94±9.36	43.15±9.54*	53.89±5.59@
HepG2-cur300	9.16±5.27	37.79±8.86	31.99±4.46
HepG2-cur3,000	-4.23±8.90	29.71±5.74	28.95±9.14 [#]

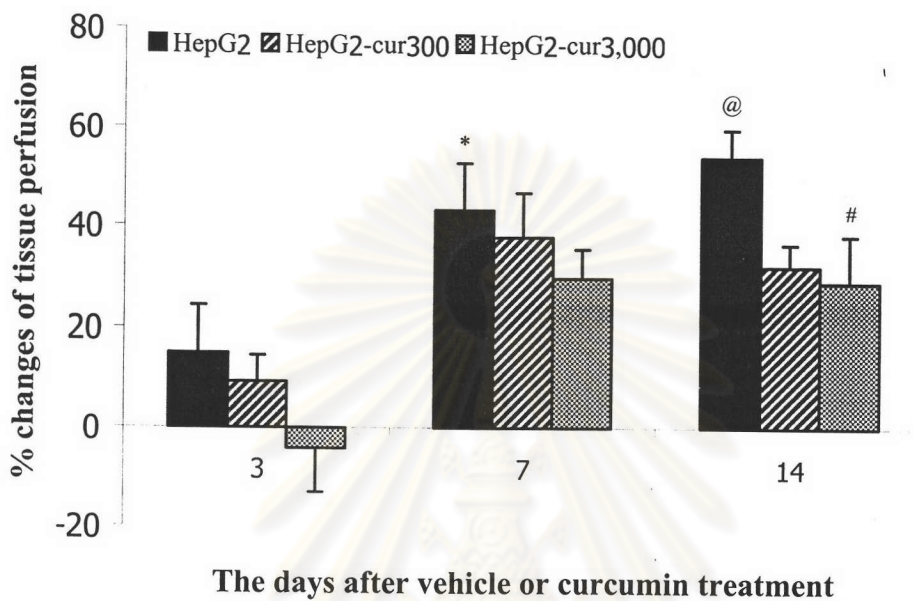
*P < 0.01 significant difference compared to 3 days HepG2.

@P < 0.001 significant difference compared to 3 days HepG2.

[#]P < 0.05 significant difference compared to 14 days HepG2.

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Figure 4.2. Changes in tissue perfusion of HepG2, and HepG2-cur groups during any periods as compared to aged-matched control, manifested as per cents of the control values.



- *P < 0.01 significant difference compared to 3 days HepG2.
 @P < 0.001 significant difference compared to 3 days HepG2.
 #P < 0.05 significant difference compared to 14 days HepG2.

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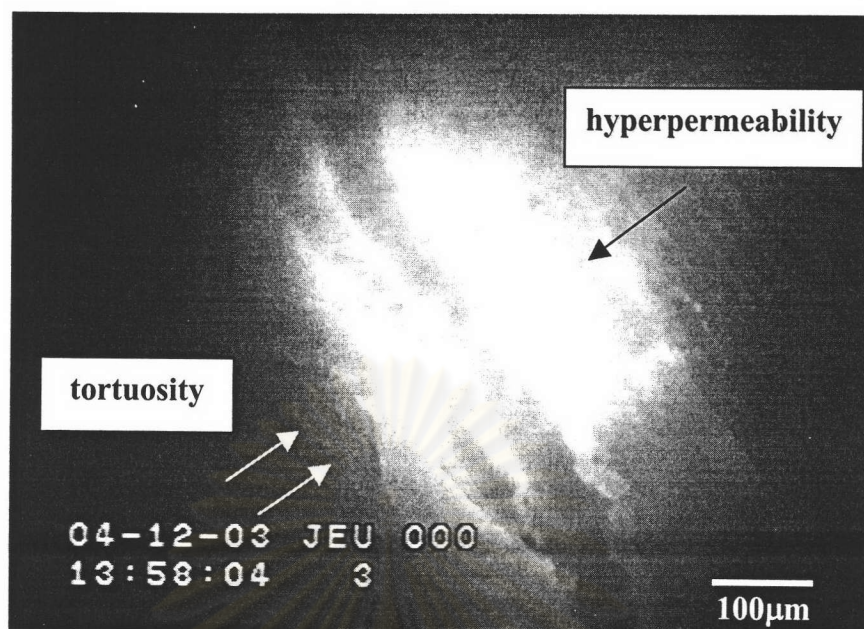


Figure 4.3. At the early stage of tumor angiogenesis onset, 3 days period, the results of tumor chemotaxis caused the native configurationally changed, which were characterized by tortuosity, dilatation, and hyperpermeability. Bar, 100 μm .

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A



B

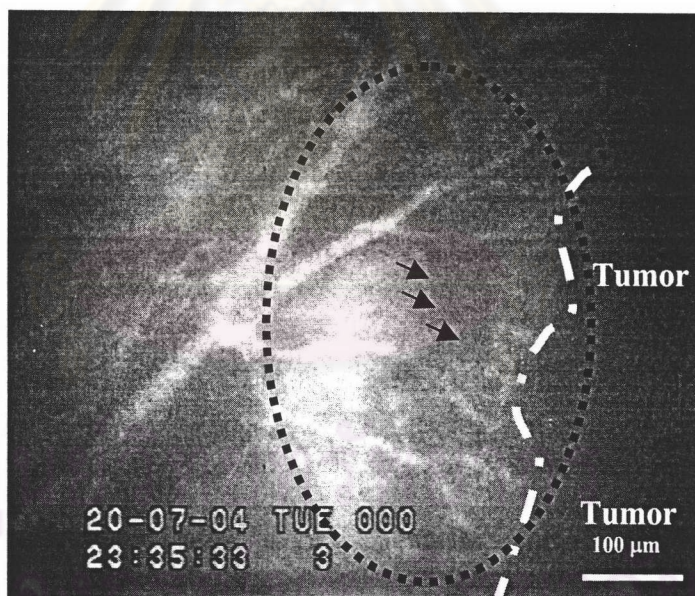


Figure 4.4. At the early stage of tumor angiogenesis onset, 7 days period, the endothelial cell sprouting was observed from the native vessel (A). Some of the proliferating neovessels appeared to migrate out, approaching to the tumor area (B). Bar, 100 μ m.

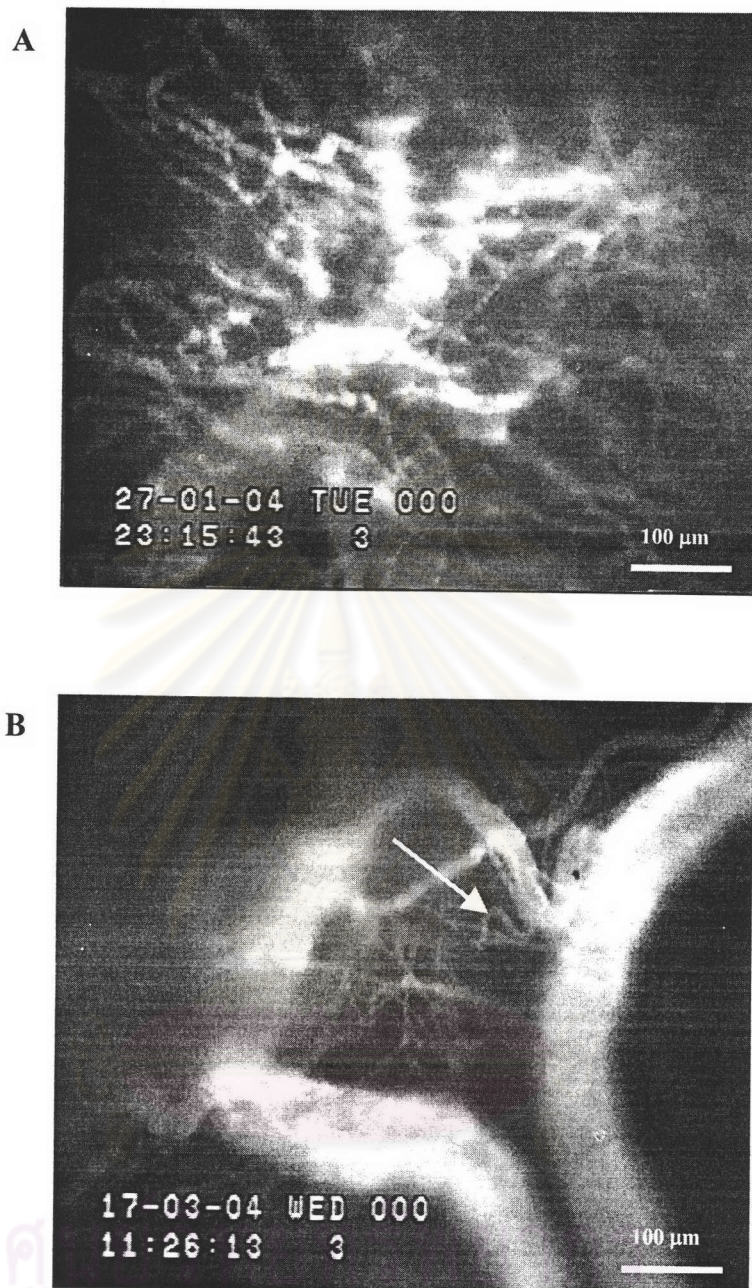


Figure 4.5. Fluorescence videoimages of neocapillaries 14 days after the inoculation of tumor cells. Note a numerous of neocapillary network (A) and shunts/loops form (indicated by arrow in B). Bar, 100 μ m.

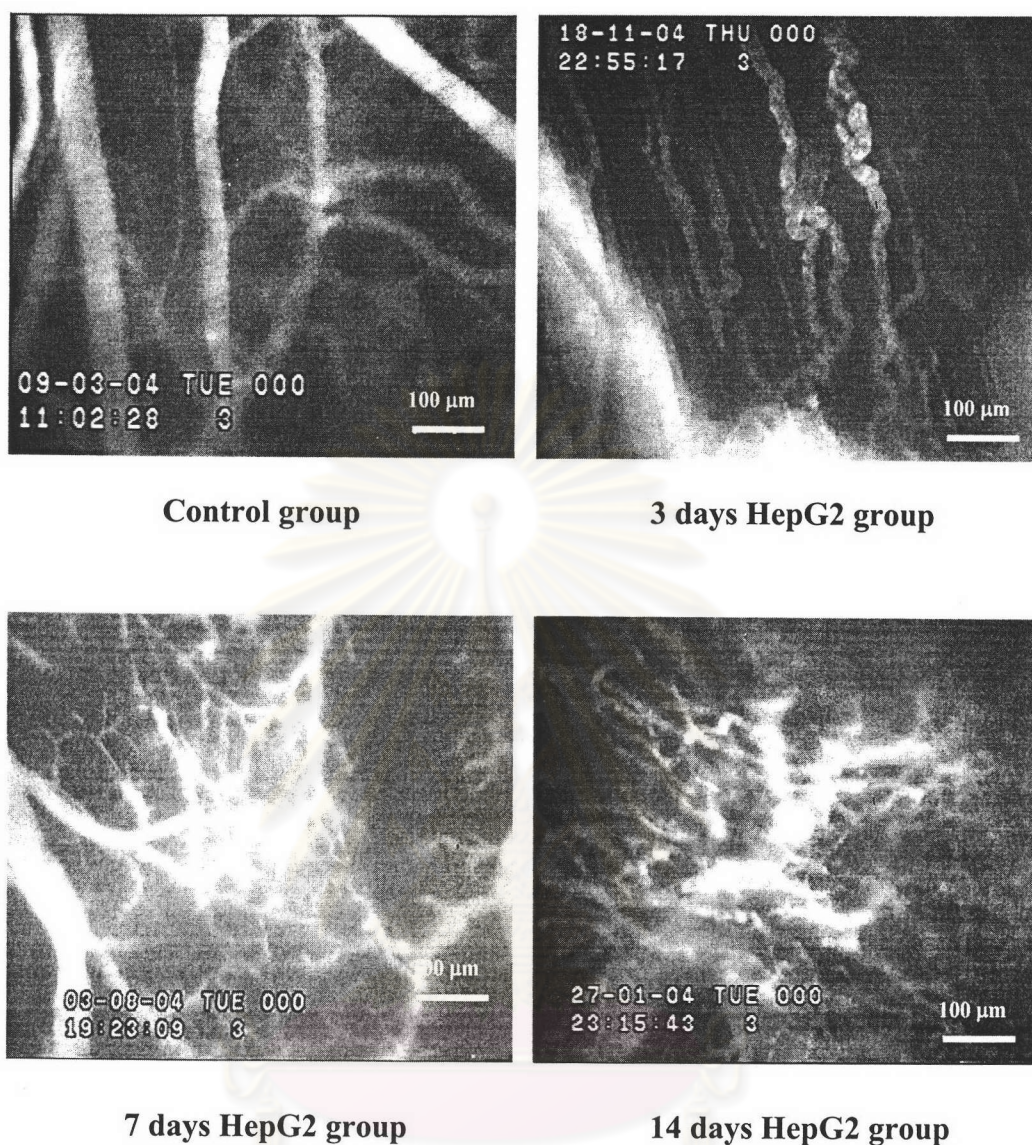


Figure 4.6. Fluorescence videomicrographs of neocapillaries (contrast-enhanced by RITC-extran) induced in the dorsal skin-fold chamber on day 0 (Control), 3, 7 and 14 after the implantation of tumor cells. Bar, 100 μm .

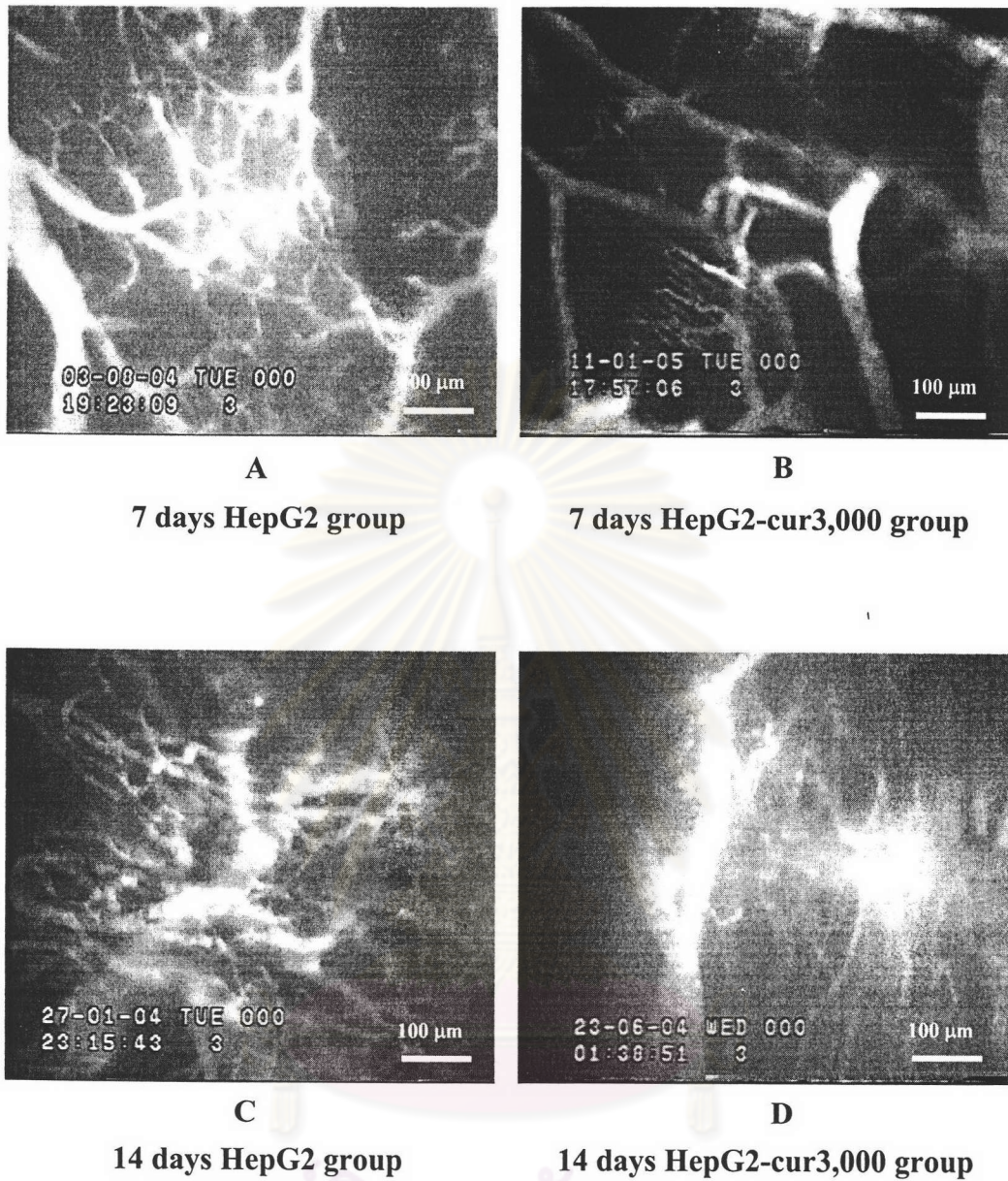
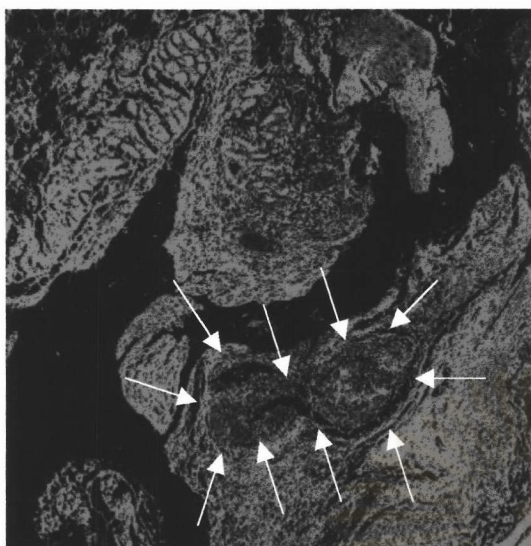
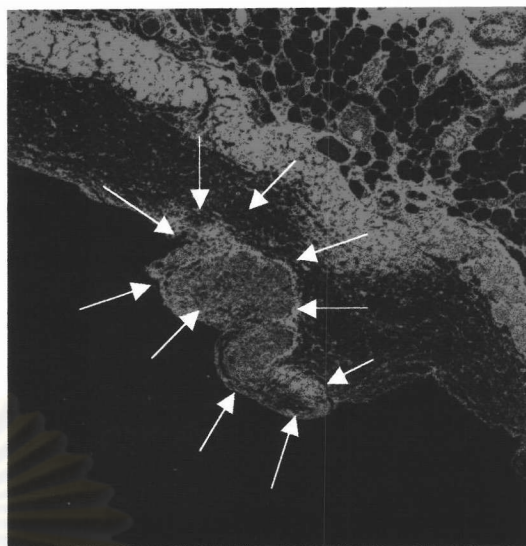


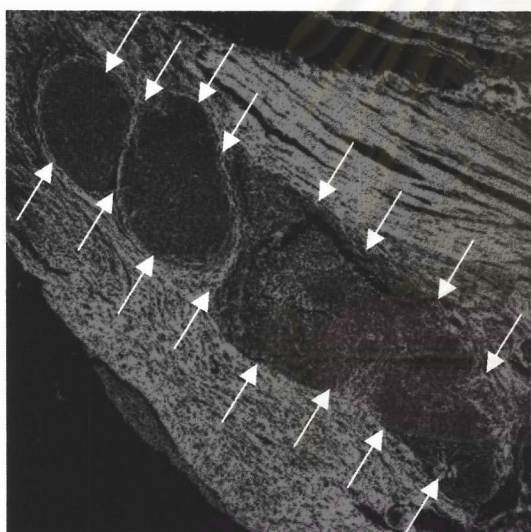
Figure 4.7. Fluorescence videoimages of neocapillaries on 7 and 14 days after the implantation of tumor cells without and with treatment of curcumin (3,000 mg/kgBW) for HepG2 (A, B) and HepG2-cur3,000 groups (C, D). Bar, 100 µm.



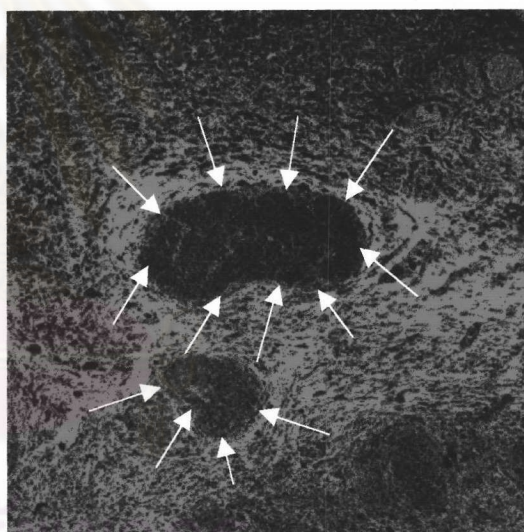
7 days HepG2 group



7 days HepG2-cur- 3,000 group



14 days HepG2 group



14 days HepG2-cur-3,000 group

Figure 4.8. Confocal laser image of an area of tissue in the dorsal skin-fold chamber (stained with hematoxylin-eosin) in 7 and 14 days HepG2 groups with and without treatment of curcumin

Table 4.3. Capillary density (%) of the tumor tissue in the dorsal skin-fold chamber on 3, 7 and 14 days after the implantation of HepG2 with and without curcumin treatment (mean \pm SEM, all n=5).

Groups	Capillary densities (%)		
	3 days	7 days	14 days
Con	28.57 \pm 1.83	29.96 \pm 0.82	27.41 \pm 0.91
Con-cur300	28.43 \pm 0.86	31.63 \pm 1.00	28.39 \pm 1.19
Con-cur3,000	28.98 \pm 1.87	28.49 \pm 0.73	27.36 \pm 0.83
HepG2	33.57 \pm 1.28	51.67 \pm 4.26 ^{*,+}	69.18 \pm 2.41 ^{a,++,b}
HepG2-cur300	31.38 \pm 1.62	48.19 \pm 0.78 ^{ns}	59.86 \pm 2.87 ^{NS}
HepG2-cur3,000	30.54 \pm 1.15	39.88 \pm 1.61 ^{#, @}	42.91 \pm 1.76 ^{c,d}

* P < 0.001 significant difference compared to 7 days Con.

+P < 0.01 significant difference compared to 3 days HepG2.

++P < 0.001 significant difference compared to 3 days HepG2.

ns no significant difference compared to 7 days HepG2.

NS no significant difference compared to 14 days HepG2.

#P < 0.01 significant difference compared to 7 days HepG2.

@P < 0.05 significant difference compared to 7 days HepG2-cur300.

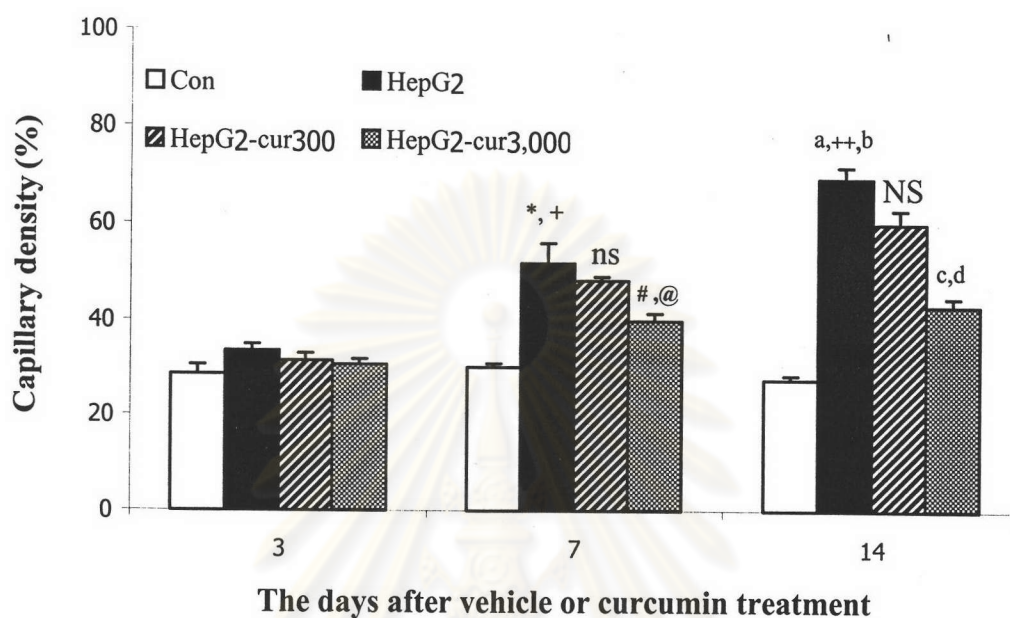
^aP < 0.001 significant difference compared to 14 days Con.

^bP < 0.01 significant difference compared to 7 days HepG2.

^cP < 0.05 significant difference compared to 14 days HepG2.

^dP < 0.01 significant difference compared to 14 days HepG2-cur300.

Figure 4.9. Capillary density (%) of the tumor tissue in the dorsal skin-fold chamber on 3, 7 and 14 days after the implantation of HepG2 with and without curcumin treatment (mean \pm SEM, all n=5).



*P < 0.001 significant difference compared to 7 days Con.

⁺P < 0.01 significant difference compared to 3 days HepG2.

⁺⁺P < 0.001 significant difference compared to 3 days HepG2.

ns no significant difference compared to 7 days HepG2.

NS no significant difference compared to 14 days HepG2.

[#]P < 0.01 significant difference compared to 7 days HepG2.

[@]P < 0.05 significant difference compared to 7 days HepG2-cur300.

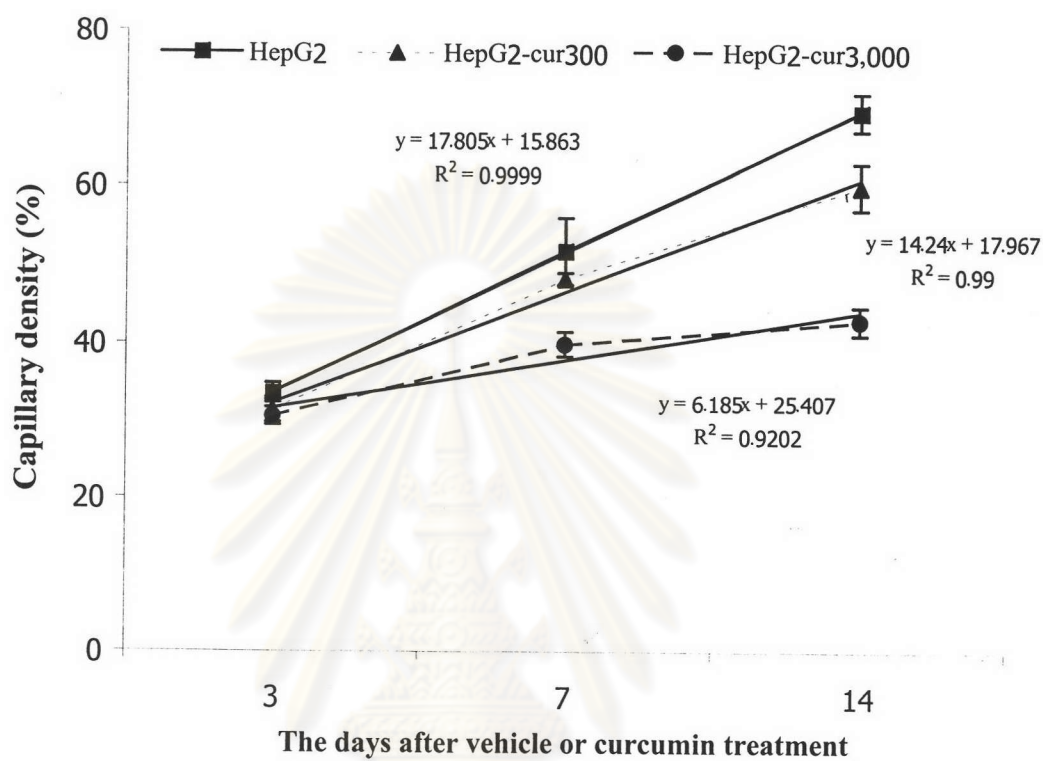
^aP < 0.001 significant difference compared to 14 days Con.

^bP < 0.01 significant difference compared to 7 days HepG2.

^cP < 0.05 significant difference compared to 14 days HepG2.

^dP < 0.01 significant difference compared to 14 days HepG2-cur300.

Figure 4.10. Relationship between capillary density (mean \pm SEM) and time courses of vehicle or curcumin treatment in HepG2, HepG2-cur300, and HepG2-cur3,000 groups.



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Table 4.4. Capillary diameter (μm) of the tumor tissue in the dorsal skin-fold chamber on 3, 7 and 14 days after the implantation of HepG2 with and without curcumin treatment (mean \pm SEM, all n=5).

Groups	Capillary diameter (μm)		
	3 days	7 days	14 days
Con	7.51 \pm 0.33	7.33 \pm 0.50	7.50 \pm 0.50
Con-cur300	7.09 \pm 0.56	7.62 \pm 0.26	7.26 \pm 0.34
Con-cur3,000	6.84 \pm 0.49	7.19 \pm 0.35	7.27 \pm 0.53
HepG2	8.84 \pm 1.30	10.32 \pm 0.41 [*]	10.89 \pm 0.65 ^a
HepG2-cur300	8.59 \pm 0.43	8.87 \pm 0.52	8.77 \pm 0.57 ^b
HepG2-cur3,000	7.61 \pm 0.42	7.70 \pm 0.36 [#]	8.47 \pm 0.29 ^c

^{*}P < 0.01 significant difference compared to 7 days Con.

[#]P < 0.05 significant difference compared to 7 days HepG2.

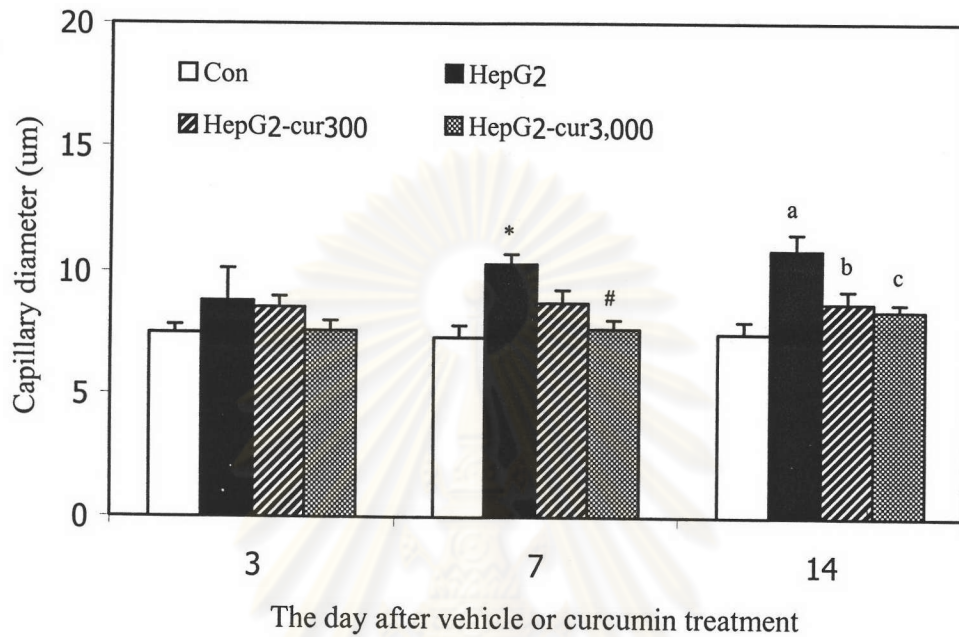
^aP < 0.01 significant difference compared to 14 days Con.

^bP < 0.05 significant difference compared to 14 days HepG2.

^cP < 0.01 significant difference compared to 14 days HepG2.

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Figure 4.11. Capillary diameter (μm) of the tumor tissue in the dorsal skin-fold chamber on 3, 7 and 14 days after the implantation of HepG2 with and without curcumin treatment (mean \pm SEM, all $n=5$).



- *P < 0.01 significant difference compared to 7 days Con.
 #P < 0.05 significant difference compared to 7 days HepG2.
^aP < 0.01 significant difference compared to 14 days Con.
^bP < 0.05 significant difference compared to 14 days HepG2.
^cP < 0.01 significant difference compared to 14 days HepG2.

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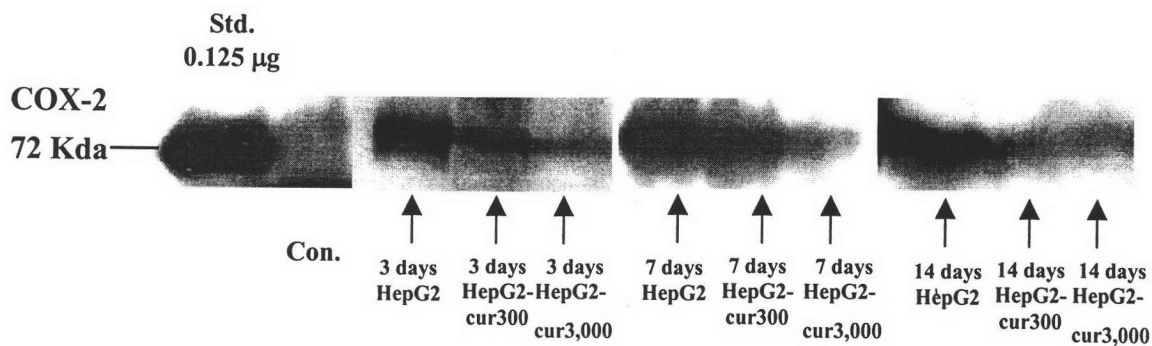


Figure 4.12. Western blot analysis of COX-2 protein. Expression of COX-2 in HepG2 groups after 3, 7 and 14 days with and without curcumin treatment. Standard COX-2 control (0.125 µg).

Table 4.5A. COX-2 protein levels by using image analysis (Global LabII software) measurement in 3 days HepG2 group with and without curcumin treatment.

(mean \pm SEM).

Groups n	COX-2 protein level ($\mu\text{g}/10 \mu\text{g}$ of total protein)		
	3 days HepG2	3 days HepG2- cur300	3 days HepG2- cur3,000
1	0.104	0.055	0.039
2	0.090	0.041	0.060
3	0.079	0.052	0.049
4	0.148	0.107	0.045
Mean	0.1053	0.0638*	0.0483⁺
SE	0.0151	0.0147	0.0044

*P < 0.05 significant difference compared to 3 days HepG2

⁺P < 0.001 significant difference compared to 3 days HepG2.

Each sample pooled all 5 specimens.

Table 4.5B. COX-2 protein levels by using image analysis (Global LabII software) measurement in 7 days HepG2 group with and without curcumin treatment.

(mean \pm SEM).

Groups n	COX-2 protein levels ($\mu\text{g}/10\mu\text{g}$ of total protein)		
	7 days HepG2	7 days HepG2- cur300	7 days HepG2- cur3,000
1	0.098	0.046	0.025
2	0.101	0.081	0.045
3	0.096	0.084	0.065
4	0.128	0.115	0.099
Mean	0.1058	0.0765	0.0585[#]
SE	0.0075	0.0150	0.0158

[#]P < 0.005 significant difference compared to 7 days HepG2.

Each sample pooled all 5 specimens.

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Table 4.5C. COX-2 protein levels by using image analysis (Global LabII software) measurement in 14 days HepG2 group with and without curcumin treatment. (mean \pm SEM).

Groups n	COX-2 protein levels ($\mu\text{g}/10\mu\text{g}$ of total protein)		
	14 days HepG2	14 days HepG2 -cur300	14 days HepG2- cur3,000
1	0.121	0.085	0.068
2	0.094	0.045	0.030
3	0.087	0.062	0.046
4	0.125	0.072	0.054
Mean	0.1068	0.0610^a	0.0445^b
SE	0.0095	0.0057	0.0051

^aP < 0.01 significant difference compared to 14 days HepG2.

^bP < 0.001 significant difference compared to 14 days HepG2.

Each sample pooled all 5 specimens.

Table 4.6. Comparisons of COX-2 protein levels by using image analysis (Global LabII software) measurement in 3, 7, and 14 days HepG2 group with and without curcumin treatment. (mean \pm SEM).

Group	COX-2 protein level ($\mu\text{g}/10\mu\text{g}$ of total protein)		
	3 days	7 days	14 days
HepG2	0.1053 \pm 0.0151	0.1058 \pm 0.0075	0.1068 \pm 0.0095
HepG2-cur300	0.0638 \pm 0.0147 ⁺	0.0765 \pm 0.0150	0.0610 \pm 0.0057 ^a
HepG2-cur3,000	0.0483 \pm 0.0044 [*]	0.0585 \pm 0.0158 [#]	0.0450 \pm 0.0051 ^a

⁺P < 0.05 significant difference compared to 3 days HepG2

^{*}P < 0.001 significant difference compared to 3 days HepG2.

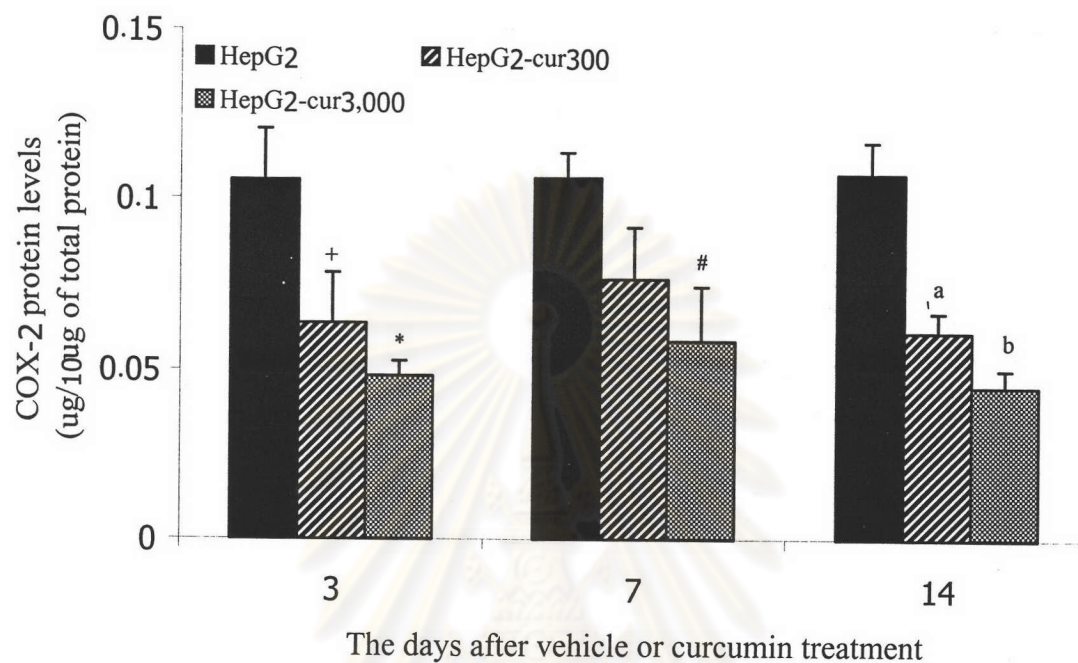
[#]P < 0.005 significant difference compared to 7 days HepG2.

^aP < 0.01 significant difference compared to 14 days HepG2.

^bP < 0.001 significant difference compared to 14 days HepG2.

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Figure 4.13. Comparisons of COX-2 protein levels by using image analysis (Global LabII software) measurement in 3, 7, and 14 days HepG2 group with and without curcumin treatment. (mean \pm SEM).



⁺P < 0.05 significant difference compared to 3 days HepG2

^{*}P < 0.001 significant difference compared to 3 days HepG2.

[#]P < 0.005 significant difference compared to 7 days HepG2.

^aP < 0.01 significant difference compared to 14 days HepG2.

^bP < 0.001 significant difference compared to 14 days HepG2.

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Figure 4.14. Relationship between COX-2 protein levels (mean±SEM) and time courses of vehicle or curcumin treatment in HepG2, HepG2-cur300, and HepG2-cur3,000 groups.

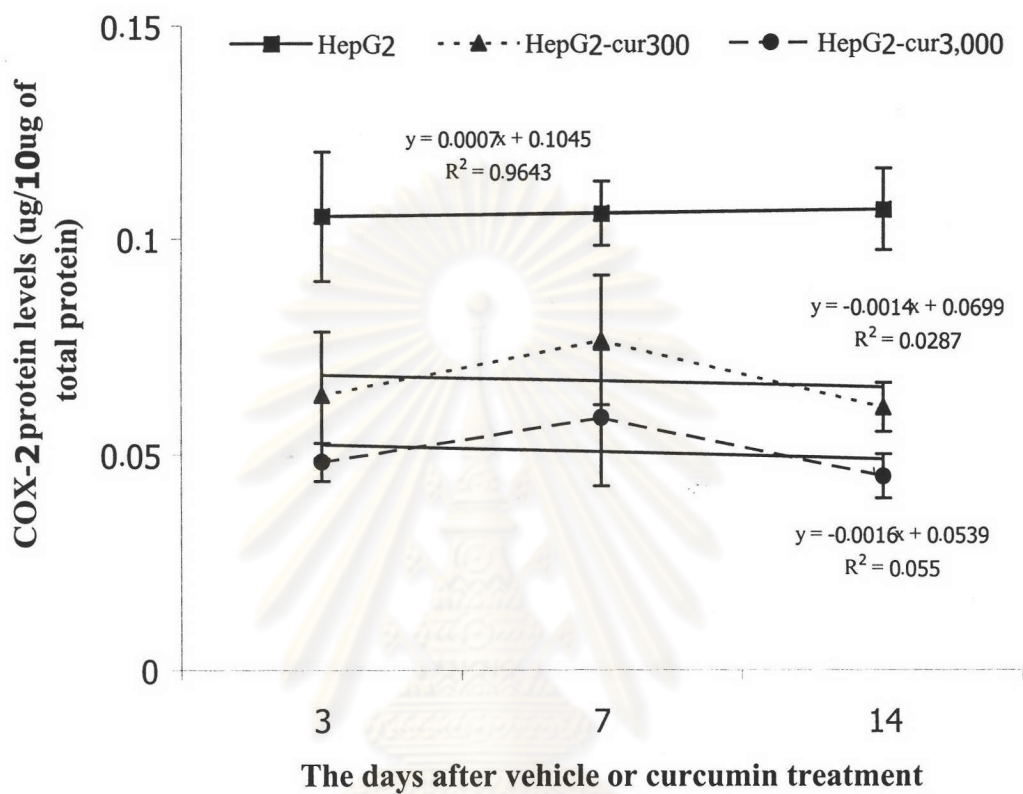


Table 4.7. Serum VEGF (ng/ml) of control and HepG2 groups after 3, 7 and 14 days with and without curcumin treatment (mean \pm SEM, all n=5).

Groups	Serum VEGF (ng/ml)		
	3 days	7 days	14 days
Con	7.20 \pm 0.75	6.38 \pm 0.68	6.53 \pm 1.24
Con-cur300	7.31 \pm 0.55	7.62 \pm 1.81	5.58 \pm 0.71
Con-cur3,000	7.79 \pm 1.16	5.46 \pm 0.89	6.69 \pm 1.79
HepG2	16.80 \pm 2.43	49.52 \pm 5.18 ^{*,+}	49.96 \pm 5.74 ^{a,+}
HepG2-cur300	10.85 \pm 1.55	23.43 \pm 4.92 [#]	31.30 \pm 7.84 ^b
HepG2-cur3,000	10.16 \pm 1.00	18.56 \pm 3.34 [#]	18.18 \pm 2.76 ^{c,d}

*P < 0.001 significant difference compared to 7 days Con.

+P < 0.001 significant difference compared to 3 days HepG2.

#P < 0.001 significant difference compared to 7 days HepG2.

^aP < 0.001 significant difference compared to 14 days Con.

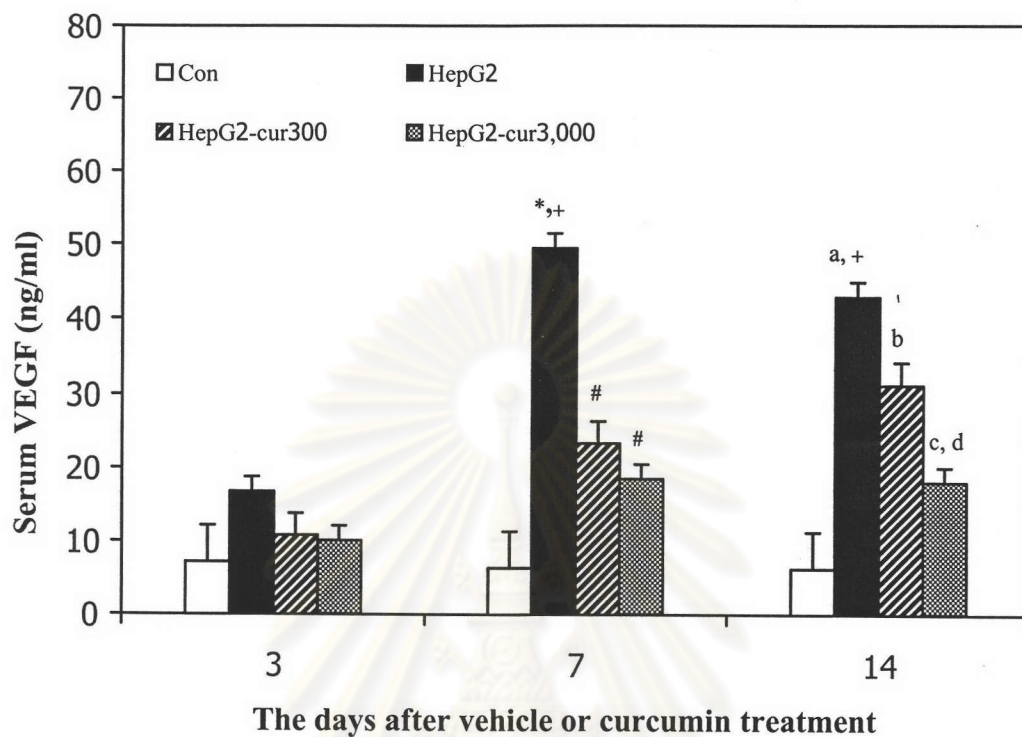
^bP < 0.01 significant difference compared to 14 days HepG2.

^cP < 0.001 significant difference compared to 14 days HepG2.

^dP < 0.01 significant difference compared to 14 days HepG2-cur300.

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Figure 4.15. Serum VEGF (ng/ml) of control and HepG2 groups after 3, 7 and 14 days with and without curcumin treatment (mean \pm SEM, all n=5).



*P < 0.001 significant difference compared to 7 days Con.

⁺P < 0.001 significant difference compared to 3 days HepG2.

[#]P < 0.001 significant difference compared to 7 days HepG2.

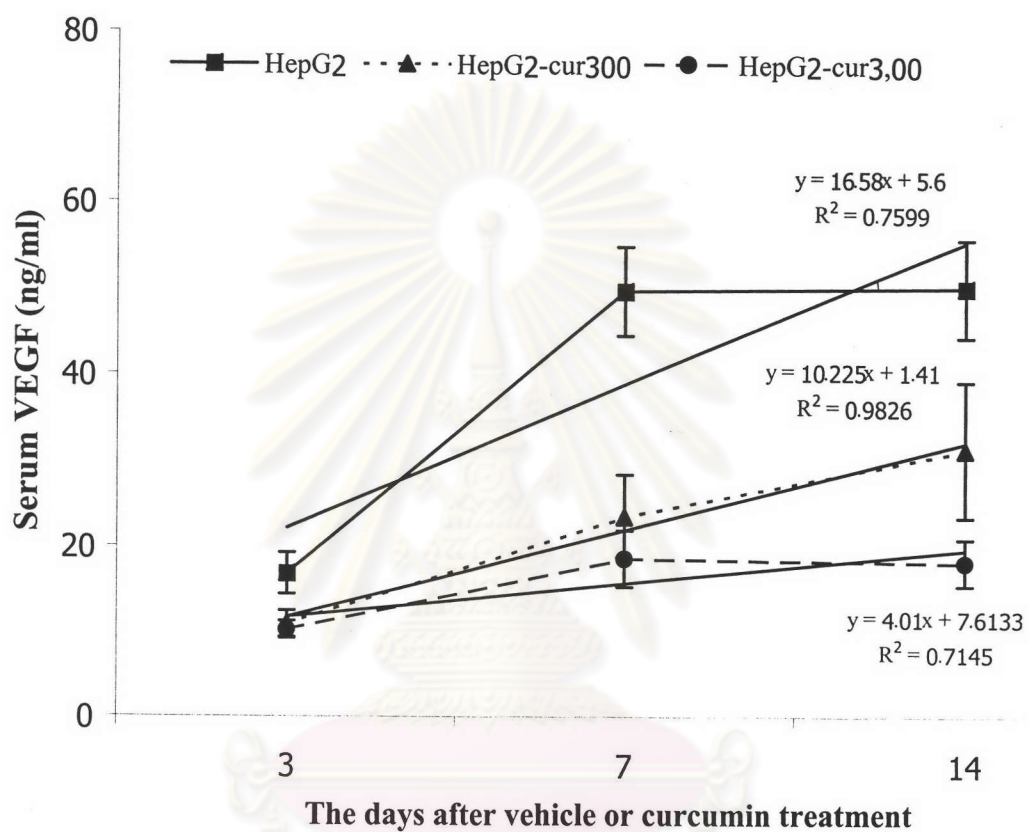
^aP < 0.001 significant difference compared to 14 days Con.

^bP < 0.01 significant difference compared to 14 days HepG2.

^cP < 0.001 significant difference compared to 14 days HepG2.

^dP < 0.01 significant difference compared to 14 days HepG2-cur300.

Figure 4.16. Relationship between serum VEGF (mean±SEM) and time courses of vehicle or curcumin treatment in HepG2, HepG2-cur300, and HepG2-cur3,000 groups.



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Table 4.8. Relationship between capillary density (mean±SEM) and COX-2 protein levels of HepG2 groups (mean±SEM) during any periods.

Parameters	The days after inoculation		
	3	7	14
Capillary density (%)	33.57±1.28	51.67±4.26	69.18±2.41
COX-2 protein levels (µg/10µg of total protein)	0.1053±0.0151	0.1058±0.0075	0.1068±0.0095

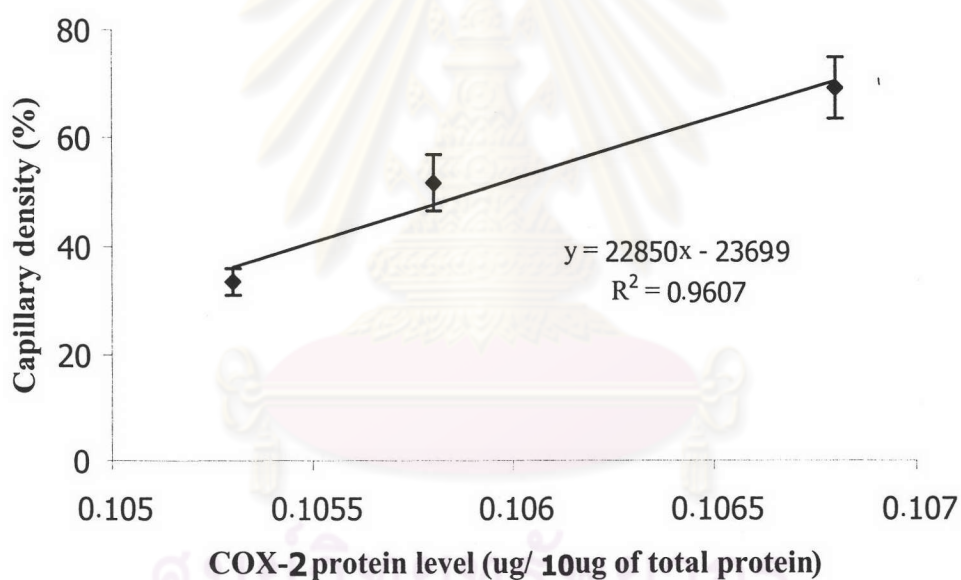


Figure 4.17. Relationship between capillary density and COX-2 protein levels of HepG2 groups during any periods.

Table 4.9. Relationship between capillary density (mean±SEM) and serum VEGF levels of HepG2 groups (mean±SEM) during any periods.

Parameters	The days after inoculation		
	3	7	14
Capillary density (%)	33.57±1.28	51.67±4.26	69.18±2.41
Serum VEGF levels (ng/ml)	16.80±2.43	49.52±5.18	49.96±5.74

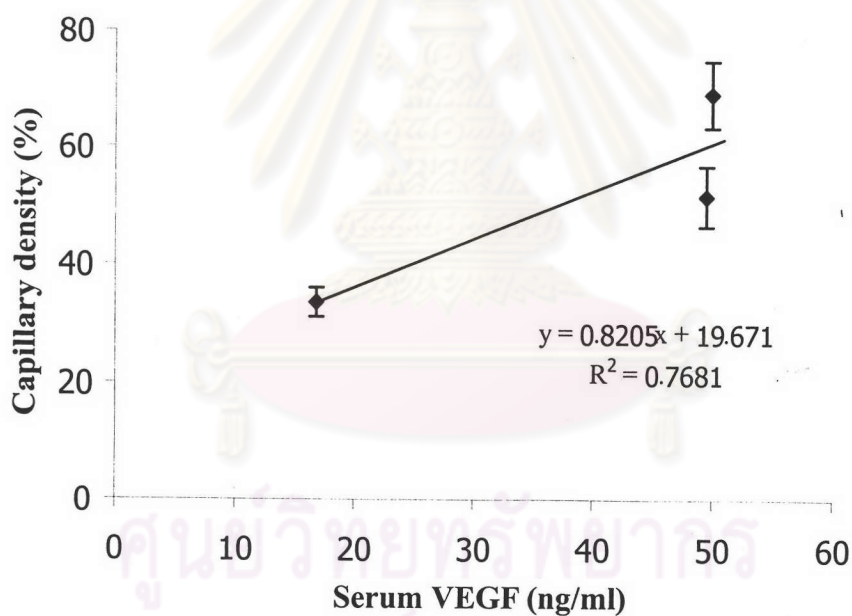


Figure 4.18. Relationship between capillary density and serum VEGF levels of HepG2 groups during any periods.

Table 4.10. Relationship between COX-2 protein levels (μg) (mean \pm SEM) and serum VEGF levels of HepG2 groups (mean \pm SEM) during any periods.

Parameters	The days after inoculation		
	3	7	14
COX-2 protein levels ($\mu\text{g}/10\mu\text{g}$ of total protein)	0.1053 \pm 0.0151	0.1058 \pm 0.0075	0.1068 \pm 0.0095
Serum VEGF levels (ng/ml)	16.80 \pm 2.43	49.52 \pm 5.18	49.96 \pm 5.74

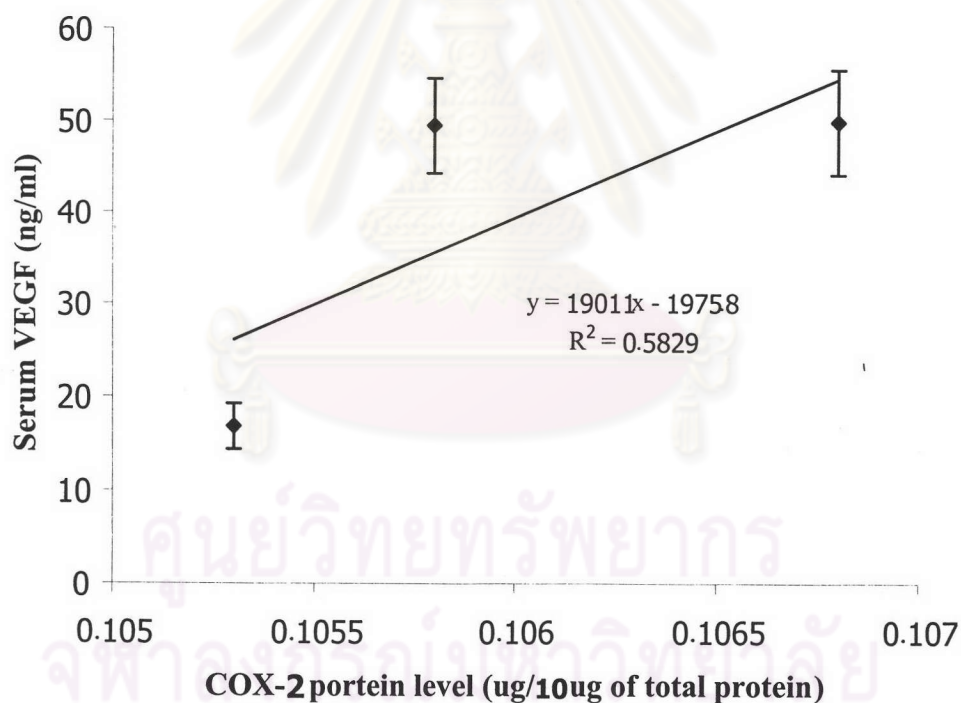


Figure 4.19. Relationship between COX-2 protein levels ($\mu\text{g}/10\mu\text{g}$ of total protein) and serum VEGF levels during any periods.