L-15 Antithrombotic and Antimetastatic Substance from Some Medicinal Plants

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The studies for antithrombotic substances from medicinal plants in my laboratory were started from the studies on PAF-antagonistic substances from Korean medicinal plants.

The screening studies of PAF-receptor binding antagonistic activity were conducted on the extracts of 300 Korean medicinal plants, 37 tropical medicinal plants, 20 mushrooms, and 30 vegetables. From the results of screening studies, it was possible to select two Korean medicinal plants, i.e. 1) the leaf of Biota orientalis and 2) the seed of Arctium lappa, and two tropical medicinal plants, i.e. 3) the rhizome of Alpinia officinarum and 4) the leaf of Ardisia crispa as the candidates for the activity guided isolation of PAF-antagonistic substances.

The potent PASF-antagonistic substances could be successfully isolated from all candidate plants mentioned above by activity guided isolation prodcess;

Compound-Vl, $C_{21}H_{30}O_4$, mp. 82-3°C IC_{50} ; 2.56×10^{-7}M from the methanol extract of Biota orientalis ,

Compound-X, $C_{21}H_{24}O_6$, mp. 96-8 C ,IC_{50} ; 5.0×10^{-6}M from the methanol extract of Arctium lappa,

Compound-A, .$C_{21}H_{26}O_4$, oily, IC_{50} ; 4.5×10^{-6}M from the methanol extract of Alpinia officinarum, and

Compund AC7-1 ,$C_{20}H_{32}O_3$, mp. 78-80 C IC_{50} ;5.0×10^{-7}M from the methanol extract of Ardisia crispa.

Chemical structures of four PAF-antagonists were established by spectro-chemical analysis as followings;

1) Compound-Vl from Biota orientalis was established to be pinusolide , a known labdane type diterpene-lactone,

2) Compound-X from Arctium lappa was established as arctigenin an α-.β.-disubstituted lactonic lignan,

3) Compound-A from Alpinia officinarum was established as diaryl-heptanoid and

4) Compound AC7-1 from Ardisia crispa was established as a *kind of quinonoid compound.*

The chemical structures of above four potent PAF antagonists showed completely different natures of chemical skeleton. .However, when we compare the structures of pinusolide and arctigenin we can easily find that both compounds are lactonic derivatives, i.e. pinusolide is α–monosubstituted lactone and

arctigenin is α-,β-disubstituted lactone. Some studies on structure activty analysis were conducted by modification of major functional groups in the pinusolide and arctigenin. The results of SAR-analysis showed followings; 1) Lactonic ring group is essential for the PAF-antagonistic activity of pinusolide and arctigenin, 2) α-.substituent on lactone ring is more essential compared to β-substituent and 3) the lipophilicity of α-substituent is better than hydrophillic group for the PAF-antagonistic activity. 4) Chiral carbons in the bicyclo-terpenoid substituent on pinusolide may not be essential for the PAF-antagonistic activity of pinusolide.

Based on this SAR-studies, somewhat simplified new PAF-antagonistic substances could be deviced. In this approach aromatic-rings having various combination of methoxy or hydroxy substituents on 2.3.4-positions were linked to α-position of lactone via various length of methylene group. Once again the structure activity analysis on the simplified synthetic substances were conducted and it was found that maximum PAF antagonistic activity was found from the compound having four methylene group and two methoxyl group on aromatic ring.

We studied the effects of the above mentioned PAF-antagonists on the rabbit platelet aggregation in order to clarify whether the above mentioned PAF antagonists are antagonist or agonist to the platelet aggregation. The results showed clearly that all four PAF-antagonists are also potent antagonists to platelet aggregation.

On the other hand, the platelet antiaggregatory activity of three PAF-antagonists, i.e. pinusolide, arctigenin and diarylheptanoids are responding very specifically to PAF induced aggregation only, however, the platelet antiaggrgatory activity of Ac7-1 is responding nonspecifically to all aggregations induced by collagen, thrombin PAF and ADP.

This nonspecific anti-aggregatory properties of AC7-1 drew our deep interests for further studies, since it may suggest indirectly that this compound may have integrin-binding antagonistic property.

It is well known facts that rabbit and human platelets show two-phase aggregation profile when the aggregation was induced by ADP, the first phase being aroused due to direct activation of integrine by conformational change of integrin and second phase being aroused by indirect activation of integrin through a series of biochemical reactions including degranulation, resorption and activation of cyclooxygenase mediated pathway leading to the production of thromboxane and finally activation of intergrine.

Based on this knowledge it was possible to develop a new simple assay method for the evaluation of integrine binding activity of AC7-1. The essential part of our new assay method for integrin

binding activity is assaying the ADP-induced platelet aggregation under the presence of potent cyclooxygenase inhibitor indomethacin. Under the presence of indomethacin the secondary phase of ADP-induced aggregation of platelet was completely disappeared and the first phase aggregation remained intact. .This remaining first phase aggregation was again strongly inhibited by the presence of AC7-1, hence integrin antagonistic property of Ac7-1 was identified indirectly.

Ac7-1 was found at first as a potent PAF-antagonist, but it is now clear that AC7-1 has strong integrin antagonistic property(IC_{50}.; 6.4 x 10^{-6}M) too ..The integrin is cell surface receptor which is present on all cell membranes, and which play a majour role in the various cell-cell interactions and cell-ECM interactions such as in platelet aggregation and cancer cell metastasis etc.

Based on this backgrounds, our research interest was suddenly transfered from studies on antithrombotic substances to anti-metastatic substances of cancer cell. It is well known facts that all biological phenomena concerned with cancer cell metastasis such as penetration of cancer cell through ECM, angiogenesis and proliferation are started in consequence of cancer cell attachment to extra-cellular matrix of organs of remote place. Therefore we conducted an experiment to see whether Ac7-1 has inhibitory activity on the attachment of B16F10-melanoma cell to various extra-cellular matrix proteins.

The results was very exciting that AC7-1 showed very strong inhibitory effect to the ECM- binding of B16F10-melanoma cells, such as fetal bovine serum(IC_{50}; 1.9 x 10^{-5}M), collagen(IC_{50}; 3.1 x 10^{-5}M), laminin(IC_{50}; 3.3 x 10^{-5} M), fibronectin(IC_{50}; 5.0 x 10^{-7}M). We tested also the effect of Ac7-1 to the invasiveness of B16F10-melanoma cell through Matrigel coated filter membranes and found that Ac7-1 inhibited also the invasiveness of B16F10-melanoma cell(IC_{50}; 2.1 x 10^{-6}M).

In the proliferation test of B16F10-melanoma cell , AC7-1 inhibited completely the cell proliferation at the concentration of 10μg/ml, although it doesn't show any significant cyto-toxicity on MTT-test at the same or four times higher concentration of AC7-1.

Based on this preliminary observations by in vitro experiments on melanoma cell we could arrive to a conclusion that Ac7-1 may have antimetastatic activity.

Therefore, we examined the in vivo anti-metastatic activity of AC7-1 by injection of B16F10-melanoma cell and AC7-1 to black mouse. When AC7-1 was injected intravenously in a dose of 5mg/kg mouse concomittantly with or before one minutes of i.v.-injection, of B16F10-melanoma cell , none of four animal showed cancerous black spots in lung tissue when the animals were tested by autopsy after two weeks.

We tested also the effects of AC7-1 on cancer cell metastasis by the experimental model in which AC7-1 was administered after the subcutaneous injection of B16F10 melanoma cells.

In the first experimental group, Ac7-1 was administered intraperitoneally for 15 days with daily dosage of 5mg/kg mouse starting one day after the subcutaneous injection of B16F10 melanoma cells. After 15 days we found a surprisng results. In the experimental group only three mouse out of five animal showed solid tumor, whereas five mice out of six in the control group showed solid cancer. In the second experimental group AC7-1 was administered 5mg/kg doses starting two days after the melanoma cell injection and the results after 15 days was three mice out of eight mice were affected to solid tumor. In the third experimental group, Ac7-1 was administered with 10mg/kg doses in a same way as that of second group, the result showed that only two out of seven animal showed solid tumor. In the fourth experimental group the AC7-1 was injected starting ten days after the melanoma cell injection, and we found that five mice out of six mice were affected to solid tumor whereas all five mice of control group. produced solid tumor.

We are observing for long time whether the cancer cell unaffected animals due to AC7-1 administration continue healthy state after the cease of drug administration. At present , three month after the cessation of Ac7-1 administration , all unaffected animals are still alive in healthy, whereas all tumor affected animals have been died. It was difficult to obtain LD_{50}-value , since mice were healthy at the dose of 160mg/kg and the intravenous administration of Ac7-1 above this concentration was practically impossible due to solubility problem.

In conclusion we found a promising nonpeptide low molecular antimetastatic substance AC7-1 from tropical medicinal plant starting from the screening studies of PAF antagonistic activity of medicinal plants. This substance may be recommended as a candidate and also lead compound for the new drug development research.