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# Synthesis of Poly(maleimide-co-2-ethylacrylic Acid) and Its Properties of Suppressing Metastasis and Growth of Carcinoma

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**Abstract**—A series of copolymers is prepared using maleimide and 2-ethylacrylic acid as comonomer. This kind of copolymer shows low toxicity ($LD_{50}$: 601–798 mg/kg) and significant curative effect on Lewis lung carcinoma and S180.


It is well known that the metastasis of carcinoma is the main cause for high mortality of tumor patients. It was discovered that some compounds showed the ability to prevent the metastasis of carcinoma. For example, as an inhibitor of blood vessel regeneration, fenretimide 4-HPR can suppress the growing and metastasizing of prostate tumor of rats,[1] the other examples are razoxane and taxol, the former is able to affect and prevent the drop of tumor cell[2] from the organs, and the latter has the antimetastasizing efficiency for the melanoma B16-BL6 as high as 50%.[3] In addition, it was reported that some natural macromolecules, such as arabic semi-lactose and heparin have the ability to prevent the metastasis of carcinoma.[4,5]

Recently, the manmade polymers with the antitumor and antimetastasizing activities are attracted by scientists all over the world. Compared with the small molecular drugs, the polymeric drugs have the features of low toxicity, long half-life and wide suitability for individual difference.[6,7] Maleimide (MI) and its derivatives are very useful monomers. Sakurai et al.[8] reported that MI and its *N*-substituted derivatives have physiological activity, they can inhibits the growth of chick fibroblasts in tissue culture at a concentration of $10^{-3}$ mol/L. Augustin et al.[9] found that *N*-*m*-chlorophenylmaleimide and *N*-*o*-chlorophenylmaleimide can be used as bactericides. In addition, some poly(acrylic acid) also showed excellent curative effect against carcinoma. For instance, poly(methacrylic acid) exhibit significant inhibition of Sindbis and vesicular viruses,[10] and poly(ethacrylic acid) is much effective against Lewis lung carcinoma.[11]

Based on these facts, recently, our research group designed and prepared a series of copolymers containing imino-ring and 2-substituted acrylic acid units. Investigation was also carried out on their properties of suppressing metastasis and growth of carcinoma.

## Chemistry

According to our previous work,[12,13] the copolymerization of MI with 2-ethylacrylic acid (EAA) was carried out in dioxane at 60 °C initiated by AIBN (Scheme 1). By means of evaluating the monomer reactivity ratios $r_1$ and $r_2$ of the monomers MI and EAA, respectively, it was found that the copolymerization of MI with EAA showed a strong alternating tendency by the formation of contact CTC (charge transfer complex). The products with different composition could be obtained by changing the concentration of initiator, the reaction time and feed ratios of MI and EAA as we mentioned previously. The copolymer compositions under different conditions were determined by elemental analysis, which is listed in Table 1.

The homopolymers poly (2-ethylacrylic acid) (PEAA) was prepared by bulk polymerization of EAA using 2,2′-azobisisobutyronitrile as initiator at 60 °C,[14] the yield was 20% and molecular weight is about 11,000; and polymaleimide (PMI) was obtained using benzoyl

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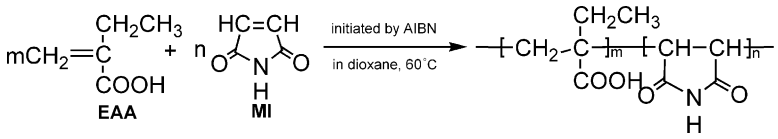


**Scheme 1.**

peroxide as initiator and chlorobenzene as solvent at 90°C,[15] the yield is about 90% and molecular weight is about 22,300. All characterization data are the same as the literatures reported.

**Table 1.** Copolymerization data of MI ($M_1$) with EAA ($M_2$)[a]

| Run no. | Copolymer composition MI (mol%) | $[\eta]$[b] ($cm^3/g$) |
|---|---|---|
| **1** | 63.8 | 19.2 |
| **2** | 26.2 | 22.4 |
| **3** | 50.0 | 31.2 |
| **4** | 65.4 | 21.9 |
| **5** | 23.0 | 31.8 |
| **6** | 24.0 | 21.9 |

[a]Experiment was carried out in dioxane using AIBN as initiator at 60 °C.
[b]Intrinsic viscosity $[\eta]$ in DMF at 30 °C.

## Results and Discussion

### Lewis lung carcinoma[16]

The effect of these copolymers on Lewis lung carcinoma planted in mice was tested.[17] The copolymers **1**–**6** were evaluated in each assay using a consecutive (qd×7) schedule, and the parameters: inhibition against Lewis lung carcinoma metastasis and that of growth were used to assess their efficacy. As Table 2 showed, whether low or high dose for these copolymers was used, the inhibition against Lewis lung carcinoma metastasis is more than 40%. Meanwhile, these copolymers could also significantly inhibit the growth of Lewis lung carcinoma cells, and most of the inhibition are greater than 30%. These data established that copolymers containing more EAA in polymer chains as **2**, **5** and **6** are more effective than **1**, **3** and **4** with less EAA shown in Table 2. For example, when mice were treated at the dose of 1/10

**Table 2.** In vivo activities of copolymers against Lewis lung carcinoma

| Run no. | Dose (mg/kg) | Wt change[a] (%) | Metastatic colonies number ($X\pm$SD) | Metastasis inhibition[b] (%) | Tumor WT ($X\pm$SD) (g) | Growth inhibition[c] (%) |
|---|---|---|---|---|---|---|
| **1**[d] | 80 | +9.4 | 2.33±2.49 | 66.99 | 0.502±0.12[d] | 37.01 |
| **1**[e] | 80 | +18 | 2.87±3.13 | 59.35 | 0.520±0.07[e] | 34.09 |
| **1**[d] | 16 | +11 | 2.88±2.36 | 59.21 | 0.565±0.11[d] | 29.11 |
| **1**[e] | 16 | +22 | 3.50±2.97 | 50.42 | 0.570±0.09[e] | 27.76 |
| **2**[d] | 64 | +6.1 | 2.00±2.06 | 71.67 | 0.462±0.07[d] | 42.03 |
| **2**[e] | 64 | +21 | 2.62±2.38 | 62.89 | 0.491±0.11[e] | 37.77 |
| **2**[d] | 12.8 | +11 | 3.85±2.47 | 45.46 | 0.526±0.09[d] | 34.00 |
| **2**[e] | 12.8 | +23 | 4.00±2.26 | 43.34 | 0.550±0.09[e] | 30.29 |
| **3**[d] | 71.5 | +8.7 | 2.21±2.55 | 68.72 | 0.492±0.02[d] | 40.33 |
| **3**[e] | 71.5 | +16 | 2.79±2.86 | 60.55 | 0.505±0.12[e] | 38.35 |
| **3**[d] | 14.3 | +10.5 | 2.91±2.73 | 58.73 | 0.559±0.12[d] | 30.90 |
| **3**[e] | 14.3 | +20 | 3.15±2.04 | 55.39 | 0.586±0.07[e] | 30.82 |
| **4**[d] | 78.4 | +9.8 | 2.73±2.95 | 61.28 | 0.512±0.14[d] | 35.76 |
| **4**[e] | 78.4 | +19.2 | 3.14±2.62 | 55.51 | 0.502±0.12[e] | 37.01 |
| **4**[d] | 15.7 | +12 | 3.18±2.90 | 54.94 | 0.567±0.08[d] | 28.85 |
| **4**[e] | 15.7 | +25 | 3.47±3.08 | 50.87 | 0.573±0.07[e] | 28.10 |
| **5**[d] | 62.6 | +5.9 | 2.10±2.65 | 70.22 | 0.458±0.11[d] | 42.57 |
| **5**[e] | 62.6 | +20 | 2.18±2.11 | 69.10 | 0.490±0.04[e] | 38.51 |
| **5**[d] | 12.5 | +10 | 3.48±2.83 | 50.74 | 0.512±0.09[d] | 35.78 |
| **5**[e] | 12.5 | +21 | 3.37±2.42 | 52.33 | 0.530±0.09[e] | 33.46 |
| **6**[d] | 60 | +6.2 | 2.03±2.39 | 71.28 | 0.462±0.05[e] | 41.99 |
| **6**[e] | 60 | +23 | 2.11±2.63 | 70.05 | 0.484±0.13[e] | 39.28 |
| **6**[d] | 12 | +12 | 3.41±3.12 | 51.62 | 0.529±0.12[e] | 33.63 |
| **6**[e] | 12 | +22 | 3.70±2.76 | 47.58 | 0.563±0.07[e] | 29.35 |
| PMI | 101 | +28 | 5.68±3.49 | 19.55 | 0.693±0.04[d] | 13.05 |
| PEAA | 31 | +7.2 | 2.53±2.63 | 64.16 | 0.288±0.09[d] | 38.77 |
| Control[d] | Solv.[f] | +13 | 7.06±3.33 | | 0.797±0.12 | |
| Control[e] | Solv.[f] | +25 | 7.06±4.58 | | 0.789±0.102 | |

[a]Percent weight change on day 8.
[b]Inhibition against tumor metastasis = 100%×[(mean metastatic $nodes_{control}$−mean metastatic $nodes_{treated}$)/mean metastatic $nodes_{control}$].
[c]Inhibition against tumor growth = 100%×[(mean foot pad $weight_{control}$−mean foot pad $weight_{treated}$)/mean foot pad $weight_{control}$].
[d]This series use the same control group.
[e]This series use the same control group.
[f]Correspondent solvent.

**Table 3.** In vivo antitumor activity of copolymers against Solid sarcoma S180

| Run no. | Dose (mg/kg) | Wt change[a] (%) | Tumor WT ($X\pm$SD) (g) | Growth inhibition[b] (%) |
|---|---|---|---|---|
| **1**[c] | 80 | +22 | 1.77±0.42[e] | 37.89 |
| **1**[d] | 80 | +19 | 1.84±0.20[e] | 35.21 |
| **2**[c] | 64 | +23 | 1.73±0.28[e] | 39.30 |
| **2**[d] | 64 | +21 | 1.71±0.43[e] | 39.79 |
| **3**[c] | 30 | +13 | 1.56±0.26[e] | 30.04 |
| **3**[d] | 30 | +21 | 1.70±0.24[e] | 34.36 |
| **4**[c] | 100 | +23 | 1.69±0.17[f] | 24.22 |
| **4**[d] | 100 | +25 | 1.84±0.24[f] | 28.96 |
| **5**[c] | 100 | +21 | 1.37±0.19[e] | 38.57 |
| **5**[d] | 100 | +26 | 1.61±0.33[e] | 37.84 |
| **6**[c] | 100 | +22 | 1.70±0.16[f] | 40.35 |
| **6**[d] | 100 | +22 | 1.72±0.26[f] | 39.65 |
| PMI | 10 | +29 | 2.52±0.15[f] | 11.58 |
| PEAA | 30 | +16 | 1.29±0.19[f] | 54.73 |
| Control[c] | Solv.[g] | +31 | 2.85±0.37 | |
| Control[d] | Solv.[g] | +28 | 2.85±0.37 | |

[a]Percent weight change on day 8.
[b]Inhibition against tumor growth = 100% × [(mean tumor weight$_{control}$ − mean tumor weight$_{treated}$)/mean tumor weight$_{control}$].
[c]This series used the same control group.
[d]This series used the same control group.
[e]Compared with control group $P<0.01$.
[f]Compared with control group $P<0.05$.
[g]Correspondent solvent.

**Table 4.** In vivo antitumor activity of copolymers against Ascites sarcoma S180

| Drug | Dose (mg/kg) | Mean life span (day) | ILS[a] (%) |
|---|---|---|---|
| **1**[b] | 30 | 19.1±4.04[d] | 38.90 |
| **1**[c] | 30 | 18.5±3.4[d] | 34.55 |
| **2**[b] | 30 | 20.1±2.85[d] | 46.18 |
| **2**[c] | 30 | 19.1±3.58[d] | 38.90 |
| **3**[b] | 30 | 18.8±4.23[d] | 36.73 |
| **3**[c] | 30 | 17.3±3.1[d] | 34.11 |
| **4**[b] | 100 | 18.6±4.19[d] | 35.27 |
| **4**[c] | 100 | 17.7±2.8[d] | 37.21 |
| **5**[b] | 100 | 19.3±4.48[d] | 40.36 |
| **5**[c] | 100 | 18.4±2.7[d] | 42.61 |
| **6**[b] | 100 | 21.2±3.93[d] | 54.18 |
| **6**[c] | 100 | 19.3±2.9[d] | 49.61 |
| PMI | 100 | 14.5±4.16[d] | 5.45 |
| PEAA | 30 | 17.9±3.23[d] | 30.18 |
| Control[b] | Solv.[e] | 13.75±2.29 | |
| Control[c] | Solv.[e] | 12.9±2.0 | |

[a]Increasing in life span = 100% × [(mean life span$_{control}$ − mean life span$_{treated}$)/mean life span$_{control}$].
[b]This series used the same control group.
[c]This series used the same control group.
[d]Compared with control group $P<0.01$.
[e]Correspondent solvent.

$LD_{50}$, that is, 80 and 64 mg/kg for copolymer **1** and **2** were used, respectively, the growth inhibition of copolymer **1** was ranged from 34.09 to 37.01%, whereas that of for copolymer **2** was increased to 37.77–42.03%. In addition the metastasis inhibition rate from 59.35 to 66.99% also increased to 62.89–71.67%, respectively. In our experimental procedure the assay was carried out in the conditions of equal toxicity, and the data presented here showed that the copolymers with 23–26% MI has the optimum effect.

## Sarcoma S180[18,19]

The copolymers **1**–**6** were then evaluated for the growth inhibition against implanted sarcoma S180.[17] Two types of sarcoma S180, Solid sarcoma S180 and Ascites sarcoma S180, were chosen to assess our copolymers by using a consecutive (qd×7) schedule. Table 3 indicates that all the copolymers have desirable effect against Solid sarcoma S180, and the inhibition for copolymers **4** and **6** are nearly to 30%, and those for copolymers **1**, **2**, **3** and **5** are more than 30%. During the experiment, test of copolymers **1** and **2** was carried out on equal toxicity, so it is clear that copolymer **2** has better curative effect than **1**. Table 4 indicates that the survival time of the ascites sarcoma S180-bearing mice was prolonged by using copolymers. The increasing in life span (ILS) of these copolymers is all above 35%, and the growth of ascites sarcoma S180 was delayed. The data listed in Tables 3 and 4 shows that the maximum inhibition rate on solid sarcoma S180 for copolymers **2**, **5** and **6** are 39.79, 38.57 and 40.35%, respectively, meanwhile copolymer **6** has the preferential curative effect against ascites sarcoma S180, whose average ILS is more than 54%.

**Table 5.** $LD_{50}$ of test compounds (ip in mice)[a]

| Compd | $LD_{50}$ (mg kg)[b] |
|---|---|
| **1** | 798.17 |
| **2** | 636.17 |
| **3** | 715.23 |
| **4** | 784.79 |
| **5** | 626.32 |
| **6** | 601.58 |
| PMI | 1010.65 |
| PEAA | 316.41 |

[a]See References and Notes for details; 10 mice/group.
[b]$P<0.05$.

## Toxicity[20]

The acute toxicity of copolymers were investigated in mice and the results were listed in Table 5. Mice, both male and female, were treated with drug using a single dose. Both compounds showed some degree of dose-dependent toxicity. The mortality occurred mainly within 48 h, and the maximum appeared in 24 h. The autopsy was carried out on all organs, and no visible and significant morphologic lesions were found. The living mice all survived with normal activity after 2 weeks. In summary, copolymers **1** and **2** have low toxicity as we expected.

In our experiments, the homopolymers PMI and PEAA were used as positive control polymers. It was found that in all cases the more the contents of MI in copolymers, the higher the value of $LD_{50}$ and the lower the activity; in contrast, the more the contents of EAA in copolymers, the smaller the value of $LD_{50}$ and the higher the activity.

In summary, a series of copolymers with different contents and composition is prepared using maleimide and 2-ethylacrylic acid as comonomer. It was confirmed that all the copolymers showed low toxicity, the activities of antitumor and antimetastasizing against Lewis lung carcinoma and S180. The copolymers with 23–26% MI appears to be the potential drugs against the metastasis and growth of Lewis lung carcinoma.

## Acknowledgements

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16. Lewis lung carcinoma cells were maintained by intraperitioneal passage at weekly intervals in C57BL/6 mice. In experiments with tumor cells, $1\times10^6$ cells in 0.05 mL were transplanted intracutaneously on the foot pad of C57BL/6 mice (10 mice per group) on day 0. The mice were administered intraperitoneally with a consecutive (qd×7) schedule at various doses from day 1. The mice were weighed twice a week to monitor the toxic effects. The foot pad bearing Lewis lung carcinoma were weighed on day 8 or 10. The mice were killed 4 weeks later, livers were removed and metastatic nodes were calculated.
17. The test copolymers were suspended in 0.5% sodium carboxymethyl cellulose containing 3.5% Tween 80. Results are expressed as the mean ± SD. The significance of difference between groups and/or drugs was assessed by using Student's *t*-test. $p<0.05$ was taken as significant.
18. The mouse solid sarcoma S180 cell line was maintained by intraperitioneal passage at weekly intervals in male TA1 mice. In experiments with solid S180 cells, $2–4\times10^6$ cells in 0.2mL were transplanted intracutaneously into the right axilla of TA1 mice (10 mice per group) on day 0. The mice were administered intraperitoneally with a consecutive (qd×7) schedule at various doses from day 1. The mice were weighed twice a week to monitor the toxic effects. The mice were killed on day 14, and the tumors were removed and weighed.
19. The mouse ascites sarcoma S180 cell line was maintained by intraperitioneal passage at weekly intervals in male TA1 mice. In experiments with solid S180 cells, $2–4\times10^6$ cells in 0.2 mL were injected intraperitioneally into abdominal cavity of TA1 mice (10 mice per group) on day 0. The mice were administered intraperitoneally with a consecutive (qd×7) schedule at various doses from day 1. The survival time was recorded and the mean life span was calculated.
20. TA1 mice were randomly divided into five groups (20/group and female:male = 1:1) and treated with drugs of copolymer **1**–**6**, respectively, using ip×1 schedule, with the groups receiving 491, 614, 768, 960, and 1200 mg/kg dose level (this dose level was the maximum tolerated dose level) on day 0. The behaviors and the death distribution were recorded. Necropsies and autopsy were completed on all mice. $LD_{50}$ was calculated by using Bliss method.