We can find several informations, though very few, in which the usage of compounds derivated further acetic or α-methylacetic radical of acidic antiinflammatory drugs is mentioned. Boltze, et al.(1) developed Indomethacin (IDM)-glutarate (TV 1322). Gurpegui, et al.(2) and Paris, et al.(3) synthesized hydrazides and glycerides of IDM, respectively and found the inhibitory effect of the former on monoamine oxidase. Orzalesi, et al.(4,5) developed Ibuprofen (Brufen, BF)-hydroxamate and recommended its excellence. Tsuji, et al.(6) developed 2-2-pyridinemethanol ester of BF named BE-100 which was said to be usable to inflamed skin by the external use. Prior to the publication by Orzalesi, et al.(4), we synthesized hydrochloride of BF-diethylaminoethylester (BF-DEAE). Masumoto and his research group of Kaken Pharm. Co. examined the pharmaco-dynamic spectrum of this derivative. The results were only shortly reported on LSM(7).

On the other hand, Minauchi(8) reported the efficacy of Carbamazepine on leprous neuritis. This finding suggested to us the possibility to treat this neuritis through a neural accommodation from central side. However, a problem may still remain whether the systemic neural accommodation is single remissive means to inflamed peripheral nerves.

In this reason, we have been continuing a series of studies on search for a substance possessing both of perineuropotrophic and antiinflammatory properties. In this report, the results hitherto examined about the former property is presented.

**Materials and Methods**

1. **Starting materials**: BF and Carprofen (CPF) were kindly supplied by kaken Pharm. Co. and Nippon Roche Co. under licences from Boots Co., England and Roche Co., Switzerland respectively. Mefenamic acid (MA) was also kindly supplied by Sankyo Pharm. Co. under licence of Parke-Davis Co., U. S. A.

2. **Synthesis of derivatives** from acidic antiinflammatory drugs: The chemical conformation are shown in Fig. 1.

   2-diethylaminoethylester. **HCl salt of BF or MA (BF-DEAE or MA-DEAE)**: The dried Na salt was refluxed for 2hrs. with excess 2-chlorotriethylamine in toluene. After washed with 5 % Na2CO3, acidic extract (10 % HCl) was made alkali-side with 20 % Na2CO3 and
extracted with Et₂O. The extract dried with Na₂SO₄ was saturated dry HCl gas under cooling. The monohydrochloride separated or remained as the residue after evaporated Et₂O was recrystallized from benzene-ligroin. The yields were 60-68% (BF-DEAE) and 55-62% (MA-DEAE) of theoretical values.

**Anal. Calcd. for**

<table>
<thead>
<tr>
<th></th>
<th>C %</th>
<th>H %</th>
<th>N %</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF-DEAE</td>
<td>66.74</td>
<td>9.43</td>
<td>4.10</td>
</tr>
<tr>
<td>MA-DEAE</td>
<td>66.91</td>
<td>7.75</td>
<td>7.43</td>
</tr>
</tbody>
</table>

**BF-diethylaminoethylamide·HCl salt (BF-DEAEAM):** BF was refluxed for 1 hr. at 90°C with excess SOCl₂. After evaporated SOCl₂ in vacuo, the residue was dissolved in Et₂O. N,N-diethylethylenediamine (4×BFmol)/Et₂O was dropped under stirring and cooling, and the reaction mixture was washed with 10% Na₂CO₃ and 1 N HCl. After alkalification of the acidic extract, it was extracted with Et₂O. The dried Et₂O layer was introduced dry HCl gas and the resultant precipitate was recrystallized 3 times from n-hexane-benzene. The yield was 71% of theoretical value. mp 110-113°C, colorless needles. **Found:** C, 66.71; H, 9.84; N, 8.08%.

**CPF-diethylaminoethylamide (CPF-DEAEAM):** CPF was changed to its chloride with each slight excess of ClCOOEt-Et₃N in tetrahydrofuran (THF) at -15°C. To this THF solution, a slight excess of diethylethylenediamine/THF was dropped very gradually at -15°C under stirring and cooling. After 2 hrs., THF was evaporated below 40°C. The EtOAc extract of the residue was washed with 5% Na₂CO₃ saturated KCl. The residue of EtOAc extract was recrystallized from a small volume of 70% EtOH. Yield 1.8 g (a). A part (1 g) in 8 ml of THF was saturated dry HCl gas. After evaporated THF-HCl below 15°C, the yellow pasty residue was 2 times washed with Et₂O and kept at -20°C for 48 hrs. in Et₂O. The solidified material was once recrystallized from benzene-CHCl₃. The precipitate was refluxed with CHCl₃. The colorless insoluble powder (b) was separated. The addition of benzene to the filtrate of (b) resulted colorless precipitate. It was recrystallized from dichloroethane (c).

**CPF-DEAEAM**

```
<table>
<thead>
<tr>
<th></th>
<th>C %</th>
<th>H %</th>
<th>N %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 131-133.5</td>
<td>67.82</td>
<td>7.05</td>
<td>11.30</td>
</tr>
</tbody>
</table>
```
CPF-DEAEAM·HCl

(b) 160-163 colorless small needles C21H27O3N3Cl2 61.77 6.66 10.29 61.58 6.54 10.28
(c) 159-162 colorless small needles 61.56 6.91 10.19

The mixed mp of (b) and (c) was 156-159°C. IR spectrum of (b) were quite coincident with that of (c). Therefore, (b) and (c) are both monohydrochloride of CPF-DEAEAM (a).

3. Blink reflux test (surface local anesthetic effect): Even the isotonic buffered solution (0.8 % NaH2PO4-0.947 % Na2HPO4, pH 7.2) of soluble Procaine·HCl or Xylocaine·HCl was once filtered through a sterilized cotton. BF, BF-DEAE, CPF and CPF-DEAEAM were tested by touching with 1/4 mandolin line of syringe needle on the cornea of guinea pigs (Hartley, male, weighing 300-400 g, 5 animals per group) where the isotonic drug solution had been dropped. MA, MA-DEAE and Xylocaine·HCl as the comparative control were tested further by using the cornea of rabbits (Japan white, male, weighing 3-3.5 kg, 4 animals per group) by touching with 1/4, 1/3 mandolin line or the mustache of rabbit. Before the test, the blink was once examined and the eyes shown immediate and constant response to touching alone were used.

4. Skin reflux test (infiltration local anesthetic effect): To the 3 sites of depilated dorsal skin of guinea pigs (Hartley, male, weighing about 300 g, 5 animals per group), 0.2 ml of MA-DEAE or Xylocaine·HCl physiological saline solution was intracutaneously injected. The convulsive contracts to the gentle and momentary 2 touchings per each site with 1/2 mandolin line of syringe needle were scored with lapse of time. In the other cases, the drug solution was injected to one site on the front half of depilated dorsal skin and another site on the hind half. When a concentration of drug was injected to the former site, another concentration of the drug was tested by hind half. Always, 6 animals per group were used. Therefore, 2 concentrations of a drug could be examined by 3 sites on the front half and 3 sites on the hind half per each concentration in each group. The touching was done with 23 X 1" syringe needle. At every touching, the normal skin closed to the touched site was examined in the same time as a comparative control.

Results

Effect on blink reflux of cornea (surface local anesthetic effect): No blink time after dropping BF-DEAE or BF-DEAEAM on the cornea of guinea pigs was compared with the anesthetic effect of Procaine·HCl. The results shown in Table I indicate that the effects of the former two at the concentration of 0.1 % are comparable to that of 2 % Procaine·HCl solution. The effect of BF-DEAE seemed to be 2 or 20 fold stronger than that of BF-DEAEAM or Procaine·HCl, respectively. On the other hand, DEAE itself exhibited only weak effect and BF itself showed no influence. However, in spite of the prolonged no blink time longer than 1 hour, both of 1 % and 5 % BF-DEAE solutions caused a slight corneal clouding, suggested the corrosive side-effect in very high concentrations.

The anesthetic effect of 0.2 % MA-DEAE solution on guinea pig cornea and that of 0.1 % solution on rabbit cornea, which are both expressed by scores, are shown in Table IIa b, res-
Table 1 Surface Local Anesthetic Effects of BF-DEA and BF-DEAEAM on Blink Reflux of Cornea of Guinea Pigs

<table>
<thead>
<tr>
<th>Drug</th>
<th>%</th>
<th>No. Reflux Time min.±S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procaine · HCl</td>
<td>0.5</td>
<td>3.0±0.41*</td>
</tr>
<tr>
<td>1.0</td>
<td>4.1±0.09</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>10.5±0.15</td>
<td></td>
</tr>
<tr>
<td>Procinamide</td>
<td>2.0</td>
<td>0±0.0</td>
</tr>
<tr>
<td>HCl·(C₂H₅)₂NCH₃CH₂OH</td>
<td>10.0</td>
<td>0.7±0.67</td>
</tr>
<tr>
<td>BF-DEAE</td>
<td>0.01</td>
<td>0±0.0</td>
</tr>
<tr>
<td>0.05</td>
<td>5.3±0.13</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>13.3±0.20</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>79.0±0.23</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>120&lt;</td>
<td></td>
</tr>
<tr>
<td>BF-DEAEAM</td>
<td>0.1</td>
<td>7.3±1.86</td>
</tr>
<tr>
<td>0.5</td>
<td>43.3±8.67</td>
<td></td>
</tr>
</tbody>
</table>

* Averaged the times of 5 cases
Touching : 1/4 mandolin line of syringe needle

The effect of CPF-DEAEAM was compared with those of Xylocaine·HCl and Na-salt of CPF using the cornea of guinea pigs. The results are shown in Fig. 2. In this case, each totaled score of 5 animal was subtracted from that of control group (2×5=10).

Although CPF-DEAEAM caused no blink reflex completely last for 60 minutes in the concentrations no lower than 0.2 %, the apparent corneal clouding was observed. It may be due to the strong surfactant ancivity of CPF-DEAEAM, thus caused blindness such as corneal leukoderma. Whereas, the complete recovery of blink reflex could be seen in the concentra-

pectively. Though these experiments lacked in the comparative control group of a standard anesthetic drug, the few blinks of guinea pig cornea and the apparent response of rabbit cornea suggested that the effect of MA-DEAE seemed to be weaker than that of BF-DEAE.

Table 2 Surface Local Anesthetic Effect of MA-DEAE on Blink Reflux of Corneas of Guinea Pigs (a) and Rabbits (b)

<table>
<thead>
<tr>
<th>Dropping : MA-DEAE 100 µg/0.05 ml Time (min.)</th>
<th>Touching 1/4 Mandolin Line</th>
<th>Dropping : MA-DEAE 100 µg/0.1 ml Time (min.)</th>
<th>Touching strong</th>
<th>Medium</th>
<th>Weak</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0</td>
<td>0*</td>
<td>10</td>
<td>11</td>
<td>9</td>
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<td>45</td>
<td>2</td>
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<tr>
<td>60</td>
<td>1</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

+2, immediate blink; +1, delayed blink; 0, no response

Each score is the total of 5 animals, and those of control and MA groups were both 0.

*just before dropping MA-DEAE
Strong: 1/3 mandolin line
Medium: 1/4 mandolin line
Weak: mustache of rabbit

Each score is the total of 4 animals.
Fig. 2 Surface Local Anesthetic Effect of CPF-DEAEAM on Blink Reflux of Guinea Pig Cornea

Dropping of drug solution: One drop (0.02 ml) was gently massaged for 10 sec. inside the closed eyelid and another drop was added.

Scoring: +2, immediate blink reflex; +1, delayed blink; 0, no or slight response of eyelid.

Each score is the total of 5 guinea pigs. The total scores of control group were nearly always 10. Each totalized score was subtracted from 10 and plotted on this figure.

Table 3 Infiltration Local Anesthetic Effect of MA-DEAE on the Convulsive Contract Reflux of Guinea Pig Dorsal Skin

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>MA-DEAE</th>
<th>Xylocaine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0*</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>15</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>20</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>25</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

Dose: Injected intracutaneously to 3 sites of each guinea pig weighing about 300 g. Five animals per group were used.

Touching: 1/2 mandolin line, 2 times/site each time.

Scoring: +1, immediate convulsive contract; 0, no response. The total score with no anesthetic action was $1 \times 2 \times 3 \times 5 = 30$.

Effect on skin reflux (infiltration local anesthetic effect): As it will be mentioned later, BF-DEAE was found to be hydrolyzed rapidly in vivo. In this reason, this effect was examined mainly regarding CPF-DEAEAM. The effect of MA-DEAE was compared with that of Xylocaine•HCl. The result shown in Table III indicates only that weaker than Xylocaine•HCl.

The effect of CPF-DEAEAM is shown in Fig. 3. In the Fig. 3, no response scores are: CPF-DEAEAM, 24±1.2 (at 0.04 %), 31±1.9 (at 0.1 %), 36±0.5 (at 0.25 %); Xylocaine•HCl, 12±2.1 (at 0.04 %), 23±2.4 (at 0.1 %), 24±2.4 (at 0.25 %). Therefore, the effect of CPF-DEAEAM seemed to be at least twice stronger than that of Xylocaine•HCl in spite of narrow safety range shown in the test of blink reflex.

Discussions

In spite of the nearly no anesthetic action of diethylaminoethylethanol, all of the compounds exhibited a notable anesthetic effects. However, as observed in the cases of BF-DEAE and especially CPF-DEAEAM, these compounds showed the side-effects on cornea in their very high concentrations. Though vaguely, these side-effects seemed to be caused by a chemico-physical properties of them such as the surfactant activity of CPF-DEAEAM. The too nar-
row safety range of CPF-DEAEAM reminded us of the risk in its use at mucous membrane, even though not so small part of this compound was presumed to be hydrolyzed to CPF or even to the metabolites of CPF\(^9,10\) due to the metabolic activity of cornea.

In comparison with diethylaminoethylamides (-DEAEAM), diethylaminoethylesters (-DEAE) were presumed to be hydrolyzed rapidly in vivo. The research group of Masumoto proved also that BF-DEAE was hydrolyzed very rapidly in a rat heparinized plasma by the first order equation with the half-life of 1.5 minutes, when examined by gas chromatography\(^11\).

If associating the efficacy of BE-100\(^9\) with BF-DEAE or BF-DEAEAM, BF seems to be an interesting starting drug for our purpose. However, at the starting point, we selected BF by an indifferent image such that the chemical structure of BF-DEAE seemed to be resembling to alkylated Procaine.

Another problem remaining still is the toxicity of BF-DEAE. Masumoto, et al. noticed that the acute toxicity of BF-DEAE was comparable to that of Procaine-HCl, somewhat higher than that of FBF, and 10 fold higher than that of BF when intravenously dosed to ddY male mice, whereas it was comparable to BF when dosed orally. The low toxicity of the latter case was presumed to be due to the rapid hydrolysis of BF-DEAE.

On the other hand, the toxicity of BF-DEAEAM was found to be far lower than that of BF-DEAE and LD\(_{50}\) value reached to about 1250 mg/kg when orally dosed, though its hydrolysis to BF in a rat plasma was negligible (Fig. 4).

The strong local anesthetic actions of tested compounds suggested their perineurotropic properties, though yet, they have not been examined by a local tracer technique.

Moreover, it should be considered that the infiltration of acute bactericidal chemotherapeutics...
Fig. 4 Stability of BF-DEAE or BF-DEAEAM in the Solution Containing a Rat Heparinized Plasma

After incubated the mixture of BF-DEAE or BF-DEAEAM/H2O (1ml) and a heparinized rat plasma (1ml), 0.5ml of 1M Na2CO3 and 50μg of anthracene/15ml of benzene were added and shaken for 20 mins. After centrifuged at 3000 rpm for 5 mins., benzene layer containing either BF-DEAE or BF-DEAEAM was separated. The metabolized BF was separated in aqueous layer. Then, each layer was further purified and analyzed gas chromatographically. GC, Hitachi Model 073; Column, 1m×3mm glass column packed with 1.5 % OV-1 on Shimalite W, 80-100 mesh; Carrier, N2 35ml/min. (BF-DEAE or BF-DEAEAM), or 40ml/min. (BF); Internal Std., 4-(3,4,5-triethoxybenzoyl) morpholine such as RFP(12-14) to be the drug for the final and causal treatment of this neuritis may scatter the bacterial toxic materials even locally inside Schwann cell to cause a temporary exacerbation in stead of the eradication of bacilli. Thus, the overlap of host reactivity on the infectious disease, leprosy presents a complication to the therapy by drugs as the third factor. In this reason, the discovery of a drug adequate to the symptomatic and peripheral use, and to be remissive the inflammation in peripheral nerves will be needed.

Summary

1. Diethylaminoethylesters (DEAE) of Brufen (BF) and Mefenamic acid (MA) were synthesized together with diethylaminoethylamides (DEAEAM) of BF and Carprofen (CPF).

2. Their local anesthetic effects were examined by blink and skin reflux tests. All of them showed notable effects. Especially, BF-DEAE and BF-DEAEAM exhibited the surface local anesthetic effects 20 fold and 10 fold stronger than that of Procaine•HCl, respectively. CPF-DEAEAM also exhibited the infiltration local anesthetic effect twice stronger than that of Xylocaine•HCl.

3. However, several problems remained. BF-DEAE hydrolyzed very rapidly in a heparinized rat plasma. Its acute toxicity was 10 fold higher than that of BF when intravenously dosed to mice whereas it was comparable to that of BF when dosed orally. Both of BF-DEAE and CPF-DEAEAM, especially the latter caused the corneal clouding in the high concentrations, which may be due to its surfactant activity.

4. BF-DEAEAM was stable in the rat plasma and its LD₅₀ value nearly reached to 1250 mg /kg by mice.

5. Standing on the contradiction between the eradication of leprosy bacilli inside peripheral nerves and the resultant possible undesirable host reactions in the nerves, the necessity to discover a drug remissive the inflammation in the peripheral nerves through its local use was discussed.
Acknowledgement

We sincerely express our thanks to Dr. S. Masumoto and the researchers of his group of Kaken Pharm. Co. for their active leadership in the animal experiments of BF-DEAE and BF-DEAEAM. The animal experiments of MA-DEAE were also cooperated by Sankyo Pharm. Co. We also express our thanks to every Pharm. Co. for their cooperation to elementary analysis of synthesized compounds. This study was supported by national fund and partly by the fund of U. S.-Japan Cooperative Medical Science Program.

References

11) Masumoto, S.: Personal communication.
らい性末梢神経炎治療の可能性を目途とした
酸性消炎剤の麻酔作用を発現する
誘導体の研究

儀 同 政 一  堤 貞 衛
（国立多摩研究所）

1. Brufen (BF), Mefenamic acid (MA) のジチルアミノエチルエステル (BF-DEAE, MA-DEAE), BF, Carprofen (CPF) のジチルアミノエチルアミド (BF-DEAEAM, CPF-DEAEAM) を合成した。

2. モルモット及びウサギ (MA-DEAE のみ) の角膜反射で表面麻酔作用、モルモット背部皮膚の撫でで浸潤麻酔を調べた結果、全誘導体ともかななり強い局麻作用を示し、BF-DEAE, BF-DEAEAM に塩酸プロカインの各約20倍と約10倍の表面麻酔作用を、CPF-DEAEAM に塩酸キシロカインの約2倍の浸潤麻酔作用を認めた。

3. しかし、BF-DEAE 特に CPF-DEAEAM は高濃度で角膜混濁の副作用を示し、また BF-DEAE をラットへ尾静脈注射した際の急性毒性は BF の約10倍強かった。一方、BF-DEAE はヘパリン加マウス血しよう中で速やかに BF へと加水分解し、その結果、経口投与での急性毒性は BF と同等だった。BF-DEAEAM はマウス血しよう中で安定で、マウスへ経口投与時の急性毒性は約 LD_{50} 1,250 mg/kg だった。

4. 末梢神経内らい薬剤とその結果起りうることが心配される好ましくない宿主反応との矛盾に起因し、末梢神経炎症を局所使用により対処しうる薬物発見の必要性を討議した。