Enhancement by Carprofen or Indomethacin of Interferon Induction by 10-Carboxymethyl-9-Acridanone in Murine Cell Cultures

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SUMMARY

Non-steroidal anti-inflammatory drugs such as carprofen or indomethacin enhanced interferon (IFN) production induced by suboptimal concentrations of 10-carboxymethyl-9-acridanone (CMA) in murine cell cultures. This effect was observed in fibroblasts and in different populations of leukocytes as in peritoneal exudate and spleen cells, and was most pronounced in bone marrow-derived macrophages. Carprofen was the most effective compound causing an up to 500-fold increase of CMA-induced IFN production in pure bone marrow-derived macrophages. In these macrophage cultures the potentiating effect on CMA-induced IFN production by carprofen and indomethacin did not depend on inhibition of cyclooxygenase.

A variety of agents, i.e. polyinosinic-polycytidylic acid [poly(rI)-poly(rC)], RNA and DNA viruses, and lipopolysaccharides, stimulate both synthesis of interferon (IFN) (Stewart, 1979) and of prostaglandins (PG) (Yaron et al., 1977; Gemsa, 1981). A relationship between the inductions of these two groups of substances has been proposed since endogenously induced or exogenously added IFN stimulated PG formation in several systems (Fitzpatrick & Stringfellow, 1980; Yaron et al., 1977). In contrast, however, there are reports showing that in murine peritoneal macrophages addition of IFN did not stimulate PG formation (Schultz et al., 1979) or that even a depression of PG synthesis was observed (Boraschi et al., 1984).

We have investigated the effects of inhibitors of PG synthesis on IFN production by different murine cell cultures, especially bone marrow-derived macrophages. Induction of IFN was stimulated by 10-carboxymethyl-9-acridanone (CMA), an efficient inducer of IFN in murine cell cultures (Storch & Kirchner, 1982). Carprofen, indomethacin, diclofenac and aspirin (Gaut et al., 1975; Flower, 1974; Ku et al., 1975) have been utilized as non-steroidal inhibitors of PG synthesis.

Cultures of pure macrophages were initiated from the bone marrow of C57BL/6 mice (Zentrum für Versuchstiere, Hannover, F.R.G.) according to the protocol of Meerpoohl et al. (1976) and further cultured as previously described (Storch & Kirchner, 1982). For IFN induction experiments, macrophages (3 × 10⁵ per ml) and peritoneal exudate cells (1 × 10⁶ per ml) were cultured in 24-well plates (Nunclon, Nunc) and spleen cells (3 × 10⁶ per ml) in tissue culture tubes (2058, Falcon). JLS-V9R cells, murine embryo fibroblasts (MEF) or L929 cells were seeded in 24-well plates. For IFN induction, cells were pulsed for 1.5 h with the compounds to be tested, subsequently washed twice and cultured for 18 h in 1 ml RPMI 1640 medium supplemented with antibiotics and 2.5% foetal bovine serum. IFN activities in the supernatants were determined by the inhibition of the cytopathic effect of vesicular stomatitis virus on L929 cells as previously described (Marcucci et al., 1984).
Table 1. Effects of carprofen on CMA-induced IFN production by different murine cell types*

<table>
<thead>
<tr>
<th>Cell type</th>
<th>IFN induced by CMA†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>− carprofen</td>
</tr>
<tr>
<td>Bone marrow-derived macrophages</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Peritoneal exudate cells</td>
<td>5</td>
</tr>
<tr>
<td>Spleen cells</td>
<td>15</td>
</tr>
<tr>
<td>L929 fibroblasts</td>
<td>&lt;3</td>
</tr>
<tr>
<td>MEF</td>
<td>&lt;3</td>
</tr>
<tr>
<td>JLS-V9R</td>
<td>&lt;3</td>
</tr>
</tbody>
</table>

* Bone marrow-derived macrophages (3 × 10⁴ per ml), spleen cells (3 × 10⁶ per ml), peritoneal exudate cells and three fibroblast lines (1 × 10⁶ per ml) were pulsed for 1.5 h with CMA (250 μM) without or with carprofen (75 μM), washed twice and incubated with 1 ml medium.

† IFN activities (IU/ml) were determined in the supernatants after 18 h of culture. The values given are the means of three experiments. IFN was not detected in unstimulated cultures or upon incubation with carprofen alone.

CMA (sodium salt) was dissolved in 0.15 M-NaCl solution. The concentrations of carprofen (Hoffmann-LaRoche, Grenzach, F.R.G.), indomethacin (Sigma), diclofenac (Ciba-Geigy) and aspirin (Sigma) resulting in optimal effects on IFN induction were determined in bone marrow-derived macrophages. CMA (250 or 500 μM)-induced IFN production was about threefold in the presence of 1 μM-carprofen, indomethacin or diclofenac, then increased with the dose of the non-steroidal anti-inflammatory agents and was at a maximum at 75 μM. The optimal concentration of aspirin was 3.5 mM.

For determination of arachidonic acid (AA) metabolites, monolayers of bone marrow-derived macrophages (5 × 10⁶ in 2 ml medium) were plated in Petri dishes (3002F, Falcon) and labelled for 6 h with 2 μCi [¹⁴C]AA (New England Nuclear, sp. act. 52 mCi/mmol). After washing, the macrophages were cultured for 18 h with the indicated substances. Metabolites of AA were extracted as described by Salmon & Flower (1982) and separated by thin-layer chromatography (TLC) on Whatman silica gel LKD plates using solvent system A (upper phase of ethyl acetate:2,2,4-trimethylpentane:acetic acid:water, 110:50:20:100, by vol.) to separate PGE₂ from PGF₃α, or solvent system B (chloroform:methanol:acetic acid:water, 90:8:1:0.8, by vol.) to resolve PGE₂ from thromboxane B₂ (TXB₂, the stable product of TXA₂). The ¹⁴C-labelled AA metabolites were visualized by autoradiography. In parallel culture supernatants of bone marrow-derived macrophages (3 × 10⁵ cells per ml), PGE₂, PGF₂α, TXB₂ and 6-keto-PGF₁α (the stable product of prostacyclin) were quantified by radioimmunoassay (RIA) as described by Gemsa et al. (1980).

The effects of carprofen on CMA-induced IFN production were studied in different types of murine leukocytes and fibroblasts. As shown in Table 1, these murine cells responded to CMA (250 μM) in the presence of carprofen (75 μM) with increased IFN production. IFN was not detected when the cells had been incubated with carprofen alone. The potentiating effect of carprofen was most pronounced in bone marrow-derived macrophages.

In Table 2, the effects of the optimal doses of different non-steroidal anti-inflammatory drugs on CMA-induced IFN production in bone marrow-derived macrophages are compared. CMA alone induced IFN in a dose-dependent manner and the production was highest after incubation with a concentration of 2 mM. The maximum amount of IFN production stimulated by the optimum concentration of CMA was not influenced by the inhibitors of cyclooxygenase. IFN induction by low doses of CMA, however, was markedly enhanced in the presence of the inhibitors. Carprofen was the most effective of the drugs used, eliciting up to a 500-fold increased level of IFN whereas aspirin only weakly influenced IFN induction.

To study whether the potentiating effect of the non-steroidal anti-inflammatory agents on CMA-induced IFN production correlated with inhibition of cyclooxygenase products, metabolites of AA were determined by RIA. In all supernatants tested (control, CMA with or without carprofen or indomethacin) TXB₂ was below 0.3 ng/ml and PGE₂, PGF₂α and 6-keto-PGF₁α were near the borderline of detection. Separation of [¹⁴C]AA metabolites on TLC using solvent system A (Fig. 1) and solvent system B (results not shown) demonstrated that control
Fig. 1. Metabolites of $[^{14}C]$arachidonic acid in the supernatants of bone marrow-derived macrophages separated by TLC in solvent system A and identified by autoradiography. Mobilities of unlabelled standards were determined by detection using iodine vapours. Lane 1, control cultures; lane 2, CMA (0.5 $\mu$M); lane 3, CMA (0.5 $\mu$M) + carprofen (75 $\mu$M).

Table 2. Effects of different non-steroidal anti-inflammatory drugs on CMA-induced IFN production in bone marrow-derived macrophage cultures*

<table>
<thead>
<tr>
<th>CMA (mM)</th>
<th>No CMA added</th>
<th>Carprofen (75 $\mu$M)</th>
<th>Indomethacin (75 $\mu$M)</th>
<th>Diclofenac (75 $\mu$M)</th>
<th>Aspirin (3.5 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.25</td>
<td>&lt; 3</td>
<td>1693</td>
<td>1065</td>
<td>278</td>
<td>40</td>
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<tr>
<td>0.5</td>
<td>77</td>
<td>2133</td>
<td>1871</td>
<td>1496</td>
<td>171</td>
</tr>
<tr>
<td>1</td>
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<td>2387</td>
<td>2333</td>
<td>2123</td>
<td>1830</td>
</tr>
</tbody>
</table>

* Bone marrow-derived macrophages (3 x 10^5 per ml) were pulsed for 1-5 h with CMA in the absence or presence of the non-steroidal anti-inflammatory drugs, washed twice and cultured in 1 ml medium.

† Interferon activities were measured in the supernatants after 18 h of culture. The values given are the means of three experiments, each consisting of triplicate cultures.

supernatants contained PGE_2, TXB_2, PGF_2alpha, 6-keto-PGF_1alpha, PGA_2, PGD_2 and an additional AA metabolite migrating with PGD_2. Production of cyclooxygenase products was suppressed in cultures after incubation with CMA and CMA plus carprofen.

Enhancement of IFN induction has been described by a variety of agents, i.e. some short-chain fatty acids, DEAE-dextran, phorbol myristic acetate, theophylline or by priming with low doses of IFN (for review, see Sehgal et al., 1982). We report here that IFN production induced
by suboptimal concentrations of the low molecular weight inducer CMA was markedly increased in the presence of non-steroidal anti-inflammatory agents such as carprofen or indomethacin. This potentiating effect depended on the type of cell since it was expressed differently in the cultures studied and was most pronounced in bone marrow-derived macrophages. The enhancing effect depended also on the type of inducer, because it was found in response to CMA, but not to viruses or poly(rI)poly(rC) (data not shown), suggesting that different regulatory mechanisms for different IFN inducers exist.

The non-steroidal anti-inflammatory drugs indomethacin, carprofen, diclofenac and aspirin are known to be inhibitors of fatty acid cyclooxygenase. The observed potentiating effects of these substances, however, were not due to inhibition of cyclooxygenase because of the following results. The concentrations of the non-steroidal anti-inflammatory drugs resulting in optimal increase of CMA-induced IFN induction were markedly higher than those needed for inhibition of PG synthesis (Flower, 1974). Addition of PGE₂ to the cultures did not reverse the potentiating effect of indomethacin or carprofen on CMA-induced IFN induction (data not shown). Aspirin, well known for its ability to inhibit cyclooxygenase, was less effective in our system. Compared with the controls, the cyclooxygenase products were already suppressed in bone marrow-derived macrophage cultures incubated with CMA alone. Thus, the enhancing effect of indomethacin or carprofen on IFN production induced by suboptimal concentrations of CMA was independent of the inhibition of cyclooxygenase.

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REFERENCES

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