Short Communication

Reduction of 1-methyl-1-nitrosourea-Induced Mammary Gland Carcinoma by *in vivo* Application of Immunostimulatory CpG Motifs in Sprague-Dawley Rats

D. MACEJOVÁ[1], M. DVORČÁKOVÁ[1], R. WEISS[2], S. SCHEIBLHOFER[2], S. MOSTBÖCK[2], J. LÍŠKA[3], Ľ. SZABOVÁ[1], Š. ZÓRAD[1], J. THALHAMER[2] AND J. BRTKO[1]

1 *Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia*
2 *Institute of Chemistry and Biochemistry, University of Salzburg, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria*
3 *Institute of Histology and Embryology, Medical Faculty of Comenius University, Sasinkova 4, 811 08 Bratislava, Slovakia*

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Abstract. In the present work the role of 13-cis retinoic acid and CpG oligodeoxynucleotides (CpG-ODN) in a 1-methyl-1-nitrosourea (MNU)-induced mammary gland carcinoma animal model was investigated. Treatment with both components, applied either alone or in combination, induced a significant decrease of the tumour burden and the volume of tumours only in rats that received CpG-ODN ($p = 0.046$, compared to the MNU control group). The data indicate that the Th-1 biased immunostimulatory capacities of CpG motifs may play a significant role in induction of protective immune responses against mammary gland tumours in Sprague-Dawley rats.

Retinoids have shown some promise as chemopreventive agents against chemically induced mammary gland carcinogenesis in both mice and rats (Moon and Constantinou 1997). Their cognate nuclear receptors are known to act as transcriptional activators upon binding of a retinoid molecule and by binding to retinoic acid responsive elements (Gudas 1994). Recent data indicated that *in vivo* expression of nuclear all-trans retinoic acid receptors in mouse spleen can be influenced by genetic immunization itself, or in combination with coinjection of immunostimulatory CpG motifs *in vivo* (Brtko et al. 2000).

Correspondence to Julius Brtko, Ph D , Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárska 3, 833 06 Bratislava, Slovakia
E-mail ueenbrtk@savba.sk

Table 1. The *in vivo* effect of retinoic acid and CpG-ODN on MNU induced tumours

	Number of tumours/animal		Tumour burden [g]/animal		Tumour volume [cm^3]/animal	
	Mean ± Standard Error	Median (Range 5–95 %)	Mean ± Standard Error	Median (Range 5–95 %)	Mean ± Standard Error	Median (Range 5–95 %)
MNU-control	4 00 ± 0 63	4 0 (1–8)	22 80 ± 5 26	23 77 (2 40–53 99)	45 60 ± 10 90	48 70 (3 38–105 00)
13-cis	3 75 ± 0 96	3 5 (0-7)	12 13 ± 6 82	4 29 (0 00–54 16)	22 00 ± 12 50	7 25 (0 00–100 00)
CpG	2 38 ± 0 6	3 0 (0–5)	8 91 ± 3 20	**6.49 (0.00–23.58)**	16 30 ± 7 08	**10.80 (0.00–56.80)**
13-cis + CpG	4 25 ± 0 77	3 5 (2–8)	22 32 ± 6 68	18 48 (1 01–53 15)	39 10 ± 13 00	32 20 (1 41–102 00)
non CpG	5 50 ± 0 76	6 0 (2–9)	25 71 ± 8 62	24 02 (0 74–73 11)	41 60 ± 15 10	39 50 (0 67–131 00)

MNU-control this group received only MNU, 13-cis MNU + 13-cis retinoic acid, CpG MNU + CpG-ODN, 13-cis + CpG MNU + 13-cis retinoic acid and CpG-ODN, non CpG MNU + non-immunostimulatory oligodeoxynucleotide Significant differences ($p = 0\ 046$) to the MNU-control are shown in bold

Cpg motifs are recognized by immunocompetent cells *via* so-called "pattern recognition receptors" that represent a mechanism of the innate immune system to signal "danger" based on structural surface characteristics shared by a variety of infectious agents (Hemmi et al 2000) They act on immune cell species like professional antigen presenting cells, natural killer cells, B and T cells and induce the secretion of a panel of cytokines, in particular type-1 interferons (IFNα/β), IL-1β, TNF-α, IL-6 and IL-12 (Krieg et al 1995, Stacey et al 1996, Sparwasser et al 1997, Sun et al 1998) This inflammatory Th-1 type adjuvant effect of CpG motifs makes them potential candidates for stimulating immunity against tumours

As a prerequisite for testing components that might be involved in reduction of mammary tumours we have recently established the *in vivo* rat animal model for induction of mammary gland carcinomas by treatment with 1-methyl-1-nitrosourea (MNU, Macejova et al 2001)

In the present work we investigated the effect of retinoic acid, immunostimulatory CpG oligodeoxynucleotides (CpG-ODN) and a combination of both substances on the development and/or progression of mammary gland tumours in rats

Five groups of female Sprague-Dawley rats were given 50 mg kg^{-1} MNU on the 53rd, 82nd and 102nd day of age From the 55th day of age, the first group of rats was receiving 13-cis retinoic acid (1 mg kg^{-1}) intragastrically three times per week until the end of the experiment The second group of rats was receiving two intradermal injections of CpG-ODN (5'-GCTAGACGTTAGCGT-3', 100 μg) on the 47th and 97th day of age, and the third group of rats underwent treatment that was a combination of the above first and second group The last two groups served as controls, one receiving MNU without any further treatment, the other receiving MNU and injection of ODN with inverted CpG motifs (GpC), which exert no immunostimulatory capacity The experiment was terminated when rats reached their 146th day of age

As shown in Table 1 both mammary gland tumour burden and tumour volume was significantly reduced ($p = 0\,046$, Mann-Whitney rank sum test) in the group of rats treated with CpG motifs 13-cis retinoic acid applied intragastrically was found to cause marked reduction of tumour burden as well as the volume of tumours, however, in this experiment the response did not reach statistical significance Injection of non-immunostimulatory GpC motifs did not influence tumour growth indicating that the protective effect of CpG-ODN was specifically induced by the immunostimulatory motifs and not by the DNA in general However, contrary to our expectations of a possibly positive synergistic effect of CpG and retinoic acid, the treatment with both components elicited values of tumour burden and tumour volume which were similar to the MNU control group (Table 1)

Summing up, our data demonstrate that the Th-1 biased immunostimulatory capacities of CpG motifs may play a significant role in the induction of protective immune responses against mammary gland tumours in Sprague-Dawley rats Interestingly, the effect of this treatment disappears upon application combined with retinoic acid, which on its own is also partially protective This points to the fact that combinations of different protective therapeutical approaches (like

immunotherapy and hormone treatment) not necessarily may result in positive synergistic effects but even can lead to the loss of any benefit To elucidate these questions, further work will be done on the *in vivo* effect of CpG-ODN and/or retinoic acid on induction and/or progression of mammary gland tumours in rat

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