Department of Pharmaceutical Chemistry[1], Faculty of Pharmacy, Department of Molecular and Cellular Biology of Drugs[2], Faculty of Pharmacy, Comenius University, Bratislava, Slovakia

Persistent suppression of phagocytosis after prolonged administration of local anesthetics carbisocaine and heptacaine in mouse leukocytes

V. Vegh[1], M. Bukovsky[2]

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PharmDr. Vladimir Vegh, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University, Odbojarov 10, SK-832 32 Bratislava, Slovakia
vegh@fpharm.uniba.sk

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Effects of local anesthetics heptacaine and carbisocaine on mouse peritoneal macrophages after 5 days of peritoneal administration were observed. Both compounds in a daily dose 50 mg/kg caused significant decrease in phagocytic activity and index of phagocytosis (number of ingested particles). No significant effects on phagocytosis were observed when carbisocaine was administrated in a daily dose 5 mg/kg. No significant changes in weight gain and number of peritoneal macrophages were observed in all groups.

Local anesthetics are found not only to block the Na^+-channels, but they can affect (and in much sensitive ways) some other cellular systems. One of the most concerning effect of these drugs is the modulatory activity on the immune system. Generally, local anesthetics may cause various inhibitory effects on many functions of the immune system. The exact mechanism is not clear but probably involves regulatory pathways in priming/activation of immune system cells mediated via G-proteins (Fisher et al. 2001; Hollmann et al. 2001). As local anesthetics are often used in surgery and in open wounds, their suppressive effect on host defense after local application cannot be omitted. On the other hand, their immunosuppressive and anti-inflammatory effects, which are different from those of both nonsteroidal anti-inflammatory drugs and glucocorticoids, is now of great interest as a promising therapy in states with an overactive immune system response such as post-operative stress syndrome, ulcerative colitis, endotoxin-induced sepsis, multiorgan failure, burns etc. (Hollmann and Durieux 2000; Kiefer et al. 2003). Mechanisms of action of local anesthetics-like drugs may reveal an entirely new drug class with anti-inflammatory effects.

As some studies suggest a correlation between an immunosuppressive effect and lipid solubility (Krog et al. 2002; Hattori et al. 1997), we studied the effect of the highly lipid soluble and relatively low toxic local anesthetics carbisocaine (*N*-(2-(2-heptyloxyphenylcarbamoyloxy)-2-methylethyl)-diethylammonium chloride) and heptacaine (*N*-[2-(2-heptyloxyphenylcarbomoyloxyethyl)]-piperidinium chloride) on mouse peritoneal macrophages after prolonged period of peritoneal application. The samples were administrated intraperitonealy for 5 days at 24 h intervals in doses of 5 and 50 mg/kg, corresponding to 1 and 10% of LD_{50} (Tumova and Svec 1986; Cizmarik et al. 1978), using physiological sterile saline as a control.

The local anesthetics are thought to inhibit priming of leukocytes as an important preceding step before activation with various stimuli in the development of immune response, but not to inhibit the activation (Hollmann et al. 2001). To minimize direct effects of the tested compounds the mice were killed 72 h after the last dose and then the

Table 1: Effect of local anaesthetics on animals

Group (n = 5)	Mouse weight (g)	Relative spleen weight (% mouse weight)	Relative liver weight (% mouse weight)	Number of peritoneal macrophages ($\times 10^5$)
Control	21.72 ± 1.56	0.404 ± 0.047	4.63 ± 0.62	2.55 ± 0.75
Carbisocaine (50 mg/kg)	21.35 ± 1.20 (NS)	0.360 ± 0.040 (NS)	4.89 ± 0.68 (NS)	3.12 ± 0.93 (NS)
Carbisocaine (5 mg/kg)	20.86 ± 1.85 (NS)	0.320 ± 0.063 (NS)	5.02 ± 0.41 (NS)	1.84 ± 0.35 (NS)
Heptacaine	22.21 ± 1.80 (NS)	0.380 ± 0.019 (NS)	5.33 ± 0.20 (NS)	1.98 ± 0.85 (NS)

(NS) = non-significant

Table 2: Effect of local anaesthetics on phagocytosis

Group (n = 5)	Phagocytic activity (% phagocytic cells)	Activity (control = 1) significance	Index of phagocytosis (number of ingested particles)	Activity (control = 1) significance
Control	78.1 ± 5.9	1.00	8.83 ± 1.43	1.00
Carbisocaine (50 mg/kg)	66.0 ± 4.5	0.84 $p < 0{,}01$	6.20 ± 0.83	0.70 $p < 0{,}01$
Carbisocaine (5 mg/kg)	84.1 ± 7.5	1.07 (NS)	10.18 ± 3.2	1.15 (NS)
Heptacaine (50 mg/kg)	63.2 ± 6.7	0.81 $p < 0.01$	5.54 ± 0.43	0.62 $p < 0.01$

(NS) = non-significant

leukocytes, spleen and liver were isolated. All tests were performed in 5 parallel samples. Standard Student's t-test was used for statistical analyses of significance against the saline control.

There were no observed significant changes in weight gain, and no significant differences were observed in relative weight of spleen and liver, as important lymphoid organs. Also the number of leukocytes obtained in the peritoneal lavage was not significantly changed (Table 1).

Carbisocaine in a daily dose 5 mg/kg caused no significant differences in both assays (Table 2). Carbisocaine in a daily dose of 50 mg/kg caused a significant decrease in phagocytic activity (84% against control) and in the number of ingested particles per cell (70% against control). Heptacaine was tested only in a daily dose of 50 mg/kg and this dose caused a significant decrease in both phagocytic activity (81% against control) and in number of ingested particles per cell (62% against control). As the plasmatic half-live of the tested compounds is relatively short ($t_{1/2}$ carbisocaine = 161 min (Bezek et al. 1988), $t_{1/2}$ heptacaine = 228 min (Faberova et al. 1992)), we did not expect the presence of the tested compounds after the 72 hours interval between the last dose and phagocytosis assay. Thus, the results obtained describe persistent down-regulation of phagocytic functions rather than direct action of the compounds.

Experimental

1. Animals and reagents

Inbred female black mice C57BL/6 (Velaz, Praha, Czech Republic) with average weight 21.33 ± 1.35 g were kept under standard conditions with food and water intake *ad libitum*.

Carbisocaine and heptacaine were obtained from the Dept. of Pharmaceutical Chemistry, Pharmaceutical Faculty Comenius University (Bratislava, Slovakia), synthesized as described earlier (Benes et al. 1978; Cizmarik et al. 1978), RPMI-1640 was from SEVAPHARMA a.s. (Praha, Czech republic), fetal serum from Invitrogen/GIBCO (BRL, Germany), *Micrococcus luteus* (ATC 4698) and *Streptococcus faecalis* were from the Dept. of Molecular and Cellular Biology of drugs, Faculty of Pharmacy, Comenius University (Bratislava, Slovakia).

2. Application of samples, isolation of peritoneal leukocytes

5 or 50 mg/kg of carbisocaine or heptacaine was administrated intraperitonealy for 5 days in 100 µl of sterile apyrogenic saline at 24 h interval. The same amount of saline was used as control. 72 h after the last dose the mice were killed and weighed. Peritoneal cells from the mice peritoneal cavity were then pooled with 5 ml of sterile apyrogenic saline. Peritoneal exudate with leukocytes was taken out from the mice. Leukocytes were then microscopically counted and adjusted to 1×10^6 cells/ml with addition of RPMI 1640 and 0.5% fetal serum growth proteins.

3. Phagocytosis assays

Phagocytosis assays were performed by methods described previously (Bukovsky et al. 1996). In brief, 100 ìl of suspension of mice leukocytes (1×10^6 cells/ml) obtained from peritoneal lavage was incubated for 60 min at 37 ºC with 50 µl suspension of killed *Streptococcus faecalis* (5×10^8/ml, by heating in an autoclave). Samples were then placed on a microscope slide. The slides were dried at laboratory temperature and stained according to Wright. Phagocytic activity was calculated microscopically as percentage of the phagocytic cells from the total amount of 100 cells. The index of phagocytosis was calculated as the number of particles ingested by one phagocytic cell.

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