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## CYTOTOXIC EFFECT OF CARBOCAIN® AND MARCAIN®

by

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### INTRODUCTION

Modern local anaesthetics are of low toxicity. An 8 % Xylocain solution or a 4 % Citanest solution thus produces no irritation of the eye when instilled in the conjunctival sac (*Goldberg, 1947; Wiedling, 1960*) and an 8 % Carbocain solution injected subcutaneously into the rabbit's ear produces only mild injury (*Ulfendahl, 1957*).

The local toxicity of long-acting anaesthetics, such as Marcain, is of particular interest. It is possible that the duration of contact between Marcain and the tissue is longer than that of other anaesthetics. The long contact might be of importance in the development of tissue damage. It was therefore considered worthwhile to study the cytotoxic effect of Marcain and to compare it with that of Carbocain.

### MATERIAL AND METHODS

HeLa-cells (*Gey et al., 1952*) and human skin fibroblasts from 2—4 month old foetuses were cultured and harvested in the way described previously (*Spångberg, 1969 a*).

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The toxicity of the anaesthetic agents was assessed from their effect on HeLa-cells and human skin fibroblasts in slide cultures prepared according to Bergman's technique (Bergman, 1963; Spångberg, 1969 a) and from their effect on the oxygen consumption by HeLa cells.

*Slide culture.* In a culture chamber consisting of a glass ring fastened to a slide, 0.2 ml of the test solution and 0.5 ml of cellular suspension (150,000 cells/ml) were deposited and mixed. After the mixture had been kept at 37°C for 5 hours in the chamber the glass ring was removed and the slide was washed in lukewarm (37°C) Parker 199 (SBL). The cells that had attached to the slide were then cultured for 18 hours in Hellenthal cuvettes in a »final growth medium» consisting of Parker 199 with an addition of human and calf serum (Spångberg, 1969 a).

After the final growth the slide culture was fixed and stained and the cells and mitotic figures were counted (Bergman, 1963; Spångberg, 1969 a)

The mean number of cells per field of vision was determined and the mitotic count was calculated from counts in 20 fields of vision per slide. The average number of cells per field of vision of the slides in the experimental group and the mean number of mitotic figures per 100 cells were calculated.

In all experiments cultures for each type of test solution were made in duplicate. The experiments were repeated four times for the HeLa cells and twice for the fibroblasts. Each test solution was tried on 10 slide cultures with HeLa cells as well as on 6 with fibroblasts.

*Respirometry.* The oxygen consumption by the cells was measured with the aid of a Warburg apparatus (Braun, V 166, Melsungen, West Germany) with Warburg's direct technique. The experiments were carried out in atmospheric environments (Rueckert & Mueller, 1960) and at 37°C, and the CO<sub>2</sub> was absorbed with 0.2 ml 20 % (w/v) potassium hydroxide; a period of 75 minutes was allowed for gas and temperature equilibration before the beginning of the experiments. The reaction vessels were shaken 110 times a minute with an oscillation amplitude of 7 cm.

At the beginning of the experiment 2.5 ml of cellular suspension was added to each reaction vessel and 0.4 ml test substance to the side arms. After the end of the equilibration period the respiration was noted for every 15th minutes during a total period of 90 minutes (control period), after which the test material was mixed with cellular suspension. The oxygen respiration was then recorded in the same way during two consecutive 60 minute test periods. The resulting uptake of oxygen after addition of the test substance to the cell culture is given in the description of the results as percentages of the initially recorded control respiration of the culture. This was

calculated for the first and second test periods and for a double test period (120 minutes). Six experiments were carried out for each test substance and concentration.

*Test solution.* In the »slide culture» technique the solutions were prepared by adding to a basic medium consisting of Parker 199 and 25 % calf serum Carbocain\* and Marcain\* (0.5 % w/v mepivacaine and bupivacaine hydrochloride in physiological saline) respectively in such amounts that the final concentration of the anaesthetic agents in the slide cultures were 0.17, 0.057, 0.028, 0.014, 0.007 and 0.004 % w/v. In the controls only basic medium was used.

The test substance in the repirometric experiments was prepared in sodium bicarbonate-free Parker 199 buffered with trishydroxymethylaminomethane-HCl (*Spångberg*, 1969 b) to which was added Carbocain or Marcain (3.6 % w/v in physiological saline) in such amounts that the concentration of the anaesthetic agent in the flask cultures were 0.248, 0.124 and 0.062 % w/v. As control tris buffered Parker 199 was used.

*Statistical treatment.* Student's t-test was used for all comparisons.

Levels of significance	
almost significant	$0.05 > p > 0.01$
significant	$0.01 > p > 0.001$
highly significant	$0.001 > p$

## RESULTS

### *Slide culture*

*HeLa cells* (Table I). Carbocain in a concentration of 0.007 % in the culture produced a significant reduction of the number of cells. Higher concentrations caused highly significant deviations from the control and 0.107 % inhibited all growth of cells on the slide. At 0.007 % Marcain in the cell culture the reduction in the number of cells was almost significant. Higher concentrations produced a highly significant reduction; 0.057 % entirely inhibited growth.

Carbocain in a concentration of 0.028 % produced an almost significant reduction of the mitotic count, while Marcain in such a low concentration as 0.014 % reduced the mitotic count significantly.

No significant differences were found between equal concentrations of Carbocain and Marcain regarding their effect on the cells count and mitotic frequency.

\* AB Bofors Nobel-Pharma, Mölndal, Sweden.

Table I.

*Effect of Carbocain and Marcain on HeLa-cells. Number of cells per field of vision and mitotic figures per 100 cells [ $M \pm \epsilon(M)$ ]*

Concentration (% w/v)	Carbocain		Marcain	
	Cells/field of vision	Mitotic figures/ 100 cells	Cells/field of vision	Mitotic figures 100 cells
Control	10.8±0.8	3.4±0.5	10.8±0.8	3.4±0.5
0.004		Non tested	10.0±0.7	4.1±0.5
0.007	7.9±0.5**	3.1±0.4	8.1±0.8*	4.0±0.5
0.014	4.4±0.7***	2.4±0.5	4.2±0.6***	1.4±0.3**
0.028	3.6±0.6***	1.9±0.5*	2.0±0.5***	0.8±0.4**
0.057	0.8±0.2***	0	t.i.c.	t.i.c.
0.107	t.i.c.	t.i.c.		Non tested

\* = 0.05 > p > 0.01; \*\* = 0.01 > p > 0.001; \*\*\* = 0.001 > p.

t.i.c. = total inhibitory concentration.

*Fibroblasts.* (Table II). Carbocain in a concentration of 0.107 % in the culture significantly reduced the cell count which was, however, not affected by lower concentrations.

At 0.007 % and 0.014 % Marcain produced an almost significant reduction, while 0.028 % gave a significant reduction. In a concentration of 0.057 % Marcain inhibited all cell growth.

Table II.

*Effect of Carbocain and Marcain on human skin fibroblasts. Cells per field of vision [ $M \pm \epsilon(M)$ ]*

Concentration (% w/v)	Cells/field of vision	
	Carbocain	Marcain
Control	6.1±0.5	6.1±0.5
0.004	Non tested	6.2±0.8
0.007	5.2±0.6	4.1±0.5*
0.014	5.4±0.7	3.7±0.8*
0.028	4.0±0.4	2.9±0.6**
0.057	5.7±0.7	t.i.c.
0.107	3.3±0.5**	Non tested

\* = 0.05 > p > 0.01; \*\* = 0.01 > p > 0.001

t.i.c. = total inhibitory concentration.

Table III.  
*Effect of Carbocain and Marcain on respiration of HeLa-cells. Oxygen uptake given in per cent of respiration before addition of test substance*  
 $[M \pm \varepsilon(M)]$

Concentration (% w/v)	Test period					
	0'—60'		60'—120'		0'—120'	
	Carbocain	Marcain	Carbocain	Marcain	Carbocain	Marcain
0.248	65.2 ± 2.5***	50.9 ± 2.9***	80.8 ± 2.9***	14.6 ± 3.4***	73.0 ± 2.2***	32.7 ± 2.8***
0.124	112.3 ± 8.2	66.0 ± 2.2***	103.1 ± 3.4	57.9 ± 1.2***	107.7 ± 5.6	62.0 ± 1.3***
0.062	116.1 ± 11.4	80.1 ± 4.3***	112.9 ± 6.5	85.7 ± 5.7**	114. ± 64.6	82.9 ± 4.9**
Control	105.4 ± 1.8		105.3 ± 1.2		105.4 ± 1.4	

\* = 0.05 > p > 0.01; \*\* = 0.01 > p > 0.001; \*\*\* = 0.001 > p.

On comparison between Carbocain and Marcain the lowest concentration producing a significant reduction of the cell count was 0.028 %, at which Marcain had a stronger reducing effect than Carbocain.

On comparison between Carbocain and Marcain it was found that only at 0.028 % was there a significant difference in the cell count. At this concentration Marcain had a stronger reducing effect than Carbocain.

### *Respirometry*

Both Carbocain and Marcain in a concentration of 0.248 % had a highly significant effect on the respiration of the cells (Table III). But the effect of Marcain was significantly to highly significantly stronger than that of Carbocain.

In concentrations up to 0.124 % Carbocain had no effect on the uptake of oxygen.

In the presence of 0.124 % Marcain the respiration of the cells was highly significantly lower than in the presence of Carbocain in a corresponding concentration and than in the controls. At 0.062 % Marcain in the culture respiration differed significantly from that of the controls, and the deviation during the first and second experimental period differed almost significantly from that of Carbocain.

### DISCUSSION

Marcain is a stronger anaesthetic than Carbocain, and in dental practice a concentration of 0.25 % or 0.5 % is sufficient to produce a good effect (*Feldmann & Nordenram, 1966*), which requires a 2–3 % solution of Carbocain. It was therefore considered relevant to study the effect on cell cultures at equivalent concentration. Thus if 0.004 %, 0.007 % and 0.014 % Marcain are compared with fourfold concentrations of Carbocain, Marcain will have significantly lower toxic effect on HeLa cells studied according to the »slide culture» technique. No such difference, however, can be demonstrated in corresponding comparisons of their effect on human fibroblasts.

In respirometric experiments comparison between the effect of 0.062 % Marcain and fourfold concentrations of Carbocain revealed an almost significantly stronger effect of Carbocain during the first experimental period, but no difference during the second. The ratio found between the toxicity of the two anaesthetics was in agreement with that reported by *Henn & Brattsand (1966)*. With the use of a trypan blue-test, they compared the effect of *inter alia* Carbocain and Marcain after intracutaneous injection into rabbits and found that the threshold irritant concentration of Carbocain was 3 % and of Marcain 0.6 %.

## SUMMARY

The cytotoxic effect of Carbocain and that of Marcain were compared *in vitro*. When tested on HeLa cells in slide cultures no difference was found regarding the effect of the anaesthetics on cell count and mitotic frequency. In tests on human fibroblast cultures it was found that the lowest concentration of the anaesthetics capable of producing a reduction in cell count was 0.028 %, and that then the toxic effect of Marcain was the greater.

Analysis of the respiration of HeLa-cells showed that Marcain produced a stronger reduction than Carbocain.

Marcain is a more potent anaesthetic than Carbocain and therefore it was regarded as justified to compare the cytotoxic effect of Marcain with the fourfold concentration of a Carbocain solution. In such a comparison Marcain was found less toxic.

## RÉSUMÉ

## EFFET CYTOTOXIQUE DE LA CARBOCAÏNE ET DE LA MARCAÏNE

L'effet cytotoxique de la Carbocaïne et celui de la Marcaïne ont été comparés *in vitro*. Lorsqu'on les testait sur des cellules HeLa en cultures sur lames, on ne trouvait pas de différence en ce qui concernait l'effet des anesthésiques sur le nombre de cellules et sur la fréquence des mitoses. Lorsqu'on les testait sur des cultures de fibroblastes humains, on constatait que la plus basse concentration d'anesthésique capable de déterminer une diminution du nombre de cellules était 0,028 %, et que l'effet toxique de la Marcaïne était alors plus grand que celui de la Carbocaïne.

Lors de l'analyse de la respiration des cellules HeLa, on a constaté que la Marcaïne déterminait une plus forte réduction que la Carbocaïne.

La Marcaïne étant un anesthésique plus puissant que la Carbocaïne, on a considéré qu'il était légitime de comparer l'effet cytotoxique de la Marcaïne avec celui d'une solution quatre fois plus concentrée de Carbocaïne. Dans cette comparaison, la Marcaïne présentait une toxicité moindre que la Carbocaïne.

## ZUSAMMENFASSUNG

## DIE WIRKUNG VON CARBOCAIN UND MARCAIN AUF HELA ZELLEN UND HAUT-FIBROBLASTEN IN VITRO

Die *in vitro* cytotoxische Wirkung von Carbocain wurde mit der von Marcain verglichen. In Experimenten mit HeLa Zellen hat man keinen Unter-

schied zwischen diesen Betäubungsmitteln in bezug auf ihre Wirkung auf die Anzahl der Zellen oder die mitotische Frequenz in kulturen auf Glasscheiben gefunden. In Experimenten mit menschlichen Fibroblastkulturen erwies es sich, dass die niedrigste Konzentration der Betäubungsmittel, welche die Anzahl der Zellen reduzieren könnte, war 0.028 % und dass die toxische Wirkung von Marcain grösser war als die von Carbocain.

Eine Analyse der Respiration von HeLa Zellen zeigte, dass Marcain eine grössere Reduktion zeugte als Carbocain.

Marcain ist ein stärkeres Betäubungsmittel als Carbocain. Daher schien es berechtigt, die Wirkung von Marcain mit der einer vierfachen Konzentration von Carbocain zu vergleichen. In solchen Vergleichen erwies sich Marcain als weniger toxisch.

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