EXPERIMENTAL BIOLOGY

PAIN EFFECTS ON CELL DIVISION IN THE EPITHELIUM OF MOUSE CORNEA

I. A. Utkin and L. P. Kosichenko

From the Sukhumi Medico-biological Station (Director – Candidate in Biological Sciences I. A. Utkin) of the Academy of Medical Sciences USSR

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Indications exist that the application of stimuli which disturb an animal or pain him lead to inhibition of cell division in the corneal epithelium [2, 3, 6]. Investigating the effect of various kinds of pain stimuli and trauma on the cell division of the epithelial cells of mouse cornea, we came across some mitotic changes which had not been described in the literature.

EXPERIMENTAL METHODS AND RESULTS

The experiments were carried out on white mice (male) of approximately the same age. The methods of preparing the cornea and counting the mitoses were described in an earlier communication [5]. The mitotic index (number of cell divisions in 100 fields, corresponding to an area of 1 mm^2 of cornea) was determined for the entire cornea, as well as for separate zones (peripheral, middle and central) of it. Before the beginning of the investigations all the animals were kept several days in the building under the same conditions as would be used for the experiment. In all, 8 series of experiments were carried out on 114 mice.

In the first series, 14 mice were placed in two cages, 7 in each cage. Both cages were side by side in a room. In the first experimental group, a thread was drawn through the middle of the left hind foot in the metatarsal area and was tied tightly around half of the limb. Both groups were killed simultaneously at 11 o'clock, 2 hours after the injury. The average indexes, characterizing the mitotic activity of the cornea of the experimental and control animals in this series are shown in Table 1. The results of this experiment corresponded to those found by other authors: pain caused a distinct fall in the number of mitoses in the cornea. Inhibited cell division was found in all the corneal zones, especially in the peripheral area.

As is apparent from the presented data, the difference in the mitotic activity of the experimental and control corneas lay not only in the total number of divisions but in the correlation of the stages of mitosis. The number of metaphases was lowest in the injured animals: 2 hours after the injury, there were almost half as many on the periphery of the cornea as in the control.

In order to obtain sharper changes in the mitotic scheme of the corneal epithelium, we used a stronger traumatic stimulus in the next series: shattering the limb with injury to the integument in the metatarsal area.

In the second series of experiments (8 experimental and 8 control mice) the animals were killed 30 minutes and 2 hours after the limb injury. 30 minutes after the injury, the number of cell divisions in the corneal epithelium of the experimental animals was not found to be less than that of the controls (Table 1). The mitotic indexes of the peripheral zones of both groups almost coincide, while in the middle and central zones they were noticeably higher in the corneas of the injured animals. Two hours after the pain stimulus, the number of mitosis in the corneas of the experimental mice exceed the number of dividing cells in the corneas of the control mice by even more.

TABLE 1

Effect of Injuring a Limb on the Mitotic Activity of the Cornea

Corneal zone	Mitotic stage	Experimental series: first, 2 hr after injury, control	first, 2 hr after injury, expt.	second, 30 min after injury, control	second, 30 min after injury, expt.	second, 2 hr after injury, control	second, 2 hr after injury, expt.	third, before injury (control)	third, 30 min after injury (expt.)	fourth, before injury	fourth, 30 min after injury, control	fourth, 30 min after injury, expt.	fourth, 2 hr after injury, control	fourth, 2 hr after injury, expt.
Peripheral	P	63	43	40	68	15	22	63	34	35	42	44	20	30
	M	111	63	73	58	41	72	145	24	106	46	25	52	72
	A	33	27	13	9	17	27	38	16	28	4	10	13	26
	TL	28	28	10	7	25	33	50	25	37	6	15	23	38
	TL	34	25	10	12	23	30	29	27	23	11	18	12	21
Total		269 ±21	186 ±24	146 ±12	144 ±7	121 ±17	184 ±32	325 ±25	126 ±19	229 ±22	109 ±18	112 ±12	125 ±6	187 ±32
Middle	PC	82	67	54	115	42	47	66	36	68	73	58	32	52
	M	141	91	78	68	94	152	143	46	177	47	33	74	103
	A	44	31	20	17	34	36	35	25	33	7	21	27	44
	TE	39	35	17	20	45	55	55	50	53	17	38	38	71
	TL	44	30	21	28	34	48	33	33	37	24	36	20	36
Total		350 ±28	254 ±30	190 ±15	248 ±13	249 ±31	338 ±40	322 ±14	190 ±14	368 ±22	168 ±9	186 ±9	191 ±14	306 ±27
Central	P	87	72	48	92	55	49	56	28	99	87	23	45	63
	M	148	104	79	63	141	126	109	45	177	50	27	107	107
	A	37	34	17	19	41	43	18	18	39	17	19	24	49
	TE	36	33	18	24	44	47	35	34	55	35	30	34	73
	TL	34	29	20	36	32	26	16	24	27	37	22	22	25
Total		342 ±28	272 ±28	182 ±28	234 ±24	313 ±38	291 ±21	234 ±18	149 ±13	397 ±27	226 ±47	121 ±9	232 ±57	317 ±36
Average index for cornea as a whole		300 ±21	215 ±24	162 ±10	189 ±9	179 ±19	238 ±30	314 ±16	148 ±14	289 ±18	139 ±25	134 ±7	157 ±9	236 ±30

Symbols: P – prophase, M – metaphase, A – anaphase, TE – early telophase, TL – late telophase.

This phenomenon, it would seem, should be regarded as the result of the absence of mitotic inhibition during severe limb injury. However, this problem required clarification in connection with the fact that the indicators which characterize the mitotic scheme of the corneas of the control animals proved to be exceptionally low in this series of experiments. In view of this, the control group of mice in the third series was killed before, and the experimental one 30-40 minutes after injury (both groups were kept in different cages).

The mitotic activity of the corneas of the control animals corresponded in this case to that usual for the summer-fall period during which the experiments were carried out. At the same time, the mitotic activity of the corneas of the injured mice decreased sharply, especially on the periphery of the cornea. This decrease affected nearly all the stages of the mitotic cycles, failing to affect only the late stages of nuclear reconstruction. In the center of the cornea the mitotic inhibition was weaker and only affected the early stages of cell division. There is no doubt about the fact that there is a sharp decrease in mitosis as a result of injury.

In the fourth series, the control and experimental animals were killed before the pain stimulus and 30 minutes and 2 hours after it.

As shown in Table 1, the number of mitoses in the control and experimental groups of this series fell sharply. In the control group, after 30 minutes the average number of mitoses decreased by half. In addition, the inhibition of cell division is obvious in all the epithelial zones, but, as in the previous cases, is especially marked on the periphery. The number of metaphases in this zone decreased by more than half, the number of anaphases by 1/7, and of telophases by 1/6. The general picture of changes in the mitotic scheme of the cornea of the experimental animals is close to that observed in the corresponding control. By the end of the second hour of the experiments the number of mitoses in the corneas of the experimental animals approached the original number. At the same time, the corneas of the control group of mice remained at the previous very low level of mitotic activity. The results of this series of experiments reproduced the picture of changes in mitotic activity of the animals' corneas in the second series without serious deviations.

DISCUSSION OF EXPERIMENTAL RESULTS

On the basis of the above facts, can injury of a limb be considered as an inhibitor of cell division? We believe that such a statement is justified with regard to periods soon after injury. It follows, in particular, from the first and third series of experiments.

What determines the sharp decrease in the mitotic activity of the corneas of completely healthy, apparently intact animals in that case? First of all, the fact that the control mice, which were killed prior to injury (third and fourth series of experiments) or simultaneously with the experimental animals, but 2 hours after a slighter injury of the limb (first series of experiments), had comparatively high and coincident indexes of mitotic activity, becomes obvious. The mitotic indexes were usually low in the same control animals which were with severely injured mice under the same experimental conditions.

However, during analysis of the data of the second and fourth series it is essential to keep in mind that the killing of the mice was accompanied by changes in the numerical composition of the experimental and control groups. In addition, during the procedure of injuring the animals, they were removed from the cages where they were usually kept, although for a very short time (the same was done with the control mice). All of these circumstances, as we indicated in a previous communication [5] have a great effect on the mitotic division of corneal cells.

Nevertheless, the sharp changes in mitotic activity of the corneas of the control mice which were near the injured ones cannot be explained solely by the above-mentioned changes. First of all, the sharply decreased number of mitoses compared with the usual control indicators was found in the first group of the second series, i. e. before any change in the numerical composition of the group. Further, the mitotic inhibition in the subsequent groups of mice in the second and fourth series was so marked that both in its extent and duration it exceeded what we observed during changes in the composition of the group of mice [5].

The thought arose that the proximity of mice which had suffered pain was possibly not a matter of indifference to the mitotic activity of the normal animals; severe injury could be accompanied, for example, by sound reactions. Sound stimuli can change the mitotic regime of the corneal epithelium [4].

In order to clarify this problem several series of experiments (20 mice in each series) were set up. The animals were placed in 4 cages – 5 in each one. Two cages were placed in one room, two in another close to each other. In one room both groups of mice remained intact. In the other, one group of mice received a severe injury to a limb. After a certain length of time, 5 completely intact mice were killed and 5 mice which were next to the injured ones. In the fifth series the mice were killed 10 minutes after the injury, in the sixth one 30 minutes after it.

The decreased number of mitoses at the above times after stimulation was most evident in the metaphase stage (as in the majority of the preceding experiments). In connection with this we present the data on this stage of cell division in greater detail (Table 2).

If there was a hardly perceptible decrease in the average number of metaphases in the peripheral zone of the cornea (116 as against 133 in the control) after a 10 minute sojourn by the mice next to injured ones, a 30 minute proximity had a sharper and quite distinct effect on the control mice: the total number of metaphases in the corneas of these mice decreased 3[illegible]%. In addition, the maximum index in the experimental group (137) was considerably less than that of the control (159). This indicates the incontrovertible existence of this effect. It is again most marked on the periphery; in this zone the number of metaphases decreased by almost half.

TABLE 2

Number of Metaphases in Mouse Corneas

Expt. series	Control mice					Experimental mice				
	mouse No.	periphery	middle	center	cornea as a whole	mouse No.	periphery	middle	center	cornea as a whole
Fifth	1	104	102	61	96	1	109	91	76	100
	2	133	132	139	133	2	97	83	68	91
	3	57	59	53	54	3	105	90	48	96
	4	176	218	113	177	4	133	96	60	115
	5	197	68	24	154	5	135	183	145	149
Average		133 ±22	114 ±26	78 ±18	123 ±19		116 ±7	109 ±17	79 ±15	110 ±9
Sixth	6	171	143	131	159	6	100	95	97	98
	7	197	202	116	189	7	125	104	91	116
	8	230	122	66	183	8	126	113	61	114
	9	209	165	58	188	9	146	119	118	137
	10	182	137	114	164	10	101	125	69	102
Average		198 ±9	154 ±12	97 ±13	176 ±7		119 ±8	111 ±5	87 ±9	113 ±6

Not only the sharp decrease in the absolute number of metaphases indicates the undoubted regularity of the changes observed in the mitotic scheme, but also the disruption of the relationship of the mitotic stages usually common to intact animals. In the control, 27% were prophases, 47% methaphases, 8% anaphases and 18% telophases; in the experimental group the figures were 34, 30, 10, 26% correspondingly. In the fifth series, which only gave an indication of the initial changes in the mitotic scheme, the number of prophases also proved to be lower, although this decrease was not statistically significant.

The seventh series of experiments was carried out approximately in the same way as the sixth, with the exception that the animals of both groups were removed from their cages for some time before injury. This procedure, although it changed the mitotic scheme of the corneas to some extent, did not eliminate the differences in the number of mitoses in the animals which sat next to the injured ones and those which had intact mice as neighbors. The number of prophases in the corneas of mice of the first group compared with the second group was increased insignificantly (by 5%), while the number of meta- and anaphases was decreased considerably (the former by 23%, the latter by 39%). Material differences were not found in the number of telophases (the deviation was 1%). In other words, the inhibition of cell division which developed told on the initial phases. At later periods (an hour after injury) we observed the transposition of the wave of inhibited mitoses in the cornea through all the final phases of mitotic division.

The above facts indicate without doubt that the presence of injured mice in the next cage causes changes in the mitotic scheme of completely normal animals which suffered no direct injury. The inhibition begins at the early stages of cell division and gradually continues to the later stages. The extent of the decrease in the number of mitoses and nature of the change in the stages depends on the length of time elapsing after the injury and, apparently, on the strength of the pain stimulus.

As regards the direct effect of injury on cell division, the material obtained only allows the following thoughts to be expressed. Injury to an animal causes a decrease in the number of cells undergoing division. This is evident immediately after the injury and is connected with the general systemic reaction to pain stimulation. It is possible that one of the most important factors determining the inhibitory effect in this case is adrenalin, as some investigators suppose [1, 6]. Subsequent increases in the number of mitoses in comparison with their corresponding number in the control indicate that after greater lengths of time pain stimulation can cause an increase in the number of cell divisions. Whether this is connected with stimulation of cell division, or with an increase in the length of time for it to take place, is not as yet known.

LITERATURE CITED

[1] I. A. Alov, G. Ya. Pavlenko and M. V. Sukhinina, Byull. Eksptl. Biol. i Med. 1955, No. 4, pp 63-65.

[2] S. Ya. Zalkind, Doklady Akad. Nauk SSSR, Vol. 99, No. 6, pp. 1091-1093.

[3] G. S. Strelin, I. B. Bychkovskaya and V. V. Kozlov, Doklady Akad. Nauk SSSR Vol. 99, No. 1, pp. 165-167 (1954).

[4] I. A. Utkin, Byull. Eksptl. Biology i Med., Vol. 36, No. 11, pp. 52-55 (1953).

[5] I. A. Utkin and L. P. Kosichenko, Byull. Eksptl. Biol. i Med., Vol. 41, No. 4, pp. 62-66 (1956) (T. p. 349).

[6] J. S. Friedenwald and W. Buschke, Am. J. Physiol. 141, 5, 689-694 (1944).

*T. p. = C. B. Translation pagination.