Heightened Sensitivity to the Convulsant Corazol in Mice Adoptively Transferred with Splenocytes from Mice with Corazol-Induced Kindling

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Recipient mice adoptively transferred with splenocytes from donor mice that had undergone corazol-induced kindling showed increased sensitivity to this convulsant, as was found both in determining the threshold corazol doses required to elicit clonic convulsions or tonic seizures culminating in death and in using a model of corazol-induced kindling.

Key Words: *kindling; corazol; convulsions; lymphocytes; adoptive transfer*

Recent animal studies have provided mounting evidence that cells of the immune system are able to retain the "memory" of the pathological process occurring in certain nervous system disorders: when transferred to an intact animal, such cells induce in it some of the manifestations characteristic of the pathological process in question. For example, B lymphocytes have been shown capable of transferring reduced susceptibility to morphine from mice with low morphine sensitivity to intact recipients [4] and, as our previous study demonstrated, some signs of the parkinsonian syndrome are also reproducible through such immunological transfer [1].

In the present study, an attempt was made to transfer to intact mice the proneness to convulsions shown by the brain of mice that had undergone corazol-induced kindling. The latter has been widely used as a research tool in the study of chronic epileptogenesis, learning, memory, and other phenomena [2,3,5-7].

MATERIALS AND METHODS

For adoptive transfer to intact mice, we used splenocytes from 15 donor (CBA×C57Bl/6)F_1 mice in which kindling had been induced through daily intraperitoneal administration of corazol in a subconvulsive dose (30 mg/kg) for 30 days. Control mice (n=10) received daily injections of physiological saline in the same volume over the same period. The severity of the convulsive response was evaluated daily using a scoring system in which score 1 was assigned to momentary shuddering and nodding; score 2 to discrete clonic convulsions of the whole body; score 3 to a series of clonic convulsions of the whole body or to clonus of the forelimbs; score 4 to tonic-clonic convulsions with rearing up on the hind legs ("kangaroo" posture); score 5 to clonic-tonic convulsions that caused the mice to fall on their side and included a phase of tonic extension; and, finally, score 6 to repetitive tonic-clonic convulsions and/or death of the animal. The overall convulsive response in each group was evaluated by averaging the scores; only mice in which this response occurred were included in the analysis. After the discontinuation of corazol dosing (on day 30), the severity of convulsions had an average score of 4 to 5. On the second day after the last corazol or saline injection, the mice were killed, their thymuses and spleens were removed and weighed, the number of lymphocytes per spleen was counted, and lympho-

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TABLE 1. Sensitivity of Mice to Corazol on Day 7 after Adoptive Splenocyte Transfer ($M \pm m$)

Group	Number of mice	Corazol dose resulting in:			
		clonic convulsions		death	
		mg/kg	%	mg/kg	%
Control group 1: saline	8	23.32±0.41	100±1.76	55.65±2.80	100±5.03
Control group 2: splenocytes (saline for 30 days)	11	24.50±0.86	105.06±3.69	48.98±2.69	88.01±4.83
Test group: splenocytes after kindling	11	19.97±0.48*	85.63±2.06	38.24±2.13**	68.71±3.82

Note. *$p<0.001$ in comparison with control groups 1 and 2; **$p<0.001$ in comparison with control group 1 and $p<0.01$ in comparison with control group 2.

cyte suspensions were prepared. To remove erythrocytes from the suspensions, a hypotonic shock was induced by adding 10 volumes of distilled water for 10 sec and then 10 volumes of doubly concentrated medium 199 to a concentrated cell suspension. Only suspensions in which at least 95% of splenocytes were found to be viable by the trypan blue exclusion test were used. Intact recipient mice were each injected with 2×10^7 splenocytes intraperitoneally.

The effect of adoptive splenocyte transfer on the corazol sensitivity of recipient mice was studied in two series of tests. In the first series, recipient mice were tested 1, 7, and 14 days after the adoptive transfer for susceptibility to convulsions by injecting them intravenously with 1% corazol solution at a rate of 0.01 ml/sec. Thresholds for clonic convulsions and tonic convulsive seizures leading to death were recorded. The threshold corazol dose required to elicit such convulsions was calculated for each animal in mg/kg. Animals transferred with splenocytes in the same number from mice administered saline for 30 days as well as those given a single intraperitoneal injection of physiological saline served as controls.

In the second series, the effect of adoptive transfer on the development of corazol kindling in recipient mice was assessed. Kindling induction was started on the second day after splenocyte transfer.

The significance of differences between the control and test mice was estimated by Student's t test.

RESULTS

The mice subjected to kindling with corazol had spleens of decreased weight as compared to their control counterparts after the 30-day treatment with saline (0.337±0.017% body weight *vs.* 0.428±0.033% body weight; $p<0.05$). The two groups did not differ significantly in thymus weight.

In the first test series, the corazol sensitivity of recipient mice at 24 h after adoptive splenocyte transfer was the same as before it. On day 7 after the transfer, smaller corazol doses were required to elicit clonic convulsions or tonic seizures culminating in death (Table 1), while on day 14 only the corazol dose required to elicit clonic convulsions was lower (Table 2). It should be noted that the transfer of splenocytes from the control mice administered saline did not significantly alter the corazol sensitivity of recipients as compared to splenocytes transferred from mice given only one injection of saline (Tables 1 and 2).

In the second series, in which the development of kindling in recipient mice was followed, convulsions assigned score 1 were observed in 1 of the 11 control mice after 3 daily corazol injections and in 4 mice (36%) after 4 injections. After the transfer of splenocytes from mice that had been receiving saline for 30 days, convulsions after the

TABLE 2. Sensitivity of Mice to Corazol on Day 14 after Adoptive Splenocyte Transfer ($M \pm m$)

Group	Number of mice	Corazol dose resulting in:			
		clonic convulsions		death	
		mg/kg	%	mg/kg	%
Control group 1: saline	12	23.66±1.22	100±3.57	47.07±2.31	100±4.90
Control group 2: splenocytes (saline for 30 days)	11	26.01±0.85	117.58±3.84	52.56±4.29	111.66±9.11
Test group: splenocytes after kindling	11	22.57±1.03*	103.16±5.51	53.23±4.95	113.08±10.5

Note. *$p<0.02$ in comparison with control group 2.

testing corazol dose occurred in 2 out of 12 mice on day 2 and in 4 out of 12 (36%) on day 4. Among the 12 test mice transferred with splenocytes from animals that had undergone kindling, convulsions scoring 1 were recorded in 2 after the first injection, while those scoring 1.5 (moderate convulsions) were recorded in 4 (36%) after the second injection and in 8 (66.7%) after the fourth. During the first 10 days, convulsions given the highest score (6) occurred in 3 mice transferred with splenocytes from donors subjected to kindling and only in 1 mouse among those transferred with cells from donors administered saline for 30 days; in the control group, none of the mice was seen to develop convulsions with the highest score. Subsequently, no intergroup differences in responses to the testing corazol dose were detectable.

The results of this study point to enhanced corazol sensitivity in intact mice adoptively transferred with splenocytes from donors subjected to kindling with this convulsant. This effect was demonstrable both when determining the threshold corazol doses eliciting clonic convulsions or tonic seizures with a fatal outcome (first test series) and when using the model of corazol-induced kindling (second series). Heightened corazol sensitivity was shown by recipient mice on day 7 after the adoptive splenocyte transfer from donor mice with corazol-induced kindling, after which time the sensitivity appears to have progressively declined.

The mechanisms underlying the detected effects from the transfer of corazol sensitivity require separate study. We have shown previously that intact mice adoptively transferred with splenocytes from donor mice in which a parkinsonian syndrome had been produced with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine developed some signs of this syndrome [1]. Lymphocyte transfer from mice with genetically determined reduced susceptibility to the analgesic action of morphine to mice with normal morphine susceptibility has been reported to make the latter less susceptible to morphine-induced analgesia [4]. The lymphocytes of animals that have experienced nervous system disorders of various kinds acquire a capacity for reproducing, to some extent, certain signs of the respective disorders upon transfer to intact recipients, which indicates that this phenomenon is a general one rather than confined to some particular form of nervous system pathology. It is significant that immunocytes not only "carry" the memory of the experienced nervous system disease, but can also be implicated in the induction of that disease. It remains to be established which fractions of immunocompetent cells (whether T cells, B cells, macrophages, or their combinations), and which factors they produce or respond to, are responsible for this phenomenon.

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