BMC Pharmacology

BioMed Central

Research article

Anticonvulsant, sedative and muscle relaxant effects of carbenoxolone in mice

Hossein Hosseinzadeh*[1] and Marjan Nassiri Asl[2]

Address: [1]Pharmaceutical Research Center, Faculty of Pharmacy, Mashhad University of Medical Sciences, P.O. Box: 1365-91775, Mashhad, I. R. Iran and [2]Department of Pharmacology, Faculty of Medicine, Mashhad University of Medical Science, Mashhad, I.R. Iran

Email: Hossein Hosseinzadeh* - hosseinzadehh@yahoo.com; Marjan Nassiri Asl - nassiriaslm@yahoo.com

* Corresponding author

Published: 29 April 2003

BMC Pharmacology 2003, **3**:3

Received: 24 December 2002
Accepted: 29 April 2003

This article is available from: http://www.biomedcentral.com/1471-2210/3/3

Abstract

Background: Carbenoxolone, as an antiulcer medicine, has some pharmacological properties such as: the inhibition of gap junctional (GJ) intercellular communication. In vitro studies have shown, carbenoxolone to abolish the generation of full or partial ectopic spike generation, by 4-aminopyridine, as well as spontaneous epileptiform activity in CA_3 or CA1 regions of the rat hippocampal slices via closing GJ channels. Thus, we considered the possible anticonvulsant effects of carbenoxolone in animal seizure models.

Results: ED_{50} values of diazepam and carbenoxolone in the pentylenetetrazole model were 1.13 mg/kg and 283.3 mg/kg, respectively. In this model, carbenoxolone in doses of 200 and 300 mg/kg prolonged the onset time of seizure and decreased the duration of seizures. In the maximal electroshock model, carbenoxolone in a dose of 400 mg/kg decreased the duration of seizure producing protection against seizure but failing to protect against mortality in comparison with diazepam. In the potentiation of pentobarbitone sleep test, carbenoxolone significantly increased sleeping time and decreased latency in doses of 100, 200 and 300 mg/kg in mice dose dependently. In the traction test, carbenoxolone (400 mg/kg) showed muscle relaxant activity and in the accelerated rotarod test, carbenoxolone in doses of 200 and 300 mg/kg showed a decline in motor coordination.

Conclusion: It can be concluded that carbenoxolone possesses anticonvulsant, muscle relaxant and hypnotic effects, which could contribute to the control of petit mal and grand mal seizures.

Background

Carbenoxolone, the succinyl ester of glycyrrhetinic acid [1], is now used in clinical treatment of ulcer diseases [2]. It has some pharmacological properties such as: the inhibition of 11β-hydroxy steroid dehydrogenase (11β-HSD) [1], and inhibition of gap junctional intercellular communication [3]. Electrotonic synaptic communication between neurons via gap junctions (GJs) is increasingly recognized as an important synchronizing mechanism in the brain. The pathogenesis of abnormal neuronal synchrony underlying seizures, formerly thought to be based mainly on the chemical synaptic transmission, now includes a role of gap junctional communication. This concept has been strengthened by evidence from several in vitro models, in which pharmacological manipulations of gap junctional communication predictably affect the generation of seizures: blockers diminishing seizure, and enhancers increasing the seizures [4]. GJ channels can contribute to the prolongation of epileptiform discharges [5].

Table 1: Anticonvulsant effect of carbenoxolone in the pentylenetetrazole-induced convulsion in mice

Treatment	Dose	Onset of seizure(sec)	Duration of clonic seizure (sec)	Seizure protection (%)	Mortality protection (%)
Normal saline	10 ml/kg	50.83 ± 8.14	25.83 ± 4.47	0	0
Diazepam	0.1 mg/kg	45.58 ± 2.61	9.68 ± 0.49***	0	0
	0.5 mg/kg	121.73 ± 4.91	7.9 ± 0.52***	0	100
	1 mg/kg	439.61 ± 60.80***	4.7 ± 1.67***	42.8	100
	1.5 mg/kg	587.18 ± 12.86***	0.71 ± 0.71***	85.7	100
	3 mg/kg	600***	0***	100	100
Carbenoxolone	100 mg/kg	213.14 ± 66.59	6.35 ± 1.55***	14.3	57.14
	200 mg/kg	388.9 ± 71.74*	3.28 ± 1.06***	28.6	57.14
	300 mg/kg	464.9 ± 66.6**	3 ± 1.91***	57.14	28.6

Carbenoxolone and diazepam (i.p.) were injected 60 and 30 minutes respectively, before the administration of pentylenetetrazole (i.p. 90 mg/kg). Values are the mean ± SEM for 7 mice. *P < 0.05, **P < 0.01, ***P < 0.001, Compared to control, Tukey-Kramer Test.

Table 2: Anticonvulsant effect of carbenoxolone against seizure induced by maximal electroshock in mice

Treatment	Dose	Duration of tonic seizure (sec)	Seizure protection (%)	Mortality protection (%)
Normal saline	10 ml/kg	17.57 ± 1.72	0	14.28
Diazepam	0.25 mg/kg	11.8 ± 0.48**	0	100
	0.5 mg/kg	8.63 ± 0.21***	0	100
	3 mg/kg	6.06 ± 1.27***	14.3	100
Carbenoxolone	100 mg/kg	12.11 ± 2.28	14.3	85.7
	200 mg/kg	12.10 ± 2.04	14.3	87.7
	300 mg/kg	11.85 ± 1.61	14.3	60
	400 mg/kg	9.16 ± 1.71*	28.6	14.28

Carbenoxolone and diazepam (i.p.) were injected 60 and 30 minutes respectively, before the induction of maximal electroshock seizures, respectively. Values are the mean ± SEM for 7 mice. *P < 0.05, ***P < 0.001, Compared to control, Tukey-Kramer Test.

In in vitro studies, carbenoxolone abolished the generation of full or partial ectopic spike generation, by 4-AP, in the CA_3 region of rat hippocampal slices [6]. Also carbenoxolone depresses spontaneous epileptiform activity in the CA_1 region of rat hippocampal slices [7]. Carbenoxolone appears to cross the blood brain barrier about one hour after an intraperitoneal injection in rats as there was the inhibition of 11-β-hydroxysteroid dehydrogenase in multiple brain regions [1]. It and/or its congener glycyrrehetinic acid may also enter the eyes of humans. Ingestion of licorice has been reported to cause transient visual loss [8], a possible consequence of retinal gap junction blockade.

Thus, we considered the possible anticonvulsant effect of carbenoxolone in animal seizure models.

Results

In pentylenetetrazole (PTZ) model, ED50 values of diazepam and carbenoxolone were 1.13 mg/kg (%95 CL : 0.89, 1.44) and 283.3 mg/kg (%95 CL : 144.27, 556.29), respectively.

In PTZ model, carbenoxolone increased the latency of seizures and decreased the duration of seizures, dose dependently (Table 1).

In maximal electroshock (MES) model, carbenoxolone (400 mg/kg) decreased the duration of tonic seizures. This effect was not statistically significant in the lower doses of carbenoxolone. This agent could not completely protect the mice against mortality in a dose of 400 mg/kg (Table 2).

In the potentiation of pentobarbitone sleep test, carbenoxolone significantly increased the sleeping time in mice at doses of 100, 200 and 300 mg/kg compared to controls dose dependently (Table 3). Also, carbenoxolone in doses of 100, 200 and 300 mg/kg decreased the latency compared to controls dose dependently and this effect was statistically significant (Table 3).

In the traction test, carbenoxolone did not induce a muscle relaxant activity significantly up to a dose of 400 mg/

Table 3: Potentiation of the pentobarbital sleep with carbenoxolone in mice

Treatment	Dose	Latency (min)	Duration (min)
Normal saline	10 ml/kg	7.28 ± 2.09	28.01 ± 1.88
Diazepam	1 mg/kg	4.67 ± 0.43**	49.16 ± 4.67***
Carbenoxolone	100 mg/kg	4.33 ± 0.25**	65.55 ± 5.06***
	200 mg/kg	4.24 ± 0.31**	75.10 ± 7.34***
	300 mg/kg	3.63 ± 0.38***	93.32 ± 16.49***

Carbenoxolone and diazepam (i.p.) were injected 60 and 30 minutes respectively, before pentobarbital (30 mg/kg) respectively. Mean latency and duration of sleep in minutes ± SEM from 10 mice in each group. **P < 0.01, ***P < 0.001, compared to control, Tukey-Kramer test.

Table 4: Effect of Carbenoxolone on muscle relaxant activity by traction test

Treatment	Dose	% failure
Normal saline	10 ml/kg	0
Diazepam	0.125 mg/kg	0
	0.25 mg/kg	50*
	0.5 mg/kg	100***
Carbenoxolone	200 mg/kg	20
	300 mg/kg	40
	400 mg/kg	60**

Carbenoxolone and diazepam (i.p.) were injected 60 and 30 minutes respectively, before the test. Values are the mean ± SEM for 10 mice, *P < 0.05, **P < 0.01, ***P < 0.001, Compared to control, Fisher's exact test (two sided)

Table 5: Effect of carbenoxolone on muscle relaxant activity by accelerod test

Treatment	Dose	First trial (sec)	Second trial (sec)
Normal saline	10 ml/kg	300	300
Diazepam	1 mg/kg	7.6 ± 10.95**	24.96 ± 11.74**
Carbenoxolone	100 mg/kg	18.87 ± 6.57**	221.21 ± 37.55
	200 mg/kg	9.78 ± 1.60**	71.44 ± 38.25**
	300 mg/kg	3.55 ± 1.19**	33.46 ± 26.64**

Carbenoxolone and diazepam (i.p.) were injected 60 and 30 minutes respectively, before the first trial. Values are the mean ± SEM for 10 mice. **P < 0.01, Compared to control, Dunnett's test

kg. Carbenoxolone (400 mg/kg) showed muscle relaxant activity compared to normal saline, and this effect was more than diazepam in a dose of 0.25 mg/kg (Table 4).

In accelerod performances, carbenoxolone in the first trial in doses of 100, 200 and 300 mg/kg, showed a decline in motor function relative to normal saline (10 ml/kg) (Table 5). The second trial, carbenoxolone in doses of 200 and 300 mg/kg decreased the riding time on rotarod compare to normal saline (Table 5).

Discussion

Present results indicate that carbenoxolone has anticonvulsant activities in PTZ and MES models.

Agents affecting the PTZ model can inhibit petit mal seizure [9]. Thus, carbenoxolone may have activity on this kind of seizure. Carbenoxolone also showed activity against MES. This implies that carbenoxolone may have efficacy in grand mal seizure [9].

Less is known about the effects of carbenoxolone on GJs, although it has been proposed that glycyrrhetinic acid, to which carbenoxolone is related, binds directly to connexin molecules causing a conformational change leading to a closure of the junction [7]. Modeling studies indicate that neuronal synchronization can be mediated by low densities of GJs either between dendrites, as long as these dendrites are excitable [10], or between the axons of py-

ramidal cells [11]. Furthermore, the electrotonic coupling could be promoted during epileptogenesis [12,13].

Optical imaging of neural activity evoked by electrical stimulation in the cultured rat suprachiasmatic nucleus (SCN) revealed that the spread of depolarization was inhibited by gap junction blockers (halothane, octanol) but not by a blocker of voltage-depended Na^+ channels. Depolarization propagation was also inhibited by muscimol, a $GABA_A$ receptor agonist, in a dose dependent manner, and inhibition was reversed by bicuculline, a $GABA_A$ receptor antagonist. This indicated that the propagation of depolarization between SCN cells was inhibited by $GABA_A$ receptor activation. There were two possible explanations for this result. First, GABA might affect the permeability of gap junction channels. Secondly, muscimol might increase the chloride conductance independent of gap junctions, and might cause a decrease in the spread of depolarization. Furthermore, muscimol inhibited dye-transfer between neurons in the SCN culture in a dose-dependent fashion, so that GABA might have reduced gap junction communication between SCN neurons via the GABAA receptor [14]. In that study they could not clarify how the activation of a GABAA receptor and subsequent increase in chloride conductance impact gap junctions. In studies, it has been reported that increases in intracellular Ca^{+2} or decreases in intracellular pH can uncouple cells [15]. So they suggest that a participation of the GABA receptors in the regulation of Ca^{+2} concentration or intracellular pH could explain the uncoupling effect of muscimol. As a matter of fact it was suggested that the gap junction coupling state between neurons in SCN is not static but dynamically regulated by the $GABA_A$ receptor system [14]. Also in vitro guinea-pig brain preparation prolonged seizure-like activity elicited using a brief application of bicuculline was abolished by the GJ channel blockers octanol and glycyrrhetinic acid as well as by intracellular acidification [16].

It is suggested that carbenoxolone, by blocking GJ channels inhibits both the induction and duration of epilepsy in the in vivo models of seizures. From previous evidences we speculate that carbenoxolone, by closing GJ channels, possibly directly or via the GABAergic system, inhibits the spread of depolarization waves in the CNS and peripheral organs. These could explain the anticonvulsant, muscle relaxant and hypnotic effects of carbenoxolone. But further experiments need to establish this hypothesis.

At anticonvulsant doses, carbenoxolone produced a hypnotic effect in the pentobarbitone sleep test and motor impairment in the rotarod test, but only at a dose of (400 mg/kg), which had anticonvulsant effect, did carbenoxolone show muscle relaxant activity in the traction test.

In the potentiation of pentobarbitone sleep test, carbenoxolone significantly showed sedative effect. Glycyrrhetinic acid is an aglycone saponin extracted from licorice root [7] and saponines have hypnotic effects [17]. Carbenoxolone is also the succinyl ester of glycyrrhetinic acid. Thus, this can explain the sedative effect of carbenoxolone.

In the muscle coordination test, carbenoxolone showed a muscle relaxant effect. Synchronized firing is a widespread phenomenon in the mammalian brain [18,19] including the motor cortex [20], respiratory motor neurons [21,22] and limb motor neurons [23,24]. These studies have demonstrated the presence of gap junctions in many levels of the motor system, in both motor neurons and in premotor pattern generating circuits. Further gap junction coupling (GJC) has brought about robust coordination patterns, even in the absence of chemical synapses, and has been shown to mediate synchronization of neurons during motor behaviors [25].

On the other hand, as yet there is no information about the effects of carbenoxolone in the muscle tissues; therefore, the possibility exists that these channels may be in muscles and synchronize the firing between muscle cells. Carbenoxolone by blocking these channels causes motor impairment. However, a demonstration of whether these channels are, in fact, capable of mediating electrical transmission requires physiological experiments.

Thus, we conclude that carbenoxolone has anticonvulsant, muscle relaxant and hypnotic effects in the in vivo models, possibly via blocking GJ channels. The exact mechanism of these effects needs further investigation.

Materials and Methods

Chemicals

Carbenoxolone, pentylenetetrazole and pentobarbitone were obtained from Sigma. Diazepam was from Daru Pakhsh Pharmaceutical Co., Iran in the form of ampoule (2 mg/10 ml).

All drugs were dissolved in a physiological saline solution.

Animals

Male BALB/c mice, 25–30 g were obtained from the animal house of Pharmaceutical Research Center, Bu-Ali Research Institute of Mashhad University of Medical Sciences. The animals were housed in colony rooms with 12/12 h light/dark cycle at 21 ± 2°C and had free access to food and water.

All animal experiments were carried out in accordance with Mashhad University of Medical Sciences, Ethical Committee Acts.

Anticonvulsant activity

Pentylenetetrazole (PTZ) Seizure model

Mice were divided into groups of seven. Diazepam, carbenoxolone and normal saline were injected intraperitoneally (i.p) 30, 60 and 60 min respectively, before the administration of pentylenetetrazole (i.p, 90 mg/kg). The onset of a general clonus was used as the endpoint. The general clonus was characterized by forelimb clonus followed by full clonus of the body. The time taken before the onset of clonic convulsions, the duration of clonic convulsions, and the percentage of seizure and mortality protection were recorded [26].

Maximal electroshock seizure (MES) model

Mice were divided into groups of seven. An alternating current stimulus of 50 Hz and 150 mA through ear-clip electrodes was delivered for 0.2 sec to the experimental animals. A drop of 0.9% saline solution was poured into each ear prior to placing the electrodes. Diazepam, carbenoxolone and normal saline are injected intraperitoneally (i.p) 30, 60 and 60 min, respectively before the test. The duration of tonic convulsion (a tonic extension of the hindlimb), the percentage of seizure and the mortality protection were recorded [26].

Potentiation of sodium pentobarbitone sleep

Mice were divided into groups of ten. Carbenoxolone at doses 100, 200 and 300 mg/kg and diazepam at the dose of 1 mg/kg and normal saline (10 ml/kg) were injected interaperitonially to separate groups. 1 h after receiving carbenoxolone and normal saline and 30 min after receiving diazepam, each animal was injected sodium pentobarbitone (30 mg/ kg, i.p). The sleeping time was noted by recording the interval between the loss and regaining of righting reflex [27].

Muscle relaxant activity

This was examined by: (a) Traction test and (b) Rotarod test

Traction test

Forepaws of a mouse were placed on a small twisted wire rigidly supported above a bench top. Normal mice grasped the wire with forepaws and when allowed to hang free, placed at least one hind foot on the wire within 5 seconds. Inability to put up at least one hind foot constituted failure to the traction [28].

The test was conducted in groups of ten previously screened animals, 1 h after the injection of either saline (10 ml/kg) or carbenoxolone (200, 300 and 400 mg/kg) and 30 min after diazepam (0.125, 0.25, 0.5 mg/kg).

Rotarod test

Motor coordination and balance were tested using accelerating rotarod (TSE RotaRod System). Mice were placed on a horizontal metal coated rod with rubber (3 cm diameter) rotating at an initial speed of 10 rpm/min. Terminal speed of the rod was 20 rpm in accelerated studies and rotational velocity of the rod was linearly increased from 10 to 20 rpm within 20 s. The time each animal was able to maintain its balance walking on top of the rod was measured. Mice were given two trials with a maximum time of 300 s and a 30 to 60 min intertrial rest interval. [29]. Before the beginning of all experiments, the riding ability of the animals in the rotarod was checked. Thus, the mice were initially put on a rotating rod, and mice that immediately dropped off (within 30 s) were removed from the experiment.

Mice were divided into groups of ten. Diazepam (1 mg/kg), carbenoxolone (100, 200 and 300 mg/kg) and normal saline (10 ml/kg) were injected 30, 60 and 60 minutes respectively, before the test.

Statistical analysis

The dose of carbenoxolone to produce an anticonvulsant (ED50) effect in 50% of animals and its associated 95% confidence limits was calculated by Litchfield and Wilcoxon methods (PHARM/PCS Version 4). Data were expressed as mean values ± SEM and tested with variance analysis followed by the multiple comparison tests to Tukey-Kramer for the anticonvulsant activity and for the sleeping activity.

Fischer's exact test (two sided) in traction test and one-way ANOVA with post hoc comparisons analyzed by Dunnetts test were used in the accelerod performance.

Acknowledgments

The authors are thankful to the Vice Chancellor of Research, Mashhad University of Medical Sciences for financial support and T.W. Stone, Professor of Pharmacology, Division of Neuroscience and Biomedical Systems, University of Glasgow, Scotland, for his linguistic help.

References

1. Jellinck PH, Monder C, McEwen BS and Sakai RR **Differential inhibition of 11 beta-hydroxysteroid dehydrogenase by carbenoxolone in rat brain regions and peripheral tissues** *J Steroid Biochem Mol Biol* 1993, **46:**209-213
2. Turpie AG and Thomson TJ **Carbenoxolone sodium in the treatment of gastric ulcer with special reference to side effects** *Gut* 1965, **6:**591-594
3. Davidson JS and Baumgarten IM **Glycyrrhetinic acid derivatives: a novel class of inhibitors of gap-junctional intercellular communication. Structure-activity relationships** *J Pharmacol Exp Ther* 1988, **246:**1104-1107
4. Carlen PL, Skinner F, Zhang L, Naus C, Kushnir M and Perez Velazquez JL **The role of gap junctions in seizures** *Brain Res Rev* 2000, **32:**235-241
5. Kohling R, Gladwell SJ, Bracci E, Vreugdenhil M and Jefferys JGR **Prolonged epileptiform bursting induced by 0-Mg^{+2} in rat hippocampal slices depends on gap junctional coupling** *Neuroscience* 2001, **105:**579-587

6. Gladwell SJ and Jefferys JGR **Electrotonic ectopic action potentials in the 4-aminopyridine acute model of epilepsy in the rat hippocampal slice** *J Physiol* 2000, **523P:**194
7. Ross FM, Gwyn P, Spanswick D and Davies SN **Carbenoxolone depresses spontaneous epileptiform activity in the CA1 region of rat hippocampal slices** *Neuroscience* 2000, **100:**789-796
8. Dobbins KR and Saul RF **Transient visual loss after licorice ingestion** *J Neuroophthalmol* 2000, **20:**38-41
9. Vida JA **Anticonvulsants** *In: Principles of Medicinal Chemistry (Edited by: Foye WO, Lemke TL and Williams DA) London: Williams and Wilkins* 1995, 182-198
10. Traub RD **Model of synchronized population bursts in electrically coupled interneurons containing active dendritic conductrances** *J Comp Neurosci* 1995, **2:**283-289
11. Draguhn A, Traub RD, Schmitz D and Jefferys JGR **Electrical coupling underlies high-frequency oscillations in the hippocampus in vitro** *Nature* 1998, **394:**189-192
12. Colling SB, Man WD, Draguhn A and Jefferys JGR **Dendritic shrinkage and dye-coupling between rat hippocampal CA1 pyramidal cells in the tetanus toxin model of epilepsy** *Brain Res* 1996, **741:**38-43
13. Perez Velazquez JL, Valiante TA and Carlen PL **Mudulation mechanisms during calcium-free induced field burst activity: a possible role for electrotonic coupling in epileptogenesis** *J Neurosci* 1994, **14:**4308-4317
14. Shinohara K, Hiruma H, Funabashi T and Kimura F **GABAergic modulation of gap junction communication in slice cultures of the rat superachiasmatic nucleus** *Neuroscience 2000* 1999, **96:**591-596
15. Spray DC and Bennett MV **Physiology and pharmacology of gap junctions** *Annu Rev Physiol* 1985, **47:**281-303
16. De Curtis M, Manfridi A and Biella G **Activity-dependent pH shifts and periodic recurrence of spontaneous interictal spikes in a model of focal epileptogenesis** *J Neurosci* 1998, **18:**7543-7551
17. Hussain MM, Sokomba EN and Shok M **Pharmacological effects of *Gardenia erubescens* in mice, rats and cats** *Int J Pharmacogn* 1991, **29:**94-100
18. Engel AK and Singer W **Temporal binding and correlates of sensory awareness** *Trends Cogn Sci* 2001, **5:**16-25
19. Usrey WM and Reid RC **Synchronous activity in the visual system** *Annu Rev Physiol* 1999, **61:**435-456
20. Baker SN, Kilner JM, Pinches EM and Lemon RN **The role of synchrony and oscillations in the motor output** *Exp Brain Res* 1999, **128:**109-117
21. Bou-Flores C and Berger AJ **Gap junctions and inhibitory synapses modulate inspiratory motoneuron synchronization** *J Neurophsiol* 2001, **85:**1543-1551
22. Kirkwood PA, Sears TA, Tuck DL and Westgaard RH **Variations in the time course of the synchronization of intercostal motoneurones in the cat** *J Physiol* 1982, **327:**105-135
23. De Luca CJ, Roy AM and Erim Z **Synchronization of motor-unit firings in several human muscles** *J Neurophysiol* 1993, **70:**2010-2023
24. Huesler EJ, Maier MA and Hepp-Reymond MC **EMG activation patterns during force production in precision grip. III. Synchronisation of single motor units** *Exp Brain Res* 2000, **134:**441-455
25. Kiehn O and Tresch MC **Gap junctions and motor behavior** *Trends Neurosci* 2002, **25:**108-115
26. Vogel HG and Vogel WH **Drug Discovery and Evaluation, Pharmacological Assay** *Springer, Berlin* 1997, 260-261
27. Dandiya PC and Collumbine H **Studies on Acorus *calamus* (III): some pharmacological properties of the volatile oil** *J Pharmacol Exp Ther* 1959, **125:**353-359
28. Villar R, Laguna MR, Calleja JM and Cadavid I **Effect of *Skeletonema statum* extracts on the central nervous system** *Planta Med* 1992, **38:**398-403
29. McIlwain KL, Merriweather MY, Yuva-Paylor LA and Paylor R **The use of behavioral test batteries: effects of training history** *Physiol Behav* 2001, **73:**705-717