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Research Article

EFFECT OF FENOFIBRATE AND GEMFIBROZIL IN SODIUM NITRITE-INDUCED ANTEROGRADE AMNESIA IN MALE WISTAR RATS

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ABSTRACT

Objectives: The present study was planned to study the effect of fenofibrate and gemfibrozil on sodium nitrite-induced anterograde amnesia in male Wistar rats.

Methods: Anterograde amnesia was induced by 75 mg/kg of sodium nitrite in six groups (eight in each group) of male Wistar rats (150–180 g). Fenofibrate (21 mg/kg and 18 mg/kg) and gemfibrozil (108 and 21 mg/kg) were used as test drugs. The paradigm used was Morris water maze, where a hidden platform was kept for the rat to escape from the water. Rats were trained to locate a hidden platform by releasing them into water for 4 times a day for 4 consecutive days. The acquisition of this task was measured by noting the time taken to escape to the platform. On the 6th day of the study, retrieval of this learnt task was measured by noting the time taken to search for the missing hidden platform. The time taken by the rats during the acquisition and retrieval tasks in fenofibrate and gemfibrozil treated groups were measured and compared with disease control group. On the 6th day (retrieval trial), only vehicle (distilled water oral) was administered to the groups.

Results: Fenofibrate and gemfibrozil completely ameliorated the anterograde amnesia. The mean escape latency time of both fenofibrate and gemfibrozil administered rats was significantly reduced with respect to sodium nitrite group while, retrieval time increased significantly. However, the same group of rats showed significant retrieval of task memory.

Conclusion: In the present study, fenofibrate and gemfibrozil ameliorated chemical hypoxia-induced anterograde amnesia. Both can potentially inhibit oxidative stress induced neurodegeneration at the commonly prescribed clinical doses. In addition to their hypolipidemic effect, they can also prevent modifiable risk factors of chronic neurodegenerative disorders. Further studies are needed to substantiate these findings.

Keywords: Anterograde amnesia, Fenofibrate, Gemfibrozil, Sodium nitrite, Morris water maze.

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INTRODUCTION

The human brain controls most of the activities of the body. Among brain's various diverse functions, the most important is formation and retrieval of memory. Memory is stored in the form of immediate memory, short-term memory, and long-term memory. The memory loss may be normal or pathological. Amnesia as a symptom presents as two temporal forms, anterograde and retrograde amnesia. Anterograde amnesia is the common type of memory loss in earlier stages of dementia [1]. Elderly population is the predominantly affected group in neurodegenerative dementia, with anterograde amnesia being more common. The global burden of amnesia is on a rise due to an increase in human life expectancy. Mild cognitive impairment is frequent in older people with prevalence rates ranging from 2% to 30% [2] At present, drugs in clinical use are indicated mostly in improving symptoms of cognitive disorders and aimed at protecting from excitotoxicity, supplementing neuronal transmission of viable neurons, or selectively improve efficiency of higher telencephalic integrative activities. However, these drugs are unable to arrest the pathology of the disease or treat them to full remission and their significant adverse effects have limited their use [3,4].

Hence, there is an unmet need for more scientific research in developing more efficacious and safer drugs in the fight against amnesia. Oxidative stress and inflammation are important risk factors of neurodegenerative disease and have been associated with increased risk of cognitive impairment and dementias both individually and collectively. Fibrates are drugs used in dyslipidemia. Dyslipidemia has been observed as a risk factor of Alzheimer's dementia. Interestingly, fibrates, a peroxisome proliferator activated receptor-α (PPAR-α) agonist, have been reported to possess anti-inflammatory and antioxidant property [5]. However, there is a lack of the literature regarding the effects of fibrates on amnesia. In view of scarcity of information, the present study was planned to explore the effects of fenofibrate and gemfibrozil on anterograde amnesia induced by sodium nitrite in male Wistar rats.

Use of sodium nitrite is rationalized by the fact that this is standard pharmacological agent to induce amnesia.

MATERIALS AND METHODS

Animals

Adult male Wistar rats weighing 150–180 g were housed under standard laboratory conditions, obtained from the central animal house of tertiary care teaching hospital and medical college. They were acclimatized to 12 h light/dark cycle for 10 days before the day of experimentation. Animals were provided feed (standard chow pellet) and water *ad libitum*. The study was approved by Institution Animal Ethics Committee as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Forty-eight rats were divided into six groups (control, sodium nitrite-induced disease control, and four test groups) with eight animals in each group.

Drugs and dosage

Fenofibrate, gemfibrozil, and sodium nitrite were obtained from the pharmacy. Clinical human doses of fenofibrate and gemfibrozil were converted into rat equivalent doses with the help of the table devised by Paget and Barnes [6].

The treatment was given orally in all the groups. Control group was given 0.5 ml of distill water. All the drugs were dissolved in distilled water.

Morris water maze

It comprises of a circular water tank which has 150 cm diameter and 50 cm height with non-reflecting interior surface. The interior surface was painted in a way that made the maze opaque when filled with opaque water. It was filled with water up to 30 cm at 25±5°C. It was divided into four quadrants using threads. In it, a square (side 10 cm) platform having height of 29 cm was placed in the middle of one quadrant (goal quadrant – Qg). The position of the platform was kept fixed throughout the training and acquisition trials. The water was made opaque by dissolving 3 L of fresh milk daily [7].

For first 10 days, rats were acclimatized to the experimental room and the investigator by repeated handling. Following every trial, rats were dried thoroughly to prevent any hypothermia.

Day 1 training – rats were familiarized with the task and trials were not counted. The familiarization by the rats were confirmed when they learnt that there is an escape route from this aversive stimulus, that is, water.

Day 2–5 - Acquisition trial: Control and drug treated (on each day for four days) rats were released into water facing toward the wall in one of the quadrants (Q). They were subjected to 4 trials/day for 4 days with 5 min interval between each, with the subsequent trial occurring after finishing the ongoing trial with all the eight rats for that same quadrant. During successive trials and successive days, starting points were changed every time.

At first, the rats were trained to locate the hidden platform by "hit and trial" method. They learnt the position of the hidden platform using distal cues. Several distal cues were provided to the rats with strict adherence to their same fixity for all the days of the trial. Even the position of the investigator was fixed with respect to the distal cues.

Subsequently, they were allowed to escape to the platform and stay there for 20 s (to generate a spatial memory of the hidden platform with the help of distal cues). The time required to escape to platform (Escape Latency Time [ELT]) was noted and compared among different days and different groups. Rats unable to locate the platform within 120 s were guided to the platform by hand and again kept there for 20 s. Rats failing the task on consecutive trials for 2 successive days were excluded from the study.

Day 6 – Retrieval trial: On the 6th day, platform from goal quadrant was removed and rats (all groups were administered vehicle now) were evaluated for time spent in previously goal quadrant (Index of Retrieval). This was done only once and the farthest quadrant from the goal quadrant was chosen to release the rats. This quadrant was kept the same for all groups. The time spent in the previously goal quadrant was compared among control and amnesia-induced group and drug treated groups.

On 7th day, all animals were euthanized using overdose of Thiopentone sodium by intraperitoneal route (90 mg/kg for male Wistar rats) (as per the guidelines of CPCSEA).

Statistical analysis

The data for all the groups were expressed as mean±SEM and were analyzed using one-way ANOVA (Analysis of variance) of repeated measurement followed by *post hoc* Dunnett's test using Graph Pad Prism 5.00 Software (San Diego, USA). p≤0.05 was considered statistically significant.

RESULTS

Physical parameters (Morris water maze)

Control group

Mean ELT in seconds was measured to assess learning and acquisition of the task of locating the submerged and invisible platform using Morris water maze. The mean ELT for vehicle treated group on day 1, day 2, day 3, and day 4 was 40.56±1.602, 26.91±5.376, 23.63±3.394, and 18.13±3.280, respectively (Table 1). The change in the mean ELT on 4th and 3rd day was statistically significant (***p<0.005, *p<0.05 respectively) compared to the mean ELT of the 1st day. While acquiring the task, their trajectory toward goal quadrant changed from circumferential on 1st day to goal directed on the 4th day. On removing the platform, they could retrieve the previous location by spending more time (56.63±3.060) in the goal quadrant.

Sodium nitrite-induced anterograde amnesia

Mean ELT in seconds for disease control group on day 1, day 2, day 3, and day 4 was 66.00±3.719, 72.13±7.123, 53.38±1.358, and 56.44±1.740, respectively (Table 1). On comparison of the mean ELT among all the 4 days, there was no significant difference from the 1st day. Even on the 4th day, they were swimming circumferentially with minimal goal directed behavior. Since there was no acquisition of the trial, when released into quadrant on the 6th day, the mean ELT was recorded to be 56.44±1.740 and the time spent in the previous goal quadrant was almost similar to time spent in all other quadrants.

Fenofibrate 21 mg/kg and fenofibrate 18 mg/kg ameliorated the effects of sodium nitrite in inducing anterograde amnesia

Mean (ELT) in seconds in fenofibrate 21 mg/kg treated group on day 1, day 2, day 3, and day 4 were 46.31±3.263, 33.75±5.399, 31.28±6.396, and 22.31±3.433, respectively (Table 1). There was statistical significant difference (**p<0.01) in the mean ELT of day 4 compared to the day 1. Similarly, mean ELT in seconds in fenofibrate 18 mg/kg treated group on days (1, 2, 3, and 4) was 52.16±7.454, 40.34±3.077, 37.81±4.569, and 30.75±5.525, respectively (Table 1). There was statistical significant difference (*p<0.05) in the mean ELT of day 4 compared to the day 1.

Gemfibrozil 108 mg/kg when given before sodium nitrite ameliorate anterograde amnesia

Mean ELT in seconds in gemfibrozil 108 mg/kg treated group on days (1, 2, 3, and 4) was 55.32±6.947, 28.14±4.960, 26.50±7.645, and 11.34±1.745, respectively. There was statistical significant difference (*p<0.05, **p<0.01, ***p<0.005) in the mean ELT of day 2, 3, and 4 compared to the day 1, respectively.

Gemfibrozil 21 mg/kg when given before sodium nitrite did not ameliorate anterograde amnesia

Mean ELT in seconds in gemfibrozil 18 mg/kg treated group on days 1, 2, 3, and 4 was 31.64±7.020, 30.96±6.186, 26.89±4.370, and 16.00±1.643, respectively (Table 2). There was no statistical significant difference in the mean ELT of day 4 compared to the day 1. However, a general trend of reducing mean ELT was seen from day 1 to day 4.

Mean escape latency time of day four

A significant reduction in mean ELT on day 4 was observed in comparison fenofibrate (21 and 18 mg/kg) and gemfibrozil (108 and 21 mg/kg) treated groups as compared to disease control group.

Time for retrieval in seconds

In retrieval trial, rats spent significantly more time for locating the platform in the goal quadrant in both fenofibrate (21 and 18 mg/kg) and gemfibrozil (108 and 21 mg/kg) treated groups in comparison to disease control group (Table 2).

Table 1: Drugs and dosages used in the study

Serial number	Treatment	Rat equivalent dose
1	Control (distilled water)	0.5 ml
2	Sodium nitrite+vehicle	75 mg/kg
3	Fenofibrate+sodium nitrite	21 mg/kg+75 mg/kg
4	Fenofibrate+sodium nitrite	18 mg/kg+75 mg/kg
5	Gemfibrozil+sodium nitrite	108 mg/kg+75 mg/kg
6	Gemfibrozil+sodium nitrite	21 mg/kg+75 mg/kg

Table 2: Effect of various treatment on mean escape latency time in Morris water maze

Groups (*n*=8)	Day 1	Day 2	Day 3	Day 4
Control	40.56±1.602	26.91±5.376	23.63±3.394*	18.13±3.280***
Disease control	66.00±3.719	72.13±7.123	53.38±1.358	56.44±1.740
Fenofibrate 21 mg/kg+$NaNO_2$	46.31±3.263	33.75±5.399	31.28±6.396	22.31±3.433**
Fenofibrate 18 mg/kg+$NaNO_2$	52.16±7.454	40.34±3.077	37.81±4.569	30.75±5.525*
Gemfibrozil 108 mg/kg+$NaNO_2$	55.32±6.947	28.14±4.960*	26.50±7.645**	11.34±1.745***
Gemfibrozil 21 mg/kg+$NaNO_2$	31.64±7.020	30.96±6.186	26.89±4.370	16.00±1.643

*p<0.005, **p<0.01, ***p<0.005 compared with Group II (ANOVA followed by *post hoc* Dunnett's test). ANOVA: Analysis of variance

Table 3: Effect of various treatments on retrieval trial

Groups (*n*=8 in each group)	Index of retrieval (s)
Control	56.63±3.060
Disease control	31.22±1.572
Fenofibrate 21 mg/kg+$NaNO_2$	64.04±5.371*
Fenofibrate 18 mg/kg+$NaNO_2$	65.19±3.476*
Gemfibrozil 108 mg/kg+$NaNO_2$	54.31±2.791*
Gemfibrozil 21 mg/kg+$NaNO_2$	58.53±2.069*

*p<0.005, compared with Group II (ANOVA followed by *Post hoc* Dunnett's test). ANOVA: Analysis of variance

DISCUSSION

The present study examined the potential neuroprotective effect of fenofibrate and gemfibrozil on cognitive impairment in sodium nitrite-induced anterograde amnesia in male Wistar rats. Sodium nitrite by inducing chemical hypoxia causes hypoxic neuronal injury affecting both the learning of the trial and the retrieval of the trial. Sodium nitrite has been reported to cause oxidative injury to neurons by reducing oxygen carrying capacity of blood and by forming peroxynitrates [7]. 75 mg/kg, s.c administration of sodium nitrite has been reported to induce cognitive deficits [8]. The same dose of sodium nitrite has been selected for the present study. The paradigm used was Morris water maze [9]. Morris water maze is a "gold standard" for hippocampal function assessment. Rodents are natural swimmers and have a natural tendency to escape water. Thus, this maze serves as a natural motivation for them. Rats are better performers as they have lesser tendencies to float. As per our knowledge, this study was first of its kind in studying effect of fenofibrate and gemfibrozil on sodium nitrite-induced anterograde amnesia.

Fenofibrate at the doses of 21 and 18 mg/kg when administered before sodium nitrite showed a statistically significant decrease in mean ELT in the acquisition trial. This observation was similar to the results of the study which assessed the effects of fenofibrate on learning and memory deficits in rats following global cerebral ischemia and found a significant reduction in the mean ELT in acquisition trial.

Furthermore, in the retrieval trial, both the doses of fenofibrate have shown significant changes, as the rat showed retrieval of the platform's location. This retrieval of the task memory could have only happened after the rat learnt it and fenofibrate treated rats showed a decrease in time required to search the hidden platform on the 4th day of acquisition trial. This observation is in agreement with the previous study of Xuan *et al*. in which fenofibrate treatment increased the retrieval time [10].

Similarly, gemfibrozil 108 mg/kg dose when administered before sodium nitrite in the acquisition trial showed a decrease in mean ELT and was statistically significant. Gemfibrozil 18 mg/kg when administered before sodium nitrite showed a decrease in mean ELT on 4th day of acquisition trial, but this difference was not significant whereas day 4 inter-group comparison showed statistically significant difference.

To summarize, both fenofibrate and gemfibrozil ameliorated anterograde amnesia induced by sodium nitrite. This could mean that fenofibrate and gemfibrozil inhibited oxidation of hemoglobin by sodium nitrite in blood. This could also be explained on the basis of other roles of fibrates such as neuronal anti-oxidant or promotion of neurogenesis or by enhancing spatial memory formation. Earlier it has been reported that the physiological function of Paraoxonases 1 (PON 1) is to hydrolyze oxidized lipids and hence acts as anti-oxidant. It seems well established that PPARs are important factors in the regulation of Paraoxonases (PON 1) expression and in counteracting oxidative stress. These data suggest that PPARs play a key role in the regulation of oxidation and inflammation and as such drugs that stimulate PPARs activity may be important targets in the struggle against amnesia [11].

This study found definite role of fenofibrate and gemfibrozil in ameliorating induction of anterograde amnesia by sodium nitrite. Thus, fenofibrate and gemfibrozil when started early in the process of neurodegeneration can bring down the prevalence of cognitive impairment and will open new avenues in the management of number of neurodegenerative disorders of the brain.

CONCLUSION

In the present study, fenofibrate and gemfibrozil were explored for its effect on model of anterograde amnesia. Sodium nitrite was used to induce anterograde amnesia in male Wistar rat. The paradigm used was Morris water maze, which is a gold standard for evaluating hippocampal spatial memory formation in rodents. Both fenofibrate and gemfibrozil have shown significant decrease in mean ELT in acquisition trial when given before sodium nitrite. Fenofibrate and gemfibrozil treated rat had significant retrieval of task memory when given before sodium nitrite in the retrieval trial. Use of fenofibrate and gemfibrozil can reduce the risk of amnesia by virtue of its antioxidant and anti-inflammatory property, in addition to its dyslipidemic action. Furthermore, fenofibrate and gemfibrozil might have a role play in the treatment of other neurodegenerative disorders. However, these clinical implications need to be assessed in clinical trials.

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AUTHORS' CONTRIBUTION

Shujauddin and Nayana K Hashilkar done the study, Sanjay Kumar Mishra analyzed the data and applied statistical tests while Zafar Masood Ansari prepared the manuscript.

CONFLICT OF INETERSETS

None.

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