Tacrine can suppress immunity response to tularemia in BALB/c mouse model

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Abstract

Tacrine is an inhibitor of enzyme acetylcholinesterase (AChE). In the past, it was used for treatment of cognitive dysfunction during some vascular dementia and Alzheimer disease. Some works concluded that AChE inhibitors can modulate immune response. For the reason, we decided to investigate immune response to a model bacterial disease tularemia for which both innate and specific immunity are necessary to resolve the disease. We used 64 BALB/c mice divided into eight groups exposed to saline, tacrine in a dose 20.0 – 500 μg/kg, infected with tularemia and infected with contemporary application of tacrine. The mice were euthanized three days after experiment beginning. We proved significant reduction of interleukin-6 (IL-6) and interferon gamma (IFN-γ) level in a dose response manner in the infected animals in course of tularemia. Moreover, tacrine caused significant increase of bacterial burden in the livers and spleens. We can conclude that tacrine can aggravate tularemia. We discuss that tacrine increase accessibility of acetylcholine and in thus way stimulates cholinergic anti-inflammatory pathway. The results represent a substantial

contribution to the field of inflammation control by nerve system and it is an advancement of the inflammatory therapy issue.

Key words: inflammation; infection; Alzheimer disease; tacrine; acetylcholinesterase; innate immunity.

INTRODUCTION

Tacrine (9-amino-1,2,3,4-tetrahydroacridine) is an inhibitor of both AChE (EC 3.1.1.7.) and butyrylcholinesterase (BChE; EC 3.1.1.8.). In the cholinesterases, tacrine binds to α-anionic site in active center of the enzymes. Though it inhibits the both cholinesterases, higher affinity to AChE is reported (Ahmed et al. 2006, Pohanka 2011b). In the past, tacrine was marketed under trade name Cognex and it was used for treatment of cognitive dysfunctions in Alzheimer disease and related vascular dementias. Despite high efficacy, tacrine was withdrawn from clinical use due to metabolic activation causing adverse effects in livers (Alfirevic et al. 2007, Pohanka 2011, Pohanka 2012a).

When used for Alzheimer disease therapy, tacrine was given to improve acetylcholine insufficiency (Krall et al. 1999). In the later research, association of inflammation via IL-6 in tacrine induced hepatotoxicity was reported (Carr et al. 2007). On the other hand, tacrine was found to abolish lipopolysaccharide induced inflammation including IL-6 secretion in central nervous system (Tyagi et al. 2007). The findings are supported by our recent experiments with another inhibitor of AChE – neostigmine (Pohanka and Pavlis 2012). In the present experiment, we decided to investigate potential implication of tacrine in immunomodulation of an infectious disease. The results can allow understand tacrine effect in chronic inflammatory disorders including the Alzheimer disease. We chosen tularemia, a disease caused by *Francisella tularensis*, as a model disease since both innate and adaptive immunity are necessary for the disease resolving and macrophages play crucial role in the both immunity reactions (Fernandes-Alnemri et al. 2010, Mahawar et al. 2012). Owing to the quoted papers, we suppose modulation of immune response to tularemia by tacrine. The present experiment is devoted to elucidation of the effect. The understanding is suitable not only for identification of biological effects in organism but also to extrapolate the results to understand role of AChE inhibitors in pathogeneses associated with inflammatory processes.

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MATERIAL AND METHODS

Microorganism

F. tularensis LVS (strain ATCC 29684) was chosen for the experiment performance. The microorganism was cultivated in a standard manner on McLeod agar supplemented with hemoglobin from bovine blood (Sigma-Aldrich, Sigma-Aldrich, St. Louis, MO, USA) and Iso VitaleX (Becton-Dickinson, San Jose, CA, USA) at 37 °C. After one day, the culture was harvested using a disposable plastic scraper. The scraped cells were re-suspended in saline solution and then washed by centrifugation at 2,000 × g for 10 minutes. Exact concentration of viable cells was confirmed by a cultivation test in a way as described above.

Laboratory animals

In a total 64 BALB/c laboratory mice (Velaz, Unetice, Czech Republic) were received in their six weeks and they were used for the experiment purposes after another two weeks of quarantine. We have chosen BALB/c as an optimal animal model as the mice are frequently used for tularemia pathogenesis research by other scientists (Shen et al. 2010). Thus the experimental data are easily comparably to our previous results as well (Pohanka and Pavlis 2012). In the experiment beginning, the animals weighted 21±1 g. For the whole time of experiment, the animals were kept in an air conditioned laboratory with temperature 22±2 °C, humidity 50±10 %, and continuous 12 hours lasting light and 12 hours lasting dark period. Food and water were supplied to the animals without any limitation. The experiment was properly approved by ethical committee of Ministry of Defence (Czech Republic) and all manipulations with the animals including euthanasia were done in an accredited vivarium of the Centre of Biological Defense in Techonin (Czech Republic).

Laboratory animals experiment performance

The animals were divided into 8 groups each sized 8 specimens. Saline, bacterial suspension in saline and tacrine hydrochloride hydrate (99% purity; Sigma-Aldrich) were given in amount 100 µl subcutaneously into rear limb. Lately confirmed in cultivation test, *F. tularensis* concentration used in the experiment was 2.91×10^6 CFU/ml. Tacrine was solved in order to be given in doses 0.02–0.1–0.5 mg/kg from which the doses 0.1–0.5 mg/kg were derived from the formerly stated therapeutic doses for Alzheimer disease suffered patients (Davis et al 1992, Knapp et al 1994). In the here described experiment, the animals receiving tacrine were challenged by the same dose of the drug the second day after experiment beginning. Subsequent groups were established: 1. controls receiving saline only, 2. tacrine

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0.02 mg/kg first and second day, 3. tacrine 0.1 mg/kg first and second day, 4. tacrine 0.5 mg/kg first and second day, 5. infection by *F. tularensis*, 6. infection by *F. tularensis* and tacrine 0.02 mg/kg first and second day, 7. infection by *F. tularensis* and tacrine 0.1 mg/kg first and second day, 8. infection by *F. tularensis* tacrine 0.5 mg/kg first and second day. The animals were euthanized three days after experiment beginning using CO_2 anesthesia and heart punction. Blood was collected into tubes with adsorbed lithium heparin (Dialab, Prague, Czech Republic). Spleens and livers were sampled from cadavers immediately after blood collection.

Ex vivo assay

The freshly collected blood was centrifuged at 1,000 ×g for 5 minutes and plasma was separated. The plasma samples were processed using an indirect enzyme linked immuno-sorbent assay (ELISA) for determination of IL-6 and IFN-γ. Kits from Abcam Company (Cambridge, MA, USA) developed for standard 96 wells microplates were used. The kits were processed in compliance with printed and attached instructions.

The spleens and livers were homogenized immediately after collection by passing through nylon net with holes sized 1 mm^2. The homogenate was re-suspended into saline and spread over the McLeod agar with composition as reported above. The agar was incubated in a previously described way and colonies were counted two days after incubation beginning.

Statistical analysis

The achieved data were processed in Origin 8 Pro (OriginLab Corporation, Northampton, MA, USA) software using one-way ANOVA test with Bonferroni test. The probability level 2alpha = 0.05 was considered as a significance threshold.

RESULTS

IL-6 level is depicted in figure 1. Tacrine alone did not cause any alteration in the cytokine level when compared to the controls. The infected animals had significantly increased IL-6. Tacrine was able to significantly reduce the IL-6 level and the reduction was in a dose response manner. Three days after experiment beginning, the IL-6 level increased from 58 pg/ml (controls) up to 569 pg/ml in the tularemia infected animals (group 5). The upper dose of tacrine in the infected animals (group 8) significantly reduced IL-6 level up to 205 pg/ml.

Figure 1

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The second assayed cytokine was IFN-γ. Levels of the marker are depicted in figure 2. The cytokine level did not alter in course of tacrine when the animals were not infected (groups 2–3). Application of tacrine into the tularemia infected mice caused decrease of IFN-γ in a dose response manner. The lowest dose of tacrine, 20.0 μg/kg, did not cause significant decrease of IFN-γ. However, the doses of tacrine 100 and 500 μg/kg initiated significant decrease of IFN-γ level in course of tularemia.

Figure 2

Bacterial burden in the examined organs was influenced by application of tacrine. The total number of bacteria increased in a dose response manner in both livers and spleens. The increase was more extensive in the livers than in the spleens. When compared to the non treated and infected animals only, the highest dose of tacrine caused approximately 2.5 times increase of bacterial burden in the spleen and 5 times increase in the livers. In compliance with expectation, non infected animals (groups 1-4) had no microbiological finding in the both livers and spleens.

Figure 3

Figure 4

DISCUSSION

The time interval of euthanasia was chosen in compliance with our previous results and findings from quoted papers (Chiavolini et al. 2008; Pohanka et al. 2012). Three days period from infection starting to euthanasia was chosen as optimal as innate immunity is activated sufficiently and level of inflammatory cytokines and bacterial burden culminate (Pohanka et al. 2012). One the other hand, pertinent demises will appear later and expected level of IFN-γ already increases to be measurable (Chiavolini et al. 2008; Pohanka et al. 2012). The cytokines were chosen to provide knowledge about both innate immunity and anti-intracellular pathogen immune responses. IL-6 is a cytokine released from macrophages in a large scale during inflammation and it is useful for research on cholinergic anti-inflammatory pathway (Wilund et al. 2009). We used assay of IL-6 for the mentioned reason. IFN-γ was the second assayed marker. The cytokine is crucial part in the both innate and adaptive immunity. Moreover, it activates macrophages during an infection so a positive feed-back is constituted.

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Release of IFN-γ during tularemia is a crucial factor for resolving of the disease by macrophages (Novosad et al. 2011).

Owing to the achieved results, we can write that tacrine can aggravate tularemia progression. The process is, however, more complicated to be concluded by a simple idea. Tacrine is an inhibitor of both AChE and BChE and it is able to simply cross blood brain barrier and act not only in peripheral nervous system but in central nervous system as well (Teltingdiaz and Lunte 1993). It is unlike to carbamate inhibitors which dominantly act in peripheral nervous system and not undergo into the brain (Jokanovic 2009) or penetrating under specific conditions such as stress (Friedman et al. 1996). On the other hand, the results for tacrine appoint at a process close to the previously described findings for carbamate neostigmine (Pohanka and Pavlis 2012) and paper written by Wang and coworkers contains also findings about suppression of immunity including inflammation after application of a cholinesterase inhibitor: huperzine (Wang et al. 2012). The correlation of results indicates the same mechanism of action for the mentioned compounds. We can infer that tacrine immunomodulatory effect is based on inhibition of cholinesterases in the peripheral nervous system.

The most probable explanation of the found phenomenon is that the compounds meet cholinergic anti-inflammatory pathway. The pathway consists from nervus vagus termination in the blood system and α7-nicotinic acetylcholine receptor (α7 nAChR) on macrophages surfaces (Zitnik 2011). The stimulation of α7 nAChR is quickly terminated by an action of AChE located on erythrocytes (Pohanka 2012). Inhibition of AChE can improve accessibility of acetylcholine and induce stimulation of the cholinergic anti-inflammatory pathway in thus way. Activation of the nicotinic anti-inflammatory pathway suppresses inflammation during antigenic stimulation of immune system and the activation can be initiated by direct agonizing of α7 nAChR (Chatterjee et al. 2012) or inhibition of AChE (Sun et al. 2012).

Here, tacrine caused aggravation of tularemia disease. For the reason, application of tacrine or similarly acting compounds can be considered as a risk factor during bacterial infections. On the other hand, beneficial action of AChE inhibitors in some pathogeneses can be attributed just to the link with inflammation regulation. The idea is supported by some experimental findings such as prevention from cognitive deficits due to inflammation when AChE inhibitor galantamine is applied (Field et al. 2012). In another work, epileptogenesis induced by inflammation was suppressed by application of acetylcholine (Gnatek et al. 2012). Owing to the quoted papers and here reported results, we infer that AChE inhibitors can be

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used for suppression of inflammation including neuroinflammation. The findings are in a good compliance with our previous experiment with neostigmine (Pohanka and Pavlis 2012). Comparing to neostigmine, tacrine acts not only in the peripheral nervous system but also in the central nervous system so it might be suitable for regulation of neuroinflammation.

CONCLUSIONS

Tacrine can aggravate progression of tularemia in a mouse model. The most probable explanation is that the AChE inhibitor stimulates cholinergic anti-inflammatory pathway and the suppressed immune system is not able easily resolve the disease. On the other hand, the effect would be beneficial in several pathologies such as neurodegenerative disorders. E.g. positive effect of tacrine when used for Alzheimer disease therapy would be attributed to the anti-inflammatory action besides improving of cognitive functions.

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Figures captions

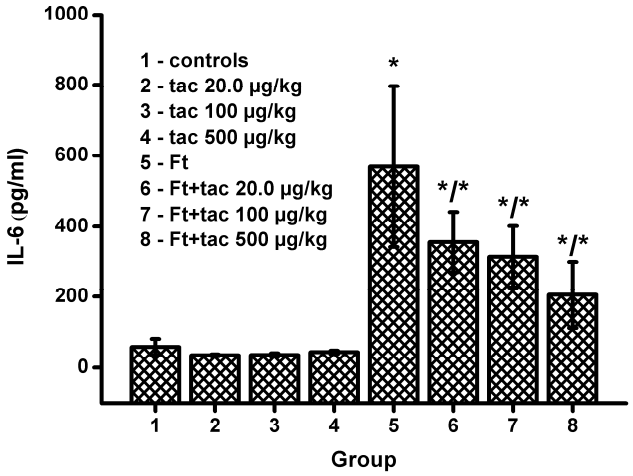

Fig. 1. **Level of interleukin-6 (IL-6) in plasma samples.** Composition of groups is depicted in the figure. Error bars indicate standard deviation for a group sized eight specimens. Asterisk in group 5 respective asterisk in numerator for groups 6–8 respond to significance against group 1. Asterisks in denominator of groups 6–7 responds to significance against group 5.

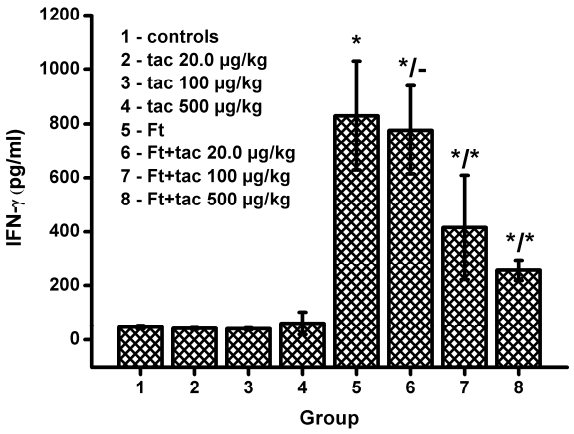

Fig. 2. **Level of interferon gamma (IFN-γ) in plasma samples.** Description is same as provided in the figure 1.

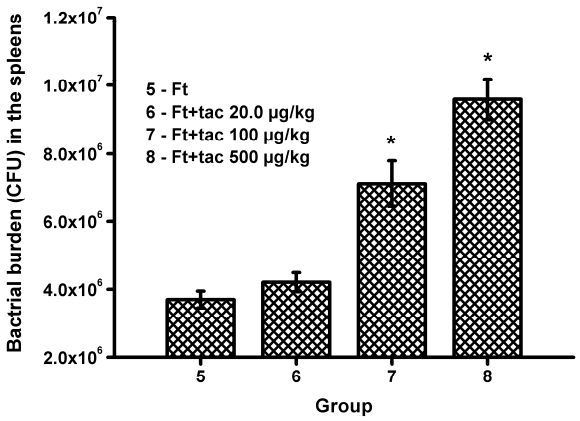

Fig. 3. **Bacterial burden in spleens of the infected animals.** Composition of groups is depicted in the figure. Error bars indicate standard deviation for a group sized eight specimens. Asterisk indicates significance against group 5.

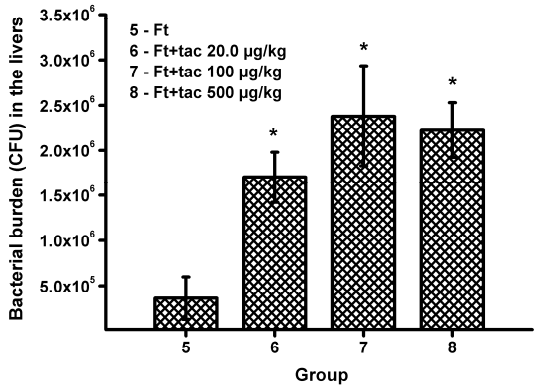

Fig. 4. **Bacterial burden in livers of the infected animals.** Description is same as provided in the figure 3.

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