INFECTION - AN AMENDMENT TO THE STROKE MODEL GUIDELINES

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ABSTRACT

Many publications show that infections have a tremendous impact on both general medical and neurological outcome. Summarizing the findings from several experimental studies we conclude that controlling infections in animal models of stroke is indispensable similar to checking body temperature in order to avoid misinterpretations of study results. In our manuscript we followed the structure used in the original article to provide a consistent outline. In brief, we reviewed the necessity of monitoring post-stroke infections, followed by a section on how to exclude the impact of infections in stroke models.

Keywords: stroke model; infection; guideline; reproducibility

INFECTION

A. THE NECESSITY OF MONITORING POST-STROKE INFECTIONS

GUIDANCE

Especially in long-term studies, it is strongly recommended to monitor for infections in blood and lung after stroke, since post-stroke infections are common even in stroke models and are known to have a negative effect on outcome after stroke.

SUPPORTING DISCUSSION

Infectious diseases are the most common medical complication after cerebral ischemia (Davenport, Dennis et al. 1996; Langhorne, Stott et al. 2000), impairing both the neurological and the general medical outcome. The most frequent complications are bacterial pneumonia and urinary tract infections (UTI) (Langhorne, Stott et al. 2000; Hilker, Poetter et al. 2003), where bacterial pneumonia is seen as the most common serious complication in stroke care (Kalra, Yu et al. 1995). This observation is not limited to stroke patients, but also mice suffer from infections after experimental stroke (Prass, Meisel et al. 2003).

Neurological deficits like dysphagia or bladder dysfunction contribute to the development of spontaneous infectious compilications by increasing the risk for colonization of the lower respiratory tract and urinary tract, respectively. However, aspiration alone is not sufficient to explain the high incidence of post-stroke pneumonia (Perry and Love 2001; Meisel, Schwab et al. 2005), and there is strong evidence that a secondary immunosuppressive state after CNS injury might be causative for the high incidence of severe bacterial infections in stroke patients (Meisel, Schwab et al. 2005; Prass, Braun et al. 2006; Chamorro, Urra et al. 2007; Emsley and Hopkins 2008; Offner, Vandenbark et al. 2009).

Following cerebral ischaemia, several groups observed lymphopenia, impaired cellular immune functions and a rapid and extensive apoptosis in lymphatic organs (Prass, Meisel et al. 2003; Liesz, Hagmann et al. 2009). This apoptosis can be prevented by application of caspase inhibitors leading to reduction of post-stroke infections and smaller infarct sizes (Braun, Prass et al. 2007).

Since the situation in the mouse resembles the situation in patients well, the mouse MCAO model can be used as a suitable model to elucidate the underlying mechanisms of post-stroke infections as well as for developing new therapeutic strategies (Meisel and Meisel 2008; Engel and Meisel 2010). However, since post-stroke infections effect

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outcome even in experimental stroke (Meisel et al. 2004), stroke studies aiming to investigate mechanisms or treatment strategies might be influenced by infections.

Commonly observed pathogens are often part of the intestinal flora, like Escherichia coli, Enterococcus faecalis and Lactococcus garviae (Meisel, Prass et al. 2004), but also Staphylococcus and Streptococcus species (Liesz, Hagmann et al. 2009) were detected.

In patients and in animals, stroke severity (Hug, Dalpke et al. 2009), impairment of protective reflexes (Nakajoh, Nakagawa et al. 2000) and neurological impairment correspond with the incidence of infections. Liesz et al. demonstrated that severe but not minor ischemic damage induces a profound immunodepression and infectious complications independent from localisation or laterality of stroke. (Liesz, Hagmann et al. 2009) However, infection also worsens neurological outcome (Hong, Kang et al. 2008) and prevention of infection not only improves survival and neurological outcome but also leads to smaller infarcts (Meisel, Prass et al. 2004). Thus, large lesion size is a prerequisite for immunodepression and post stroke infection, on the other hand infection have a negative effect on infarct maturation, at least in experimental stroke.

Further, mouse strains are known to differ in their susceptibility for post stroke infections (Schulte-Herbruggen, Klehmet et al. 2006). Despite a similar infarct size, SV129 mice have a higher bacterial load in the lung and a strongly increased susceptibility to bacteraemia compared to C57Bl/6 and Balb/C mice. Mortality is lowest in C57Bl/6 mice, followed by SV 129 and Balb/C mice. These results suggest that SV129 mice are more suitable for studying post-stroke sepsis, whereas C57Bl/6 mice are preferable for studying pneumonia (Schulte-Herbruggen, Klehmet et al. 2006).

These data suggest a close monitoring of infections to warrant a proper interpretation of results in experimental stroke research. Furthermore it is necessary to characterize each model for its immunological changes after experimental cerebral ischemia. In view of these results, characterization of infections is mandatory in establishing animal models of cerebral ischaemia.

B. PREVENTION OF INFECTIOUS COMPLICATIONS

GUIDANCE

Measures like prophylactic antibiotic treatment that can prevent infectious complications affecting outcome has to be taken into account for studies where "side effects" from infectious complications may interfere with mechanisms or treatments under research.

Example protocol for prophylactic antibiotic treatment

Drug: Enrofloxacin (Baytril 2,5% oral solution for calves, Bayer Healthcare, Germany)

Dose: daily 10 mg/kg body weight

Application: 2 times daily via feeding needle (volume ca. 0.1 ml per application)

Alternate application: Medication via drinking water (e.g. 0.35 mg/ml) starting one day before stroke. After stroke additionally one time daily application via feeding needle as above, since animals drink too less in the first days after stroke.

Duration: Starting one day before until day 6 after experimental stroke

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SUPPORTING DISCUSSION

Preventive antibiotic treatment was tested in animals (Meisel, Prass et al. 2004) and in patients (van de Beek, Wijdicks et al. 2009). In summary, three randomized controlled clinical trials on preventive antibacterial therapy after stroke have been performed. Whereas the PANTHERIS and MISS trials suggest superiority of a preventive anti-infective therapy over current standard therapy, ESPIAS is warning against this approach. (Chamorro, Horcajada et al. 2005; Harms, Prass et al. 2008; Schwarz, Al-Shajlawi et al. 2008) However, differences in study design and the chosen antibiotic may account for the differences in outcome. (Meisel and Meisel 2008). Nevertheless, the observed effects warrants evaluation of preventive antibiotic treatment in large stroke trials. (van de Beek, Wijdicks et al. 2009)

The commonly observed hyperthermia after experimental stroke (Reglodi, Somogyvari-Vigh et al. 2000) is not necessarily a "central fever" caused by stroke-induced tissue damage (e.g. in the hypothalamus) as proposed by Li et al. (Li, Omae et al. 1999). Since prophylactic antibiotic treatment prevents infections as well as fever (Schwarz, Al-Shajlawi et al. 2008), hyperthermia might be caused at least in part by infections. (Georgilis, Plomaritoglou et al. 1999; Commichau, Scarmeas et al. 2003) Likewise, the previously reported high mortality in the mice MCAO model (Schabitz, Li et al. 1999) after day 4 is less likely to be a direct effect of stroke (e.g. by brain edema) but rather a consequence of stroke associated severe bacterial infections. Hitherto, long-term studies on neuronal plasticity and regeneration in this stroke model were hampered by a generally high mortality. Thus, preventive antibacterial approaches may facilitate such studies. Furthermore, infections have to be considered in experimental stroke, since varying degrees of infection may lead to significant variability in outcomes. (Meisel, Prass et al. 2004)

Prophylactic antibiotic treatment should cover the commonly observed pathogens, and should be started within the first 12 hours after onset of cerebral ischemia and given over the first 6 days, since the risk of infections highest during this period. . (Meisel, Prass et al. 2004; Prass, Braun et al. 2006)

However, the use of prophylactic antibiotics may also influence the outcome due to neuroprotective or immunomodulatory effects of certain antibiotic drugs. Several antibiotics have well described neuroprotective effects, for example tetracyclins (Domercq and Matute 2004) like minocycline (Hayakawa, Mishima et al. 2008), or β-lactam antibiotics (Rothstein, Patel et al. 2005) like ceftriaxone (Lipski, Wan et al. 2007; Thone-Reineke, Neumann et al. 2008). However these neuroprotective effects may have limitations such as being strictly dose dependend (Matsukawa, Yasuhara et al. 2009) or gender specific (Li and McCullough 2009). For many antibiotics there is no direct neuroprotective effect observed, for example for gyrase inhibitors. Nevertheless many antibiotics have immunomodulatory effects or may interact with the inflammation cascade. (Tauber and Nau 2008) Hence the use of preventive antibiotic treatment has to be critically considered and the choice of a suitable antibiotic is one of the most critical points in planning studies with preventive antibiotic treatment.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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