ENTOMOPHARM
Insects for drug discovery

Insects as a model species of blood brain barrier permeability

The locust *ex vivo* BBB permeability model

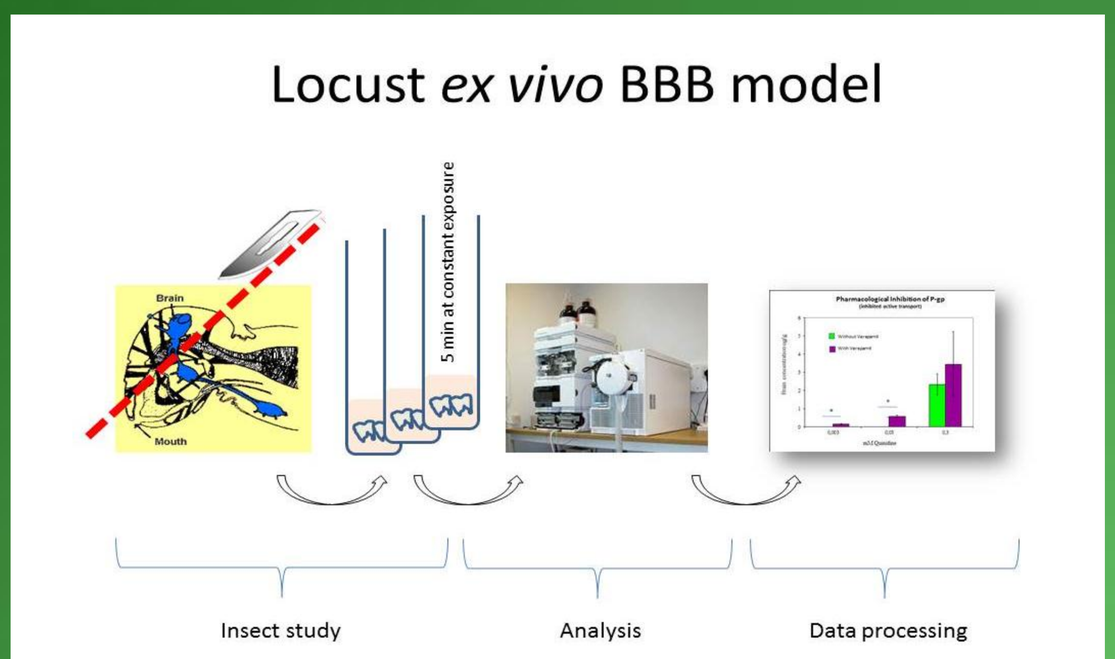

Figure 1: Brains from adult locusts (*Schistocerca Gregaria*) are exposed to test compound at constant concentration for a period of 15 min in a 3x2brains set-up within a concentration span of 1 -10 µM.

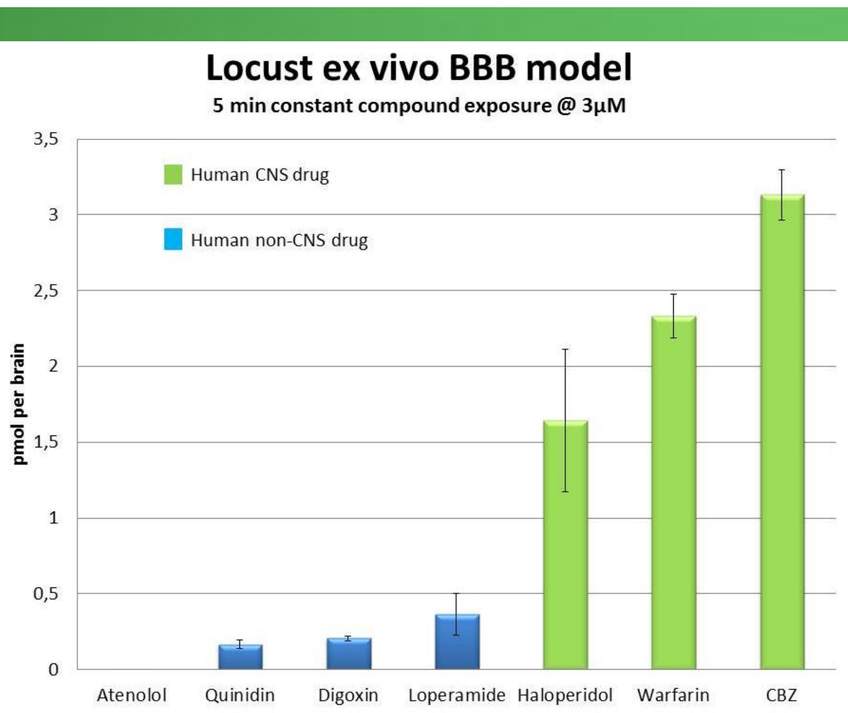

Figure 2: Locust BBB discriminates between vertebrate CNS (green shade) and non-CNS (blue shade) drugs after 5 min of constant compound exposure at 30° C. Results are mean +/- s.e.m.

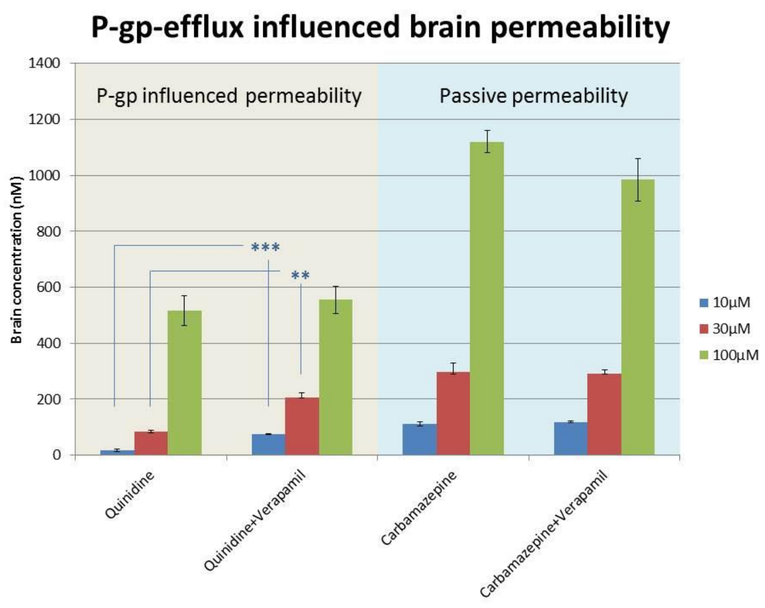

Figure 3: The P-gp substrate Quinidine has a significantly higher permeability in the 10 and 30 uM doses in co-administration with the human Pgp inhibitor verapamil. This is not the case in the 100 uM dose where Pgp efflux is expected to be saturated. Results are mean concentration per g locust brain tissue +/- s.e.m. *P<0.05, **P<0.005, ***P<0.0005 in a one-tailed equal variance students t-test (n=3).

Ex vivo BBB permeability model

EntomoPharm has developed an invertebrate *ex vivo* system, based on a natural biological brain barrier from the locust grasshopper (*Schistocerca Gregaria*), that can be used to screen and rank small-molecule compounds to identify drug leads with improved BBB properties [1] (Figure 2). The model can replace standard *in vitro* screen models with an additional opportunity for identification of P-glycoprotein (P-gp) substrates in the early screen cascade. All this whilst meeting the drug discovery demands for reliability, time and cost efficiency.

In the ***ex vivo* Locust BBB model** compound permeability is studied at constant brain exposure of 1-10 µM in a 2x3 brains set-up for a period of 15 min and is independent of degrading enzymes, elimination and plasma protein binding. The model opens possibility for testing dose/time response over an intact barrier system. Data quality is high and the study outcome is always judged towards the response of an internal positive control.

Key model advantages:

1. The locust blood brain barrier is a natural biological brain barrier that retains its biological integrity and control functions during the test procedure similar to vertebrate *in vivo* BBB models.
2. The ***ex vivo* Locust BBB permeability model** is the only existing ex vivo model of BBB permeability that is based on an intact bio-membrane.
3. The Locust brain barrier contains a P-gp efflux mechanism that can be inhibited by verapamil and the ***ex vivo* BBB locust permeability model** is a valuable tool for the identification of P-gp substrates and inhibitors. The P-gp inhibitor, verapamil, can be included in the test protocol for identification of active P-gp mediated transport (Figure 3).
4. Only a small amount of compound material is needed for the ***ex vivo* BBB permeability model** and re-synthesis of compound is rarely necessary.

P-gp identification in the model

Since P-gp mediated drug efflux is a major hurdle in CNS drug discovery, it is important to identify P-gp substrates in the early screen phase. P-gp efflux mechanisms can be confirmed in the *ex vivo* Locust BBB model. This is exemplified in a study where locust brains were exposed to three doses of either the P-gp substrate quinidine or the passive permeating compound carbamazepine and in the same three doses in co-administration with the P-gp inhibitor verapamil. At the lower concentrations (3 and 30 uM) there was a significant increase in the uptake of quinidine in co-administration with verapamil, but at higher concentrations this was not the case (Figure 3). This suggests that the P-gp efflux transporter is saturated at higher concentrations resulting in an increased uptake. This was not the case for the passive permeating drug carbamazepine.

About EntomoPharm

EntomoPharm was established in 2009 by multiyear experienced industrial professionals. The EntomoPharm vision is to become the global leader in cutting-edge insect models, delivering high-quality, high throughput and robust in vivo data in a timely and cost-efficient manner.
EntomoPharm's insect platform is designed to enable strong R&D decision making, thereby reducing the present-day 50-60% late stage ADMET failures.
EntomoPharm continually develop and optimize their pre-clinical screen models in insects to provide customers with cost-efficient, quality rich data to fit the needs of pre-clinical compound optimization programs.

1. Nielsen PA, Andersson O, Hansen SH, Simonsen KB, Andersson G.: Models for predicting blood-brain barrier permeation. Drug Discovery Today, 2011 Jun, 16 (11-12), 472-5.

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