7 Abstract

A complex system controls both procoagulant and anticoagulant reactions in hemostasis (*Ries*[1]). In normal situations, blood must remain fluid within the vasculature and yet clot quickly when exposed to nonendothelial surface, e.g. collagen. When myocardial infarction and stroke do occur, platelet aggregation activity increases. This pathological process may lead to thrombosis, a disorder in which a platelet and/or a fibrin clot occludes a blood vessel. Thrombosis may be complicated by pulmonary embolism. Such diseases are still one of the major causes of death in modern industrial nations.

Certain enzymes, e.g. **s**oluble **g**uanylate **c**yclase (**sGC**) and **p**hospho**die**sterase **5** (**PDE-5**), play an important role in platelet aggregation. Increased **c**yclic **g**uanosine-3‘,5‘-**m**ono**p**hosphate (**cGMP**) levels in thrombocytes lead to decreased coagulation.

In 1995, *Wu*[169] described **YC-1**, 3-(5‘-hydroxymethyl-2‘-furyl)-1-benzyl-1H-indazole, as a novel NO-independent activator of soluble guanylate cyclase (sGC) with antiplatelet effects. This enzyme catalyses the reaction of **g**uanosine-5‘-**t**ri**p**hosphate (**GTP**) to cyclic guanosine-3‘,5‘-phosphate (cGMP). The concentration of cGMP in platelets is also regulated by phosphodiesterase 5 (PDE-5), which degrades cGMP to **g**uanosine-5‘-**m**ono**p**hosphate (**GMP**). In 1999, *Galle*[154] demonstrated that **YC-1** also inhibits the PDE-5. Agents that elevate the cGMP level either by stimulating sGC or inhibiting PDE-5 are powerful inhibitors of platelet aggregation.

The aim of this doctoral thesis was to develop new compounds with antiplatelet (*in vitro*) and antithrombotic (*in vivo*) properties, using the sGC-activator **YC-1** and substance **A**, [4-[2-*n*-butyl-5-chloro-1-(2-chlorobenzyl)imidazolyl]methyl] acetate, as structural patterns. Substance **A** affects the cGMP metabolism by inhibiting the cGMP phosphodiesterase (PDE-5).

Ninety-nine 1-, 2-, 5-substituted 1*H*-imidazole-4-carboxylic acid derivates were prepared. The products were characterized by elemental analyses, ^{1}H-NMR-, IR- and mass spectrometry technology. Then the compounds were assayed in a Born test (*in vitro*) for their antiplatelet effects. Collagen was used to induce platelet aggregation in platelet-rich plasma. Due to solubilty problems, the incubation time was prolonged from four minutes to twenty minutes. Increased incubation time led to raised antiplatelet activity. The best result presented compound **16g** (IC_{50} = 2.5 µmol/L). Moderate IC_{50}-values (IC_{50} ~ 13 µmol/L) were observed when the substances **6c**, **16c** and **16i** were examined in the Born test.

After that the influence of the test compounds on the formation of thrombi was assayed in a laser thrombosis-model (*in vivo*) for their antithrombotic activity.
Compound **6c** showed the best inhibition of thrombus formation with 10 % in arterioles (5 % in venules) after a single oral dose of 60 mg/kg. Inhibition of thrombus formation in arterioles was also observed in substances **16b**, **16g**, **16i**, **16k**, **16n** and **19a**. They exhibited moderate (≤ 10 %) effects. Their antithrombotic activities varied from 7 % to 10 % in arterioles.
The compounds **6c**, **16c**, **16g**, **16i** and **19a** were selected for further tests because they showed the best results in a laser-thrombosis model (*in vivo*) and/or in Born test (*in vitro*).
They were investigated for more specific inducers of blood clotting agents, e.g. **p**latelet **a**ctivating **f**actor (**PAF**) and adrenaline and **a**denosine-5‘-**d**i**p**hosphate (**ADP**), instead of collagen. Different mechanisms of antiplatelet properties were found:

- Substance **6c** demonstrated an antiplatelet effect by antagonizing PAF (IC_{50} = 0.7 µmol/L).
- Compound **16c** was most active against ADP (IC_{50} = 1.9 µmol/L).
- Substance **16g** presented an antiplatelet effect by antagonizing collagen (IC_{50} = 2.5 µmol/L) and PAF (IC_{50} = 4 µmol/L).
- Compound **16i** inhibited platelet aggregation by antagonizing adrenaline (IC_{50} < 1 µmol/L).
- Substance **19a** was most active against PAF (IC_{50} = 48 µmol/L).

In addition the activation of sGC and the inhibition of PDE-5 were determined by using two different radioimmune-assays. The results of these assays demonstrated that the antiplatelet effects of the substances **6c**, **16c**, **16i** and **19a** are not mediated by increased sGC- or decreased PDE-5-activity.
Finally the compounds **6c** and **16g** were tested in a **c**yclo**ox**ygenase (**COX**)-1-assay by using a HPLC-method. These compounds exhibited potent COX-1-inhibitory activities. IC_{50}-values varied from 0.42 µmol/L (compound **6c**) to 0.94 µmol/L (compound **16g**).