Author's response to reviews

Title: Effects of Abciximab on Key Pattern of Human Coronary Restenosis in Vitro: Impact of the SI/MPL-Ratio

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Dear BMC Editorial Team,

enclosed please find the first revision of our manuscript entitled: "Effects of abciximab on key pattern of human coronary restenosis in vitro: impact of the SI/MPL-ratio." The authors would like to thank the referee for fair and constructive criticism. Due to the concerns of the reviewer with the "razor wound" migration model the Boyden chamber was used to study the effect of abciximab on cell migration.

Surprisingly, a significant stimulatory effect of abciximab on cell migration was detected: "After incubation of HCMSMC with abciximab in concentrations of 0.0002 - 2 ug/ml a stimulatory effect on cell migration was detected, statistical significance was achieved after incubation with 0.002 ug/ml (p<0.05), 0.002 ug/ml (p<0.001), and 0.2 ug/ml (p<0.05). Neither stimulatory nor inhibitory effects on cell migration were detected after incubation of HCMSMC with abciximab in a concentration of 20 ug/ml."

The queries of the referee have been carefully addressed, changes in the manuscript have been clearly marked.

We hope that the manuscript can now be accepted for publication in BMC.

With best wishes

Rainer Voisard, MD
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Reviewer I (Rudiger Blindt):
Q1: The authors analysed only the influence of abciximab on ICAM-1 expression in endothelial and smooth muscle cells. But, the effects of the substance on inflammatory cells as monocytes might be more relevant. Why did the authors not evaluate inflammatory cells?

A1: A cascade of in vitro and ex vivo models is applied by our group. If an agent is successful in in vitro studies (ICAM-1, migration, proliferation) the more complex threedimensional model of leukocyte attack (3DLA-model, Voisard et al. 2001) is applied. If the agent succeeds in the 3DLA-model ex vivo models as the human organ culture model (HOC-model, Voisard et al. 1999) will be the next step. Due to the fact that the direct effects of abciximab on ICAM-1 expression, migration, and proliferation were not very convincing the agent was not considered as candidate for further testing.

Q2: For an adequate analysis of the role of abciximab on inflammatory processes during restenosis at least some more other inflammatory pathways should be investigated (e.g. interleukin 6 and 10 expression, effects on chemokine expression).

A2: Although deeper insights in the pathophysiology of abciximab and inflammation are certainly of major interest, the authors would like to keep the design of the study on ICAM-1, migration, and proliferation.

Q3: The authors state that cell migration was not significantly altered by abciximab. In contrast, there were a few other studies that showed significant inhibition of SMC migration (Bendeck et al. 2001; Baron et al. 2000; Blindt et al. 2001). The reviewer is concerned regarding adequate use of the migration model in the study. The razor wound model used may not have enough sensitivity compared to more sensitive assays used in other studies (e.g. Boyden chamber model).

A3: In order to use a more sensitive assay the migration assay was changed. Instead of the razor wound model a Boyden chamber was used.

In methods, page 6, the Boyden chamber model was described:

Migration Studies
"Migration of HCMSMC was measured by a 24 well colorimetric assay (Chemicon, Hampshire, UK), based on the Boyden Chamber principle. HCMSMC were incubated with SmBM medium supplemented with 1% fetal calf serum (fcs) for a period of 48h. Thereafter HCMSMC were seeded on the upper side of the polycarbonate membrane (pore size 8um) of the Boyden Chamber. Migration of HCMSMC was stimulated by filling the lower chamber of the kit with SmBM medium supplemented with 10% fcs. Abciximab was added to the medium of the lower chamber in concentrations of 0.0002, 0.002, 0.02, 0.2, 2.0, and 20.0 ug/mL. After 24h of incubation HCMSMC were removed from the upper side of the membrane. Thereafter the membranes were stained for 20', airdried, and incubated with extraction buffer for 15'. The optical density of 100ul of this solution was measured at 560nm, SmBM medium supplemented with 10% fcs was used as control (100%)."

In results, page 9, the results of the Boyden chamber assay were described:

Effects of Abciximab on Cell Migration
"The effects of abciximab (0.0002, 0.002, 0.02, 0.2, 2.0, and 20.0 ug/ml) on migration of HCMSMC are shown in Figure 2 and Table 1. After a migration period of 24h a stimulatory effect was detected after incubation of HCMSMC with abciximab in concentration of 0.0002 ug/ml - 2ug/ml, no effect was found after incubation with the maximal concentration of 20ug/ml. After incubation of HCMSMC with abciximab in concentrations of 0.0002, 0.002, and 0.02ug/ml cell migration was increased by 36.29% (p<0.05), 36.68% (p<0.001), and 32.43% (n.s.). The stimulatory effect decreased after incubation with 0.2 and 2ug/ml of abciximab, cell migration was increased by 20.37% (p<0.05) and 10.81% (n.s.), respectively. After incubation of HCMSMC with the maximal concentration of 20ug/ml of abciximab no effect on cell migration was detected."

In the abstract, page 2, line 10-16 were rewritten:

"Migration: Part II of the study explored the effect of abciximab (0.0002, 0.002, 0.02, 0.2, 2.0, and 20.0 ug/ml) on migration of HCMSMC over a period of 24 h. After incubation of HCMSMC with abciximab in concentrations of 0.0002 - 2 ug/ml a stimulatory effect on cell migration was detected, statistical significance was achieved after incubation with 0.002 ug/ml (p<0.05), 0.002 ug/ml (p<0.001), and 0.2 ug/ml
(p<0.05). Neither stimulatory nor inhibitory effects on cell migration were detected after incubation of HCMSMC with abciximab in a concentration of 20 ug/ml.

In discussion, page 12, paragraph 1, line 1-6 was rewritten:

"The present in vitro study investigated the effects of abciximab on key pattern of human coronary restenosis. Three basic conclusions were determined. First, abciximab (0.0002 ug/ml - 20 ug/ml) had no effect on expression of ICAM-1 in HUVEC, HCAEC, and HCMSMC. Second, abciximab (0.0002 ug/ml - 2 ug/ml) stimulated migration of HCMSMC. Third, high concentrations of abciximab had a small but significant antiproliferative effect in HUVEC, HCAEC, and HCMSMC, SI/MPL-ratio's > 1 indicate that these effects can't be achieved after systemic infusion."

In discussion, page 15, paragraph 2, line 1-7 were rewritten:

"In the present study a stimulatory effect on cell migration was detected after incubation of HCMSMC with abciximab in concentrations of 0.0002 - 2 ug/mL, statistical significance was achieved after incubation with abciximab in concentrations of 0.0002 ug/ml, 0.002 ug/ml, and 0.2 ug/ml. Neither stimulatory nor inhibitory effects on cell migration were detected after incubation of HCMSMC with abciximab in a concentration of 20 ug/ml. In the applied migration model merely direct effects on cell migration can be studied. "

Q4: In consideration of the fact that the effect of abciximab on smooth muscle and endothelial cell migration and proliferation was extensively studied previously and that there might be some problems with the migration model used by the authors, focusing on the first part of the study (abciximab and inflammation=) might be favourable. Thus deeper insight in the pathophysiology of alphavbeta3-integrin blockade and inflammation are gained.

A4: please see A2.

Q5: The authors used a t-test to determine statistical significance. The use of an ANOVA with appropriate post-hoc analysis is more adequate because n of the analysis group is always >2.

A5: As written in Methods, page 8, Statistical Analysis, a paired t-test and not a t-test was applied:
"Statistical significance of differences between controls and drug-treated cells was determined by paired Student's t-test. Statistical significance was accepted for P < 0.05." 

Q6: The authors should state that the luminescent assay works by measuring ATP as an indicator of cell viability.

A6: The following sentence in Methods, page 7, Vitality of Cells, was rewritten, the term luciferase reaction was added:
"Luminescence of luciferase reaction as a marker of cell viability was measured in a CentroLB960 (Berthold, Technologies, Bad Wildbad, D)."

Q7: "DurchfluSZzytometer" should be translated to english.

A7: Cytoflow