CHAPTER 6
DISCUSSION

6.1. Atherosclerotic induction

In this study, hematoxilin and eosin staining showed foam cells in abdominal aorta after 12 weeks of the high-fat diet. Whereas, hyperplasion of smooth muscle formed after 14 weeks of atherosclerotic induction. Advance plaque signed by calcification of abdominal aorta formed after 17 week of the high-fat diet. Hence, we conducted experiment 18 weeks after initiation of the atherosclerotic diet. This result was different from previous study about atherosclerotic induction on animal. Madhumanthi et al. showed atherosclerosis in aorta of New Zealand rabbit after 12 week of induction (259). Li et al. showed calcium deposits in the ascending thoracic aorta after 6 weeks and atherosclerotic plaques formed in the mice aortas after 10–12 weeks of the high-fat diet (260). Srinivas et al. showed atheroscleosis of albino wistar rats after 55 days of atherosclerotic induction. The different result of time period to induce atherosclerotic may be resulted by the different type of animal, composition of diet, dose of vitamin D3, and rute of vitamin D3 administration. Considering the successful result of this study to induce atherosclerosis, it can be one of the choices methode to induce atherosclerosis.
6.2. Mesenchymal stem cell isolation, culture and identification

Method of isolation of this study was a traditional method using special characteristic plastic adherent of MSC. Umbilical cord matrix of SD rats as source of MSC. The identification of MSC was examined by STRO-1 as surface marker and was examined by the ability of MSC to differentiate into osteoblast.

Method of isolation in this study resulted in heterogeneous morphology of cells, *i.e.* fibroblast like cell and epithel like cell, but mostly fibroblast like cell. This is the weakness of the traditional method, as mentioned by Psaltis *et al.* (17) MSC resulted from passage 4 was used in this study to treat atherosclerotic rats, because higher passage of MSC increasing risk of senescence (261).

MSC have ability to differentiate into mesenchymal phenotype including bone, cartilage, and fat. Their multilineage potential can be assessed by culturing MSC in osteogenic, adipogenic, and chondrogenic media. This study assessed osteogenic potential of MSC by culturing them in osteogenic media. Formulation of osteogenic media has been developed by many researcher, but generally has common factors (262). The Formula of osteogenic media was slightly modified by researcher. This new formula was successful to differentiate MSC to be osteoblast, but it was not good to its proliferation capability. That result might have been due to a high concentration of dexamethasone. Dexamethasone is very
important to induce MSC to be osteoblas, but need to be considered in high
dose.

Another method to identify MSC is by surface marker staining. STRO-1 was
dchosen, because of some consideration. First, it is one of surface antigen profile
of MSC (17). Second, immunoselection enriched by STRO-1 is accompanied by
increased expression of cardiovascular-relevant cytokines (263). Third, immunoselection by STRO-1 enhanced tropic activity (263). This study did not
use STRO-1 for immunoselection. STRO-1 was used for immunocytochemistry
staining, and they were positive in expressing it. It means they were MSC.

6.3. Atherosclerotic Event

At the end of study, abdominal aorta was stained by hematoxilin and eosin, and
assessed by two pathology anatomy expert of The Sardjito Hospital Yogyakarta.
Atherosclerosis plaque in the positive control group was higher than that of the
MSC treatment group ($p=0.006$) and negative control group ($p=0.002$). It means
that intravenous administration of MSC ameliorate atherosclerosis in vessel. This
result support study of Wang et al. about the benefit MSC on progression of
atherosclerotic ApoE-knockout mice (30), although this study used different
animal model, Spregue Dawley rats. But, another study using rabbit had opposite
result, i.e. increasing of size lesion on vessel (29). Those different result of study
about the effect of MSC on atherosclerosis might have been due to different
sample, different dose of MSC and other factors that may not be fully understood. So, more study about MSC need to be explored.

6.4. IL-1α, IL-6 and TGF-β1 expression

Theoretically, MSCs mechanism to repair tissue injury are by paracrin action, proliferation and differentiation ability, cell fusion ability, and incorporation into newly formed vessel ability, Figure 6.1. From all those functions, mechanisms underlying tissue repair appear to be mediated predominantly through indirect paracrine actions (17). This study observed paracrin action of MSC by observing the expression of IL-1α, IL-6 and TGF-β1.

![Proposed mechanism of MSC to repair tissue injury](image)

Figure 6.1. Proposed mechanism of MSC to repair tissue injury, taken from Psaltis *et al.* (17).

IL-1α and IL-6 expression in endothel, smooth muscle, and macrophage in this study were statistically not significant. As part of tissue microenvironment,
previous studies of IL-1α and IL-6 have showed their role as proatherosclerotic. IL-1α is thought to facilitate early atherosclerotic formation by increasing leukocyte adhesion (9), mediating leukocyte transmigration (10), maintaining an inflammatory milieu (11) and adhesion molecule expression (76), and in the advanced plaque, it induced up-regulation of matrix metalloproteinases may destabilize the proteinaceous scaffold of the cap (77). On another hand, elevated levels of IL-6 are associated with increased risks of coronary and peripheral atherosclerosis (97), myocardial infarction (85), and the risk of death of patients with cardiovascular disease (98). This study showed that intravenous administration of MSC did not change IL-1α and IL-6 expression although can ameliorate atherosclerotic. It means that ameliorating of atherosclerosis was not mediated by expression of IL-1α and IL-6.

TGF-β1 is produced by both inflammatory and vascular cells (52). TGF-betas are released by immune cells and detected in wound fluid, especially during inflammation and tissue repair (264). In this study, TGF-β1 expression by macrophage inside vessel wall in MSC treatment group was lower than that of in positive control group, statistically significant (p=0.046). There is some controversial information regarding the role of TGF-β1 in atherogenesis (264). One group showed anti-atherogenic role and another group showed pro-atherogenic role. The Result of study showing the role of TGF-β1 as pro-atherogenic was published older than their role as anti-atherogenic. MSC
intravenous injection in calsification atherosclerotic plaque rats interfere TGF-β1 expression in macrophage. It means MSC seem to participate in atherosclerotic by interfering TGF-β1 expression from macrophage.

While TGF-β1 expression by endothel and smooth muscle in this study was statistically not significant ($p \geq 0.05$). But there is interesting pattern of expression. As we know that endothel is the outer layer which always interacting with element dissolve in blood flow, including MSC that injected intravena. They interfered TGF-β1 expression in endothel, and it was higher in atherosclerotic rats with MSC administration than atherosclerotic rats without MSC administration and rats without atherosclerotic, although statistically there is not significant. So, detail study about role of MSC in atherosclerotic plaque by their tropic effect, especially by TGF-β1 expression, need to be observed. However, there is potential limitation of this study, because MSCs administration were not followed by homing detection.