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Review

A new mechanism of the protamine-dependent hypotension after cardiopulmonary bypass and the role of calcium

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Abstract: Heparin and protamine are two indispensable agents of cardiopulmonary bypass surgery with effects on the cardiovascular and hematological system. Heparin is used as an anticoagulant in open heart surgery; whereas protamine is used to neutralize heparin effects when surgery is terminated. Protamine is given in order to neutralize heparin effects after cardiopulmonary bypass surgery and it causes hypotension in patients. However, the mechanism of this side effect is not clearly known. Current mechanism is that hypotension occurs after the administration of protamine due to the conformational change in the calcium channels or anaphylactoid thromboxane release or serum ionized calcium levels. The present study was to explain how protamine causes hypotension in evidence-based medicine indexed in PubMed and Web of Science. In addition to above mechanisms, possibly the infused protamine binds heparin and causes the coagulation cascade to activate heparin-AT complex on thrombin beside activating FXIa, FXa and FIXa and causing the re-use of Ca^{2+}. The re-use of Ca^{2+} at the coagulation cascade initiates an anion gap and it is assumed that hypotension develops because of the Ca^{2+} deficiency. Ca^{2+} ions are trapped in the thrombus by the resumption of thrombus formation. Ca^{2+} ions trapped in the thrombus cannot be used, so that Ca^{2+} ion deficit will develop in circulation and hypotension occurs due to the insufficiency of Ca^{2+} ions. The administration of Ca^{2+} ions together with the protamine might help to eliminate the side effect of the protamine (hypotension) while neutralizing heparin after open heart surgery in light of the information provided in the literature.

Key words: Calcium; Cardiopulmonary bypass; Heparin; Hypotension; Protamine.

Introduction

In medical history, until the 16th and 17th centuries, the heart was always regarded as an organ that must be avoided for surgery (1-3). In 1896, the perception was shattered by Ludwig Reh, when he sutured a patient's myocardium due to heart injury, and this date was regarded as the beginning of the heart surgery. But the first step starting modern heart surgery (open heart surgery) was the clinical use of extracorporeal circulation by Gibbon in 1953, the invention of heparin by Mc.Lean at the University of John Hopkins (4) and that it can be neutralized by protamine as shown by Chargoff and Olson (5). So currently more than 2 million open heart surgeries are performed annually worldwide. During cardiopulmonary bypass surgery, blood is in contact with foreign surfaces outside the vessel, thus creating a strong thrombotic effect. Cardiopulmonary bypass surgery is therefore not possible without anticoagulation. Heparin (anticoagulant) is an extremely essential anticoagulant regulator of blood clotting proteinases that are critical for maintaining hemostasis during open heart surgery. As known, body haemostasis is a complex physiological process, maintaining the fluidity of blood and must be regulated by delicate balance existing between thrombogenic and anti-thrombogenic mechanisms present in the body.

Methods

This systematic review (How protamine-induced hypotension occurred) was done through meta-analyses, reviews of clinical trials, evidence-based medicine, consensus development conferences, and guidelines in PubMed and Web of Science. Search terms were: cardiopulmonary bypass, intracellular calcium binding protein, calcium, antithrombin, protamine sulphate, heparin (anticoagulant), heparin rebound, heparin antidote, body haemostasis, anticoagulation, low-molecular-weight heparin (LMWH), heart performance, vasodilatation, hypotension, hypertension, thrombus formation, ionic complex and coagulation cascade.

Role of heparin on body haemostasis during cardiopulmonary bypass surgery

The discovery of heparin and its introduction into clinical practice have enabled the modern cardiac surgery by making cardiopulmonary bypass technology applicable (3). Heparin activates antithrombin both by inducing conformational changes in the protein that specifically enhance factor Xa binding and by providing a surface to promote thrombin or factor Xa binding alongside antithrombin in a ternary bridging complex, and rapidly inhibits thrombin, thus preventing the forma-

tion of a clot in the blood that contacts with the foreign surface. In other words, heparin requires antithrombin (AT), a plasma factor, for the anticoagulant effect of the molecule. The molecule via the pentasaccharide chain in heparin binds with AT. Thus both standard heparin and low-molecular-weight heparin (LMWH) inactivate factor Xa via antithrombin. Heparin-AT complex also inhibits FXIA, FXa and FIXA in addition to thrombin (6).

Role of protamine sulphate on body haemostasis after cardiopulmonary bypass surgery

Protamine (heparin antidote), a protein obtained from fish sperm, and a positively charged cationic peptide that contains high ratios of arginine and lysine, binds the negatively charged heparin to form a stable salt, and then this complex is removed from the circulation by the reticuloendothelial system. So that in order to neutralize the anticoagulant effect of heparin when surgery is finished, protamine sulphate is routinely used (7). The simplest and most common method is to dose protamine based on heparin administered (roughly 1 mg protamine per 100 units heparin). However, in patients with protamine allergy, other substances capable of forming a complex with heparin such as toluidine blue (5 mg / kg, intravenous slow or 0.2-0.3 gm oral) and hexadimethrine bromide (given in 15 minutes as milligrams of heparin and 1 mg / ml) should be introduced as heparin antagonists (8).

Blood should be taken after the pump and the level of heparin in the blood should be determined and the dose of protamine needed to neutralize this amount of heparin should be calculated. Protamine sulphate neutralizes all of the standard heparin (SH) and 30-70% of LMWH. For example, one milligram of protamine sulphate neutralizes about 100 IU SH, so for a patient who received 5000 IU heparin i.v. as a bolus, 50 mg of protamine sulphate is appropriate. Since the half-life of unfractionated heparin is 60-90 minutes, if the patient has been given an infusion of SH, the protocol should be calculated by taking into account the SH dose of the patient in the last 60-90 minutes. Approximately 50 mg of protamine sulphate may be given to neutralize heparin which is given in the last 2 hours in a patient receiving 2500 IU / hr heparin with continuous infusion. It should also be remembered that heparin is more slowly eliminated from circulation than protamine. Therefore, an anticoagulation status, which is called "Heparin Rebound" event, may occur after the administration of protamine. This effect is usually avoided with an additional dose of protamine. Since the administration of protamine at high doses may also cause antithrombotic effects, postoperative bleeding may also accur (9-11). Although heparin has a powerful antidote, the exact mechanism of action is still unknown. It has been suggested that protamine combines with heparin to form inactive ionic complexes with no anticoagulant activity (protamine-heparin-ATIII complex), thus preventing bleeding. This ionic complex is then cleaved after the reticuloendothelial system has been separated (5).

Potential complications protamine sulphate usage while heparin effect reversed

In order to neutralize effect heparin, protamine is widely used after cardiopulmonary bypass. However, protamine has numerous side effects such as systemic vasodilatation, bradycardia, ventricular fibrillation, and anaphylaxis. For this reason, protamine must be applied slowly. Also, beside the above well-known side effects of protamine, the most common complication after the procedure for neutralizing heparin with protamine after cardiac bypass surgery is the development of systemic hypotension in the patient because of histamine (stored especially in the basophil cells of blood and tissues) release or true anaphylaxis, along with catastrophic pulmonary hypertension due to anaphylactoid thromboxane release (5,6). Another word, the histamine released by your body during an anaphylactic reaction related with protamine administration causes blood vessels to widen which leads to a sudden and severe fall in the blood pressure, also known as hypotension.

A group of researchers have also reported that the effect of protamine sulphate on heart performance (negative inotropic effect) is due to conformational changes in ion channels and another group of investigators reported that protamine caused this effect by reducing β-adrenergic receptor sensitivity. It has also been reported that protamine partially blocks presynaptic calcium channels (5,6,12-14). If so, then some amount of Ca^{2+} ions will not enter the nerve cell. When enough Ca^{2+} ions cannot enter the nerve cell, the release of acetylcholine is decreased. If there is a decrease in the release of acetylcholine due to protamine as mentioned here, there must be an increase in blood pressure due to relaxation of intestine and bladder muscles, contraction of striped red muscles and contraction of blood vessels (12).

If so, in the light of the above paragraph, a question arises; how does protamine, which was given to neutralize heparin after cardiac bypass surgery, cause the development of hypotension in patients despite blocking acetylcholine. Acetylcholine is a neurotransmitter that causes contraction of the bladder muscles that are active in the brain and many parts of the body, relaxation of striped red muscles, palpitation and blood pressure reduction (12). It is suggested that hypotension caused by protamine, which is given in neutralizing heparin after cardiopulmonary bypass, is a result of conformational changes in calcium channels and decreased muscle contraction strength due to decreasing calcium intake or because of histamine release or true anaphylaxis, along with catastrophic pulmonary hypertension due to anaphylactoid thromboxane release. In addition to the current mechanism, the following paragraph will try to explain the probable cause of hypotension due to protamine from a different perspective. Before explaining a new possible mechanism of the protamine-dependent hypotension, we should first briefly describe the role of calcium in the biological system, including coagulation cascade and possible role in blood pressure.

Calcium

This element is essential for all living organisms (15). Ionized calcium (active form) and bounded cal-

cium (bounded to proteins e.g. albumin) are present in the human body. Ionized calcium, also known as free calcium, is the most active form. Ionized calcium binds to negatively charged sites on protein molecules, competing with hydrogen ions for the same binding sites on albumin and other calcium-binding proteins (16). This binding is pH dependent and alters the level of ionized calcium in the blood. Alkalosis (increase in pH) enhances increased protein binding, which causes a decrease in free calcium levels while acidosis decreases protein binding, resulting in increased free calcium levels (17).

Brief roles of calcium in health and diseases

In healthy people, plasma ionized calcium concentration (iCa) is maintained within the approximate reference range of 1.15-1.30 mmol/L (4.6-5.2 mg/dL). An ionized calcium level lower than 1.15 mmol/L is indicative of hypocalcemia, while an ionized calcium level higher than 1.30 mmol/L is indicative of hypercalcemia. A high level of ionized calcium (hypercalcemia) is generally associated with hyperparathyroidism, milk-alkali syndrome, Paget's disease, an overdose of vitamin D, multiple myeloma, a kidney transplant, sarcoidosis, tuberculosis, certain kinds of tumors and the use of thiazide diuretics (18). On the other hand, a low level of ionized calcium (hypocalcemia) is associated with hypoparathyroidism, vitamin D deficiency, inherited resistance to parathyroid hormone, malabsorption of calcium, magnesium deficiency, kidney failure, malnutrition, osteomalacia or rickets, high phosphorus levels (Calcium X phosphate=50), alcoholism, and acute pancreatitis (19).

Primary role of calcium in coagulation cascade

Coagulation cascade has been traditionally classified into intrinsic and extrinsic pathways, both of which converge on factor X activation (19). Under normal physiological conditions, normal vascular endothelium minimises contact between TF and plasma procoagulants. The coagulation cascade begins instantly through the activation of the factor VII – tissue factor (TF) pathway (exogenous pathways) or via the contact activation pathway (endogenous pathway), which proceeds through factors XII, XI, and IX to the assembly of a tenase complex. Both exogenous and endogenous pathways can activate factor X, which induces the formation of prothrombinase complex which converts prothrombin into thrombin, and consists of factor, Xa factor Va and $Ca^{2+,}$ on phospholipid surfaces. The tissue factor, factor VIIa complex, initiates thrombin generation and fibrin formation, and deficiency of any of the proteins within this pathway (e.g., factor IX, factor VIII, factor X, factor V, and prothrombin) decreases thrombin generation and thus, thrombus formation takes place (20,21).

Role of calcium in thrombus formation

It has been shown that intercellular calcium signaling plays a major role in regulating the rate and extent of platelet thrombus growth (19). As calcium enhances the conversion of factor X into Xa, platelet and coagulation factor-dependent thrombus formation is critical to limit posttraumatic blood loss at the sites of vascular injury (22), including cardiopulmonary bypass surgery. Most of the procoagulants and anticoagulants are produced by the liver except factor III, IV and VIII. These proteins undergo a post-translational modification (vitamin K dependent ϒ carboxylation of glutamic acid residues) which enables them to bind calcium and other divalent cations and participate in the clotting cascade (23). Platelet activation leads to intracellular calcium mobilization, where increased calcium serves as a second messenger in initiating numerous signaling pathways. So that calcium mobilization is required for stable platelet incorporation into the developing thrombus (24).

Role of calcium in blood pressure

It has been known that calcium is an excellent positive inotrope, and it increases the blood pressure regardless of the etiology of hypotension (25). For example, intravenous calcium-channel blocker administration (e.g. Verapamil) may cause hypotension via the negative inotropic and vasodilating effects (26). Therefore, it has been suggested that calcium pretreatment prior to intravenous calcium-channel blocker administration should be considered in patients in whom further reductions in blood pressure may precipitate hypoperfusion or worsen the underlying cardiovascular status. It has been noted that calcium before Diltiazem may reduce hypotension in rapid atrial dysrhythmias (27). It has also been also reported that calcium may reverse the hypotensive effects of prochlorperazine maleate and may help reverse hypotension in patients with Reye's syndrome who have been pretreated with phenothiazine antiemetics Jones. It has been overall confirmed since 1972 that hypotension could be corrected with Ca^{2+} administration (28,29). It has also been reported by another group that intravenous calcium administration is useful in the treatment of postoperative hypotension.

Based on all given information above regarding protamine, heparin and the role of calcium in coagulation cascade, here I will try to first time suggest a new possible mechanism how protamine-dependent hypotension occurs after cardiopulmonary bypass and whether the use of calcium might be helpful in case of hypotension occurring after cardiopulmonary bypass.

A new possible mechanism: The protamine-dependent hypotension after cardiopulmonary bypass surgery

Previously it was assumed that protamine-dependent systemic hypotension after cardiac surgery might occur due to histamine (stored especially in the basophil cells of blood and tissues) release or true anaphylaxis, along with catastrophic pulmonary hypertension due to anaphylactoid thromboxane release (5,6). As known, the histamine released by the body causes blood vessels to widen which leads to a sudden and severe fall in blood pressure, also known as hypotension. Another mechanism is a conformational change in Ca^{2+} channels.

Also, another mechanism is that hypotension due to protamine/heparin is unlikely to be related to changes

in serum ionized calcium levels (30). Moreover, the mechanism of calcium handling during and after bypass surgery is complex e.g. ionized and total serum calcium levels decrease significantly following the institution of cardiopulmonary bypass alone, therefore, induction of general anesthesia alone is associated with a significant decrease in total serum calcium. Ionized calcium decreases following heparinization but remains unchanged by protamine administration, and exogenously administered calcium chloride significantly increases serum ionized calcium and these changes are inversely related to the circulating pool of calcium (31).

Therefore, the above-assumed mechanisms may possibly activate systemic hypotension mechanisms. However, there should be another mechanism (possibly Ca^{2+} anion gap) other than above accepted mechanism (histamine release), due to protamine administration, not all the allergic reactions were observed (32), but almost, all the cases of hypotension developed. Therefore, there should be a different mechanism (Ca^{2+} anion gap) as explained below.

After open heart surgery operations, protamine is administered to neutralize heparin effects. This might cause calcium deficit by initiating the re-operation of the coagulation cascade. Because of protamine effects heparin in coagulation cascade by binding heparin (11), when the FXIa, FXa, FIXa and thrombin steps are re-activated, the re-use of Ca^{2+} began in the coagulation cascade. Therefore, coagulation cascade requires Ca^{2+} supports to compensate the Ca^{2+} deficits. Otherwise, Ca^{2+} deficits might result in hypotension (as Ca^{2+} increases blood pressure). Another explanation is that as thrombus formation resumes in the continuation of the operation, Ca^{2+} ions are trapped in the thrombus. Thrombus formation is a precaution against bleeding of the body, and therefore, Ca^{2+} ions that are trapped in the thrombus cannot be used, therefore, Ca^{2+} ion deficit occurs in circulation and hypotension develops due to Ca^{2+} ions gap (Figure 1). So that combining calcium-and protamine might help increase the blood pressure. Because it has been reported that calcium is an excellent positive inotrope and it increases the blood pressure regardless of the etiology of hypotension. Later, thrombolysis might cause an increase in serum Ca^{2+} concentration. So, Ca^{2+} gap should be covered on time to avoid the development of hypotension. In addition, since protamine causes clotting by binding heparin, other than this mechanism, more heparin will be released with compensatory mechanism in order to maintain the balance (33). Both heparin overdose and heparin circulating in the body coming from the pump will need more Ca^{2+} ions in order to prevent suppression on coagulation cascade due to protamine administration. In this case, therefore, the observed hypotension in patients was due to low Ca^{2+} ions (34). Taken together, the given protamine for neutralizing heparin activates coagulation cascade and causes Ca^{2+} ion deficit or histamine release or conformational change in Ca^{2+} channels might result in hypotension.

Discussion

Although often observed hypotension after bypass surgery induced by neutralizing the heparin effects by protamine is a very old story, the potential mechanisms behind the phenomenon of hypotension after neutralization of the effects of heparin by protamine after cardiopulmonary bypass surgery are not well known. This hypotension might be originated from Ca^{2+} ion deficit (re-used coagulation cascade or trapped in thrombus), histamine release or conformational change in Ca^{2+} channels or serum ionized calcium levels. Available data supports that combining calcium-and protamine can increase blood pressure. Therefore, after cardiopulmonary bypass operations, the concomitant use of calcium (calcium gluconate 1 gm or calcium chloride 0.333 gm for an average of 70 kg of human's) -protamine can be used to eliminate the side effect of the protamine (hypotension). Based on all this information, it is also predicted that Ca^{2+} preparations can be routinely used to correct hypotension in intensive care units in the future. It should be kept in mind that a sudden increase of calcium when someone injects it could trigger coronary spasm and could also cause arrhythmias.

Conclusion

The potential mechanisms behind hypotension after neutralization of the effects of heparin by protamine after cardiopulmonary bypass surgery have been explained. Calcium deficit occurs as a result of clot formation and calcium consumption driven by activation of the coagulation cascade. An anticoagulation mechanism of heparin and cause of protamine-induced hypotension after cardiopulmonary bypass (CPB) might be due to a serum Ca^{2+} decrease caused by the Ca^{2+} trapping in the thrombus. It is anticipated that the concomitant use of calcium (calcium gluconate 1 gm or calcium chloride 0.333 gm for average of 70 kg of human's) -protamine might help to eliminate the side effect of the protamine (hypotension) while neutralizing heparin after open heart surgery in light of the information provided in the literature.

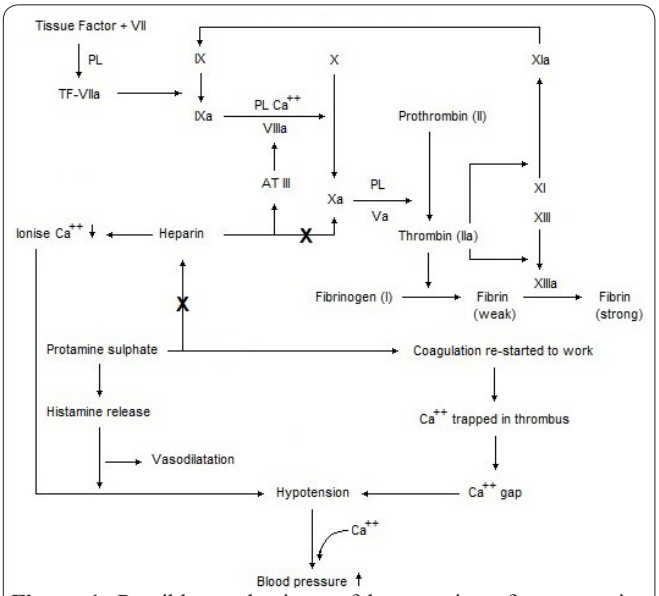

Figure 1. Possible mechanisms of hypotension after protamine administration. I: Fibrinogen. II: Prothrombin. VII: Stable factor (Proconvertin). VIII: Antihaemophilic factor A. IX: Antihaemophilic factor B (Christmas factor). X: Stuart-Power factor. XI: Plasma thromboplastin antecedent. XIII: Fibrin-stabilizing factor. AT: Antithrombin. PL: Platelet. TF: Tissue factor.

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