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ARTICLE

Effect of botulinum toxin type A on flap surgery in animal models: a systematic review and meta-analysis

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ABSTRACT

Flaps are common technical choices in aesthetic and reconstructive surgeries. However, the poor flap survival rate remains to be a difficult issue that troubles plastic surgeon. Recent research evidence supports that the use of Botulinum toxin type A (BTXA) can increase the flap survival rate. For verification, the present study was carried out to evaluate the effect of BTXA on flap surgery. Eight databases (PubMed, Cochrane Library, Ovid, Web of Science, Embase, Scopus, CBM, CNKI and WANFANG database) were searched for related published literature up to September 2020. A meta-analysis was then conducted to compare the effect of using BTXA with that of using saline or no treatment in flap surgery. Seventeen studies with a total of 565 animals were finally included in this review after strict exclusion and inclusion. Compared with saline/no treatment + flap group, BTXA + flap group showed a significantly higher flap tissue survival rate (mean difference [MD] 15.55, $p < 0.00001$), blood flow (standardized mean difference [SMD] 1.97, $p < 0.00001$) and vascular endothelial growth factor (VEGF) expression (at mRNA level: SMD 6.01, $p = 0.02$; at protein level: SMD 3.35, $p < 0.00001$). BTXA combined with flap surgery may have a positive effect on improving the flap tissue survival rate, blood flow of flaps and VEGF expression. Besides, the timing of BTXA injection may be an important factor for exerting its effect on flap surgery.

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Botulinum toxin type A; flap surgery; meta-analysis; animal models

Introduction

With the development of medical techniques, flaps have been one of the most extensively used technical means in aesthetic and reconstructive surgeries. The random-pattern skin flap was a preferred choice by surgeons at the early stage. After that, the concept of axial flap was proposed in the clinical setting considering the importance of blood supply to the survival of flap. In the later stage, perforator flaps appeared and have been improved by scientists [1,2]. The use of skin flap has an excellent effect on soft tissue injury caused by trauma or tumor resection, which may be beneficial to the reconstruction of skin structure and recovery of physical function both physiologically and psychologically. However, there may be generally a poor clinical survival rate for all flaps, which is prone to ischemic necrosis. It is a persistent issue in cosmetic reconstruction due to complex mechanisms, with continuous exploration of available drugs/methods for alleviation by scientists [3]. Surgical delay is a common choice to improve the survival rate of flap, which, however, requires two operations that may increase the risk of damage to the body and is time-consuming. Non-surgical methods, such as medical treatment (e.g. sympathetic neurolytic agents, vasodilators, calcium channel blockers and prostaglandin inhibitors), can reduce the incidence of ischemic injury, yet accompanied by potential development of systemic side effects simultaneously [4]. Under this background, botulinum toxin attracts considerable attention owing to its topically use and low side effects.

Botulinum toxin generated from Clostridium Botulinum consists of a Botulinium Neurotoxin and various non-toxic proteins. During activation, botulinum neurotoxin will proteolyze into two sub-chains, i.e. a heavy chain (100 kDa) and a light chain (50 kDa) [5]. Among the identified seven subtypes of Botulinum toxin, serotype Botulinum Toxin type A (BTXA) and type B have been extensively used in aesthetic and reconstructive surgery. In view of the broad research and application of BTXA, it has been used for treating various disease, such as facial palsy, neuropathic pain, musculoskeletal disorder, bruxism and parkinsonism [6–10].

Increasingly more evidence currently supports that BTXA can increase the survival rate of flap since it can promote anastomotic vasodilation to reach the peak velocity of blood flow [11]. BTXA can also increase blood flow through promoting angiogenesis and improving microcirculation [12,13]. Meanwhile, it can inhibit inflammatory responses and reduce the innervation of muscle related to flap, which is beneficial to improve the survival rate of flap [14,15].

In 1998, BTXA was used for the first time to flap surgery [16]. Considering a critical role of BTXA in increasing flap survival rate in animal models, its potential in clinical trial of flap surgery has been recognized to some extent. However, there has also been report to claim that BTXA has no effect on flap survival [17]. Meanwhile, most of existing studies are animal experiments with a small sample size. With respect to the aforementioned interpretation, the present systemic review was carried out to provide firm clinical basis for future clinical trials on the basis of the exclusion of unnecessary repetitive animal experiments. In our opinion, a meta-analysis of animal studies is required to promote and extend the clinical application of BTXA. It may benefit the refining and integration of data extracted from animal studies to provide

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reference for future clinical research. Moreover, it is possible to supplement innovative aspects such as VEGF and timing of treatment into analysis, which may inspire us to consider the mechanism related to BTXA. Therefore, a meta-analysis was performed here to compare the effect of using BTXA with that of saline alone or no treatment in flap surgery, in combination with qualitative analysis for outcomes based on limited data.

Methods

This meta-analysis was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement.

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) studies with animal models of any species that underwent flap surgery; (2) studies assessing the efficacy of BTXAs; (3) studies with the control group established and with flap surgery received alone; (4) studies evaluating the flap survival rate and/or blood flow and/or VEGF expression rate; and (5) studies with sufficient follow-up (at least 12 weeks).

The exclusion criteria included: (1) studies with the absence of original data, such as comments, reviews, and systematic reviews; (2) studies without primary outcomes; (3) trials performed *in vitro*; (4) studies with duplicated data reported in another study; and (5) articles with the shorter follow-up period when included studies had the same patient cohort, with articles having the longest follow-up selected only.

Data sources and search methods

All publications were searched electronically up to September 2020 from the following eight databases: PubMed, Cochrane Library, Ovid, Web of Science, Embase, Scopus, CBM, CNKI and WANFANG. Our search was restricted to animal studies, yet without any restriction to date, language, or publication status. The following combined search terms were used: (Botulinum Toxin Type A*, Botulinum Toxins*, Botulinum neurotoxin*, BTX, Botox, Kreotoixin, Creotoxin, Myobloc, Onabotulinumtoxin, Meditoxin or Onabotulinumtoxin A) AND (Surgical Flaps, Perforator Flap, Myocutaneous Flap, Free tissue flap or flaps). Meanwhile, during literature retrieval, our search terms were integrated with MeSH terms appropriately based on the use of an appropriate adjustment method for the different databases.

Selection of studies

Following the exclusion of duplicates, two authors independently assessed the titles and abstracts of all studies screened out in accordance with our pre-set retrieval strategy, followed by the exclusion of any apparently irrelevant studies. The full text of the potentially relevant articles were obtained in the next step, which were then assessed by the two authors independently to exclude irrelevant articles according to our inclusion and exclusion criteria strictly. Disagreements between the two reviewers were resolved by discussion to reach a consensus or by consulting a third author.

Data extraction and management

The following data were extracted from each study by the two reviewers independently: first author, year of publication, species of animals, sample size, age of animals, sex of animals, weight of animals, surgical procedure, flap size, intervention time, total dose/dose per injection of BTXA, evaluation time, follow-up period, tissue survival rate, blood flow and VEGF expression. If the data were not sufficient for meta-analysis, we tried to contact the authors to obtain the required information, calculated individually according to the Cochrane Handbook for Systematic Reviews of Interventions, or excluded directly if the above methods were not feasible. Disagreements between reviewers were resolved by discussion to reach an agreement or by consulting the third reviewer.

Assessment of risk of bias in included studies

Animal studies enrolled in our meta-analysis were assessed by two authors independently in accordance with the Systematic Review Centre for Laboratory animal Experimentation (SYRCLE) risk of bias tool. It included random sequence generation, similar baseline characteristics, allocation concealment, random housing, blinding of participants and personnel, random outcome assessment, blinding of outcome assessment, incomplete outcome data, selective reporting and other sources of bias. Disagreements between two reviewers were resolved by discussion to reach an agreement or by consulting the third reviewer.

Statistical analysis

All statistical analyses were performed using Review Manager (RevMan) version 5.3 software and Stata 15.1 software (Stata Corporation, College Station, TX). Meta-analyses were conducted to compare the flap survival rate, blood flow and VEGF expression of BTXA + flap group with those in saline/no treatment + flap group. The main outcomes were expressed by continuous data, mean differences (MDs) or standardized mean differences (SMDs), 95% confidence intervals (CIs), heterogenetity (I^2) and test of overall effect (Z) were used to evaluate the combined effect sizes. MD was utilized for outcomes of all studies used the same unit to measure. While SMD was applied in the case of inconsistent units. The Tau^2, chi-squared test, df and the I^2 statistic were used to assess the existence of heterogeneity. The fixed-effect model was selected for data with $p > 0.1$ or $I^2 < 50\%$. If $p < 0.1$or $I^2 > 50\%$. Data analysis was further performed to find the potential sources of heterogeneity and the random-effect model was used for subsequent assessment. Meanwhile, sensitivity analysis was further applied to evaluate the remaining studies after article exclusion in turn to assess the stability of the results. Furthermore, subgroup analysis was conducted according to the timing of BTXA injection to identify if it was the source of heterogeneity. Both Egger's test and Begg's test were used to assess publication bias, and $p < 0.05$ meant that the difference was statistically significant. The trim-and-fill method was implemented to adjust for the bias in the presence of publication bias [18]. The funnel plot was then replicated to include the 'missing' counterparts around the adjusted summary estimate.

Results

Study selection

The flow diagram of study selection is shown in Figure 1. A total of 1,060 records were identified from eight databases (PubMed, $n = 126$; Cochrane library, $n = 5$; Embase, $n = 184$; WANGFANG, $n = 33$; Ovid, $n = 309$; WOS, $n = 154$; Scopus, $n = 225$; and CNKI, $n = 24$) based on the above keywords. Subsequently, 452 records remained after the exclusion of duplicates, and 414 articles were further excluded with the review of titles and abstracts. Full-text review of the remaining 38 articles resulted in the exclusion of 21 articles, including studies with no useful data ($n = 19$), studies without primary outcome ($n = 5$), review and meta-analysis ($n = 4$), as well as letter and congress report ($n = 3$). Finally, 17 studies were included in the present meta-analysis based on the pre-set inclusion criteria [4,12,14,17,19–31].

Study characteristics

The basic characteristics of the 17 included studies are summarized in Table 1. There were a total of 565 animals, including 428 SD rats [4,12,14,17,19,21,25–31], 21 BALB/C mice [20] and 116 Wistar rats [22–24]. The sample size of these studies ranged from 10 to 132. Besides, the animal weight ranged from 240–420 g, with no available data related to age and sex of animals in some studies. There were 9 studies only using male SD rats [12,14,21,22,25,26,28–30], and 1 study performed in female BALB/C mice merely [20]. As for surgical procedure, 7 studies chose to use random pattern cutaneous flap [4,19,22–24,27,30], and myocutaneous flap was applied in Park's three studies [12,21,26]. Five studies used pedicled flap [14,17,25,29,31], one study selected expanded flap [28], and another one study chose axial pattern cutaneous flap [20]. The flap size in these studies varied from 1*1cm to 10*3cm. According to the intervention time, all studies could be divided into two subgroups: injection before flap surgery and injection after flap elevation, respectively, which was discussed in the subsequent subgroup analysis. The total dose and dose per injection of each study were different from each other. All the 17 studies compared the efficacy of BTXA + flap with saline/no treatment + flap [4,12,14,17,19–31]. The follow-up period varied from 3 days to 28 days. In terms of flap survival rate measurement, all 17 studies used the Digital photographs + Image processing software [4,12,14,17,19–31]. Four studies used Laser Doppler flowmetry to measure blood flow [4,20,27,28]. As for the assessment of VEGF expression, only one study chose to use Enzyme-Linked Immunosorbent Assay (ELISA) [29] and four studies used quantitative real time polymerase chain (qRT-PCR) [12,14,21,26], among which one study also conducted Western Blot [12].

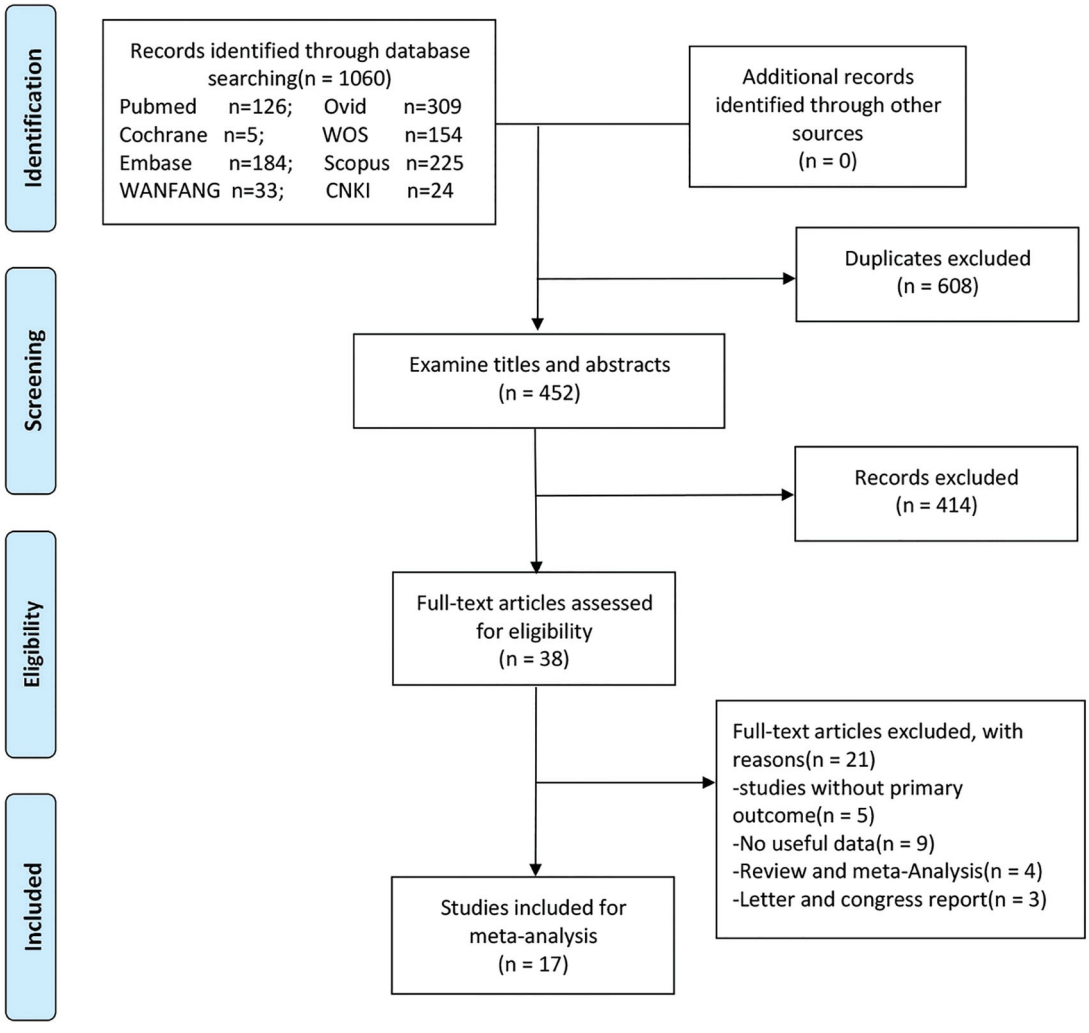

Figure 1. Flow diagram of literature selection.

Table 1. Basic characteristics of the included studies.

Author (year)	Species	Sample size	Age	Sex	Weight	Surgical procedure	Flap Size (length × width cm)	Intervention time	Total dose (IU)/ Dose per injection point (IU)	Intervention		Evaluation time (Day)
										Experiment group	Control group	
Zereyak et al. 2020[31]	SD rats	16	NA	NA	290–350g	Perforator-pedicled propeller flap	NA	4 weeks before flap elevation	16/4	BTXA	saline	8
ElShaer et al. 2019[30]	SD rats	30	NA	male	350–420g	Random pattern cutaneous flap	1 × 1, 1 × 2, 1 × 3	after flap elevation	2/0.33	BTXA	saline	10
Chen et al. 2019[29]	SD rats	132	NA	male	300g	Pedicled perforator island flap	11 × 3	after flap elevation	15/0.54	BTXA	saline	14,21,28
Liu et al. 2018[28]	SD rats	18	NA	male	250–300g	Expanded flap	8 × 2	4 weeks before flap elevation	8/1	BTXA	saline	7
Roh et al. 2017[27]	SD rats	40	NA	NA	300–400g	Random pattern cutaneous flap	9 × 3	3 days before flap elevation	10/0.37	BTXA	saline	3,7
Park et al. 2017[26]	SD rats	24	7w	male	240–280g	musculocutaneous flap	6 × 1.5	5 days before flap elevation	9/3	BTXA	saline	5
Park et al. 2016[12]	SD rats	24	8w	male	280–340g	Random pattern myocutaneous flap	6 × 5	5 days before flap elevation	8/1	BTXA	saline	5
Ghanbarzadeh et al. 2016[24]	Wistar rats	36	NA	NA	250–300g	Random pattern cutaneous flap	10 × 3	2 weeks before flap elevation	24/3	BTXA	saline	7
Camargo et al. 2016[23]	Wistar rats	60	2 m	NA	250–300g	Random pattern cutaneous flap	10 × 3	7 days before flap elevation	20/2	BTXA	saline	7
Xue et al. 2015[22]	Wistar rats	20	NA	male	250–300g	Random pattern cutaneous flap	5 × 2	after flap elevation	5/2.5	BTXA	saline	7
Park et al. 2015[21]	SD rats	24	8w	male	300–350g	Random pattern myocutaneous flap	6 × 5	5 days before flap elevation	20/2.5	BTXA	saline	5
Kim et al. 2015[25]	SD rats	24	8w	male	300–350g	Perforator flap	5 × 3	3 days before flap elevation	12/3	BTXA	saline	5
Arnold et al. 2014[14]	SD rats	36	NA	male	380–420g	Pedicled cutaneous flap	8 × 8	after flap elevation	2/2	BTXA	saline	7
Schweizer et al. 2013[20]	BALB/C mice	21	NA	female	20–24g	Axial pattern cutaneous flap	4 × 1.5	After flap elevation/ 24h before flap elevation	0.1/0.1	BTXA	no treatment	5
Kim.TK et al. 2009[19]	SD rats	30	10w	NA	350–400g	Random pattern cutaneous flap	8 × 2	After flap elevation	1.5/1.5	BTXA	saline	7
Kim.YS et al. 2009[4]	SD rats	10	NA	NA	300–400g	Random pattern cutaneous flap	10 × 3	7 days before flap elevation	20/NA	BTXA	saline	7
Arnold et al. 2009[17]	SD rats	20	NA	NA	NA	Pedicled island cutaneous flap	NA	After flap elevation	NA/NA	BTXA	saline	4

SD rats: Sprague-Dawley rats; *NA*: not available; *BTXA*: Botulinum Toxin Type A.

Risk of bias in included studies

The risk of bias of each study was independently assessed by two authors according to SYRCLE risk of bias tool [32]. There was no available information related to the method of random sequence generation in six studies [4,14,17,20,27,29]. Only one study did not provide the evidence of similar baseline characteristics [17]. None of research described the allocation concealment, random housing and random outcome assessment. Only two studies reported blinding of participant and personnel [14,24]. Six studies showed the blinding of outcome assessment [12,23,24,26,29,30]. Besides, only one study reported incomplete outcome data and other bias in included studies [23], the rest of included studies claimed no incomplete outcome data or other bias. In Camargo's study, there were 9 animals dead during research and hence corresponding data were excluded from the final result, which lead to the incomplete outcome data and possible other bias. Besides, there was no selective reporting in all studies. The summary of risk of bias of included studies is given in Figure 2.

Survival of flap tissue rate

All the 17 studies reported and compared the flap tissue survival rate between BTXA + flap group and saline/no treatment + flap group [4,12,14,17,19–31]. Data collection and analysis revealed that the flap tissue survival rate of BTXA + flap group was significantly higher than that of saline/no treatment + flap group (MD 15.55, 95%CI 12.97 to 18.14, $p < 0.00001$; $I^2 = 94\%$, $Z = 11.80$; Figure 3). Further subgroup analysis was conducted considering a high heterogeneity among all studies. Corresponding results indicated a significant heterogeneity between subgroups ($p < 0.001$; $I^2 = 92\%$, Figure 6). In this regard, difference in the flap tissue survival rate may explain the cause of high heterogeneity among all studies to some extent.

Blood flow

Blood flow was reported in four studies [4,20,27,28]. All these four studies compared the blood flow of BTXA + flap group with saline/no treatment + flap group. It was found that the blood flow of BTXA + flap group was significantly higher than that of saline/no treatment + flap group (SMD 1.97, 95%CI 1.40 to 2.55, $p < 0.00001$; $Z = 6.73$; Figure 4).

VEGF

Five studies reported outcomes of VEGF expression at mRNA level or protein level [12,14,21,26,29]. Among them, four studies [12,14,21,26] and two studies reported the difference of VEGF expression at the level of mRNA and protein, respectively [12,29]. At both levels, analysis of all the five studies showed that VEGF expression of BTXA + flap group was statistically significantly higher than that of saline/no treatment + flap group (mRNA level: SMD 6.01, 95%CI 0.89 to 11.13, $p = 0.02$; $Z = 2.30$, Figure 5(a); protein level: SMD 3.35, 95%CI 2.19 to 4.50, $p < 0.00001$; $Z = 5.69$, Figure 5(b)).

Other outcomes

All the extracted outcomes of the included studies are summarized in Table 2, and other outcomes are listed in Table 3. Outcomes included CD31, CD34, number of vessels, microvascular density and vessel diameter. In three studies [12,25,26], there was an increase of CD34 expression in BTXA + flap surgery group

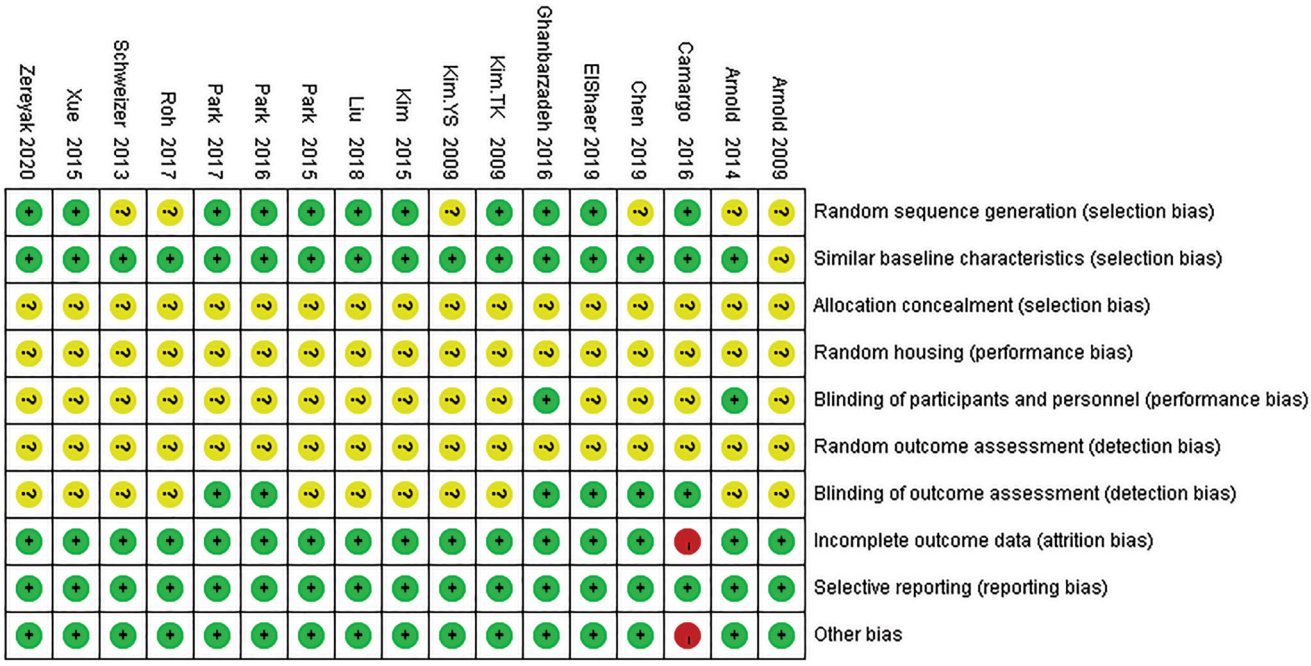

Figure 2. Risk of bias summary.

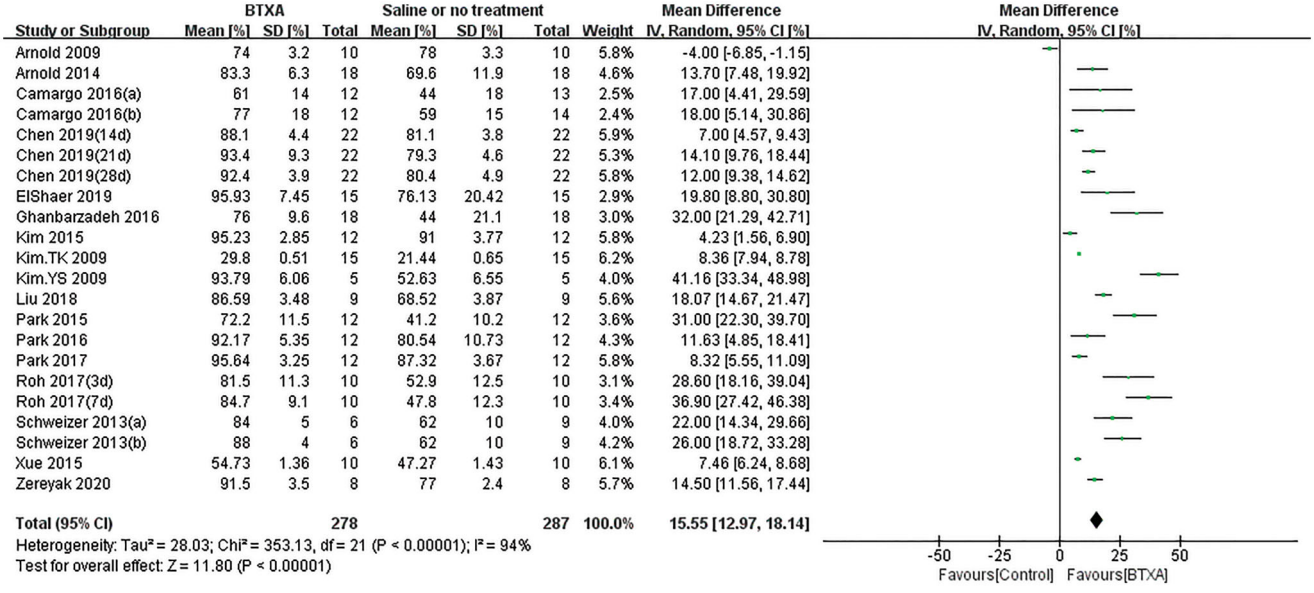

Figure 3. Forest plot of flap tissue survival rate.

when compared with saline/no treatment + flap surgery group. For CD31, Roh et al. [21] found an increase in BTXA + flap surgery group [27], while Park et al. gained the opposite result. As reported by Chen et al. [29], both the number of vessels and microvascular density showed an increase in BTXA + flap surgery group when compared with saline + flap surgery group. As for vessel diameter, three studies [21,22,25] claimed an increase in BTXA + flap surgery group than that in saline/no treatment + flap surgery group.

Subgroup analysis

Subgroup analysis was performed according to the intervention time. The enrolled 17 articles were divided into two subgroups: injection before the flap surgery group (pretreatment group) and injection after flap elevation group. Subgroup analysis showed that the flap tissue survival rate of pretreatment group (MD 21.17, 95%CI 15.54 to 26.80, $p < 0.00001$; $Z = 7.37$, Figure 6) was statistically significantly higher than that of intervention after flap elevation group (MD 9.83, 95%CI 7.05 to 12.60, $p < 0.00001$; $Z = 6.94$, Figure 6). Moreover, there was significant heterogeneity between subgroups, suggesting the presence of difference between subgroups ($p < 0.001$; $I^2 = 92\%$, Figure 6). It may explain that the intervention time of BTXA may be the cause of high heterogeneity among all studies to some extent.

Sensitivity analysis and publication bias

Further sensitivity analysis was made based on the results of flap tissue survival rate. No significant change was found in the results

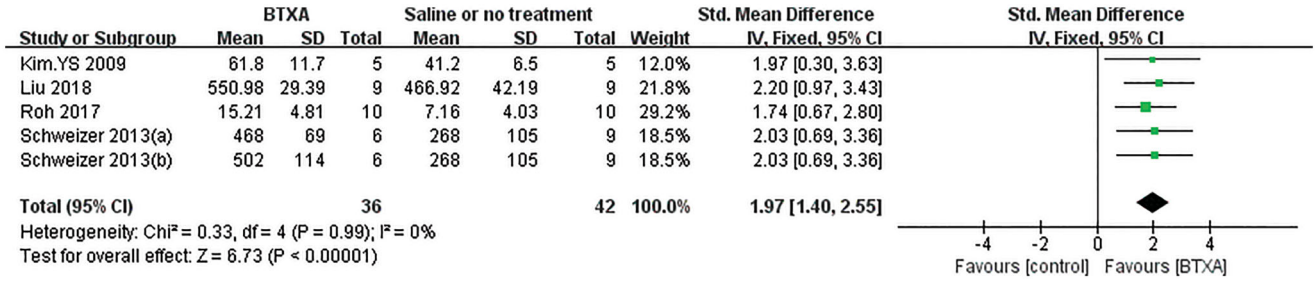

Figure 4. Forest plot of blood flow.

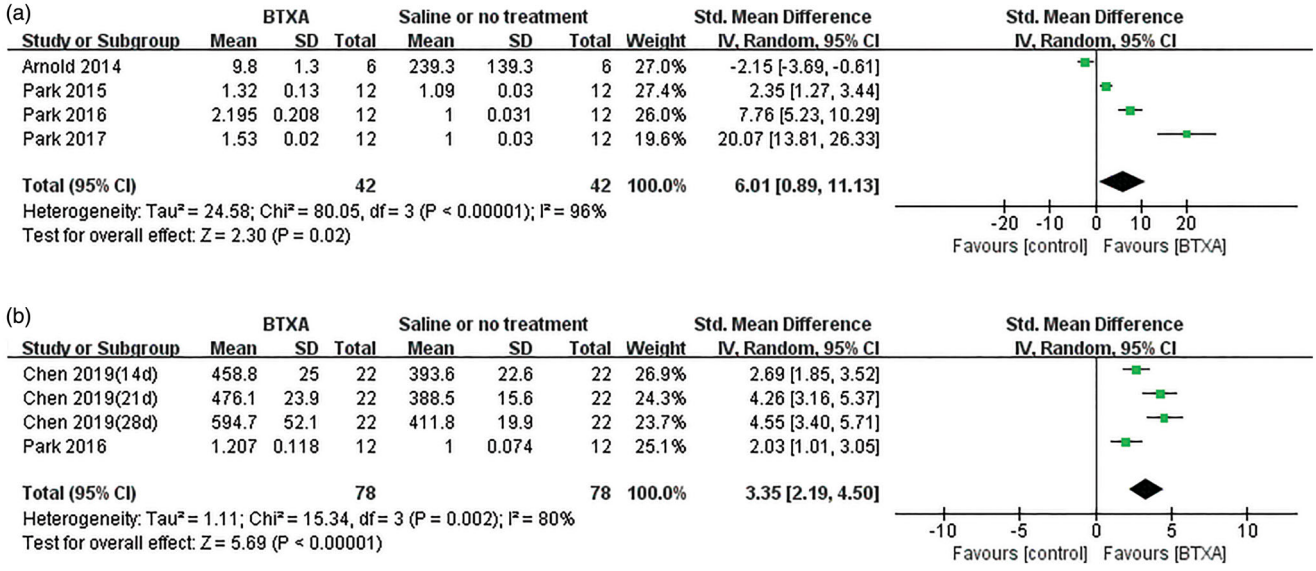

Figure 5. Forest plot of VEGF at mRNA level (a) and at protein level (b).

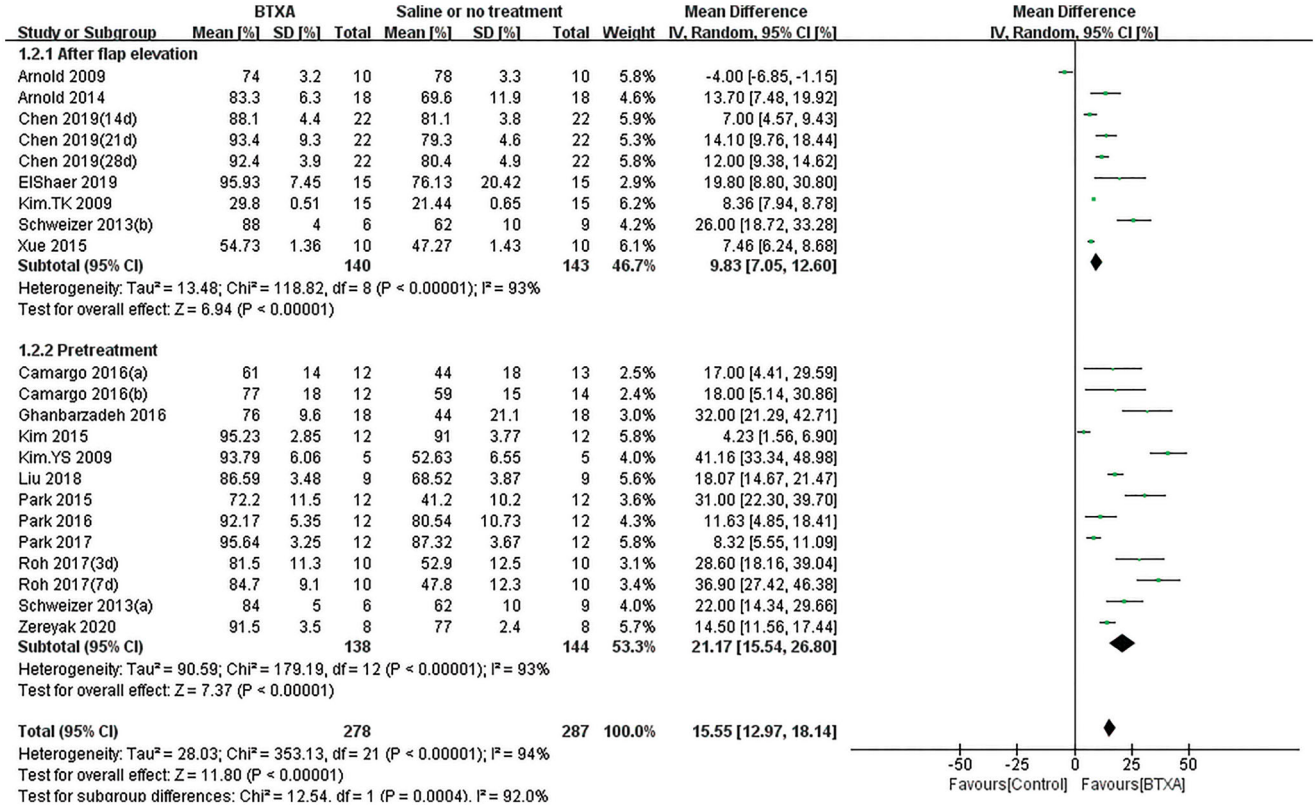

Figure 6. Subgroup analysis of flap tissue survival rate.

Table 2. Outcome data of the included studies.

Author (year)	Tissue survival rate (%)	Blood flow	VEGF expression	Measurement method
Zereyak et al. 2020[31]	8d: 91.5(3.5)vs77(2.4)	NA	NA	A
ElShaer et al. 2019[30]	10d: 95.93(7.45)vs76.13(20.42)	NA	NA	A
Chen et al. 2019[29]	14d:88.1(4.4)vs81.1(3.8) 21d: 93.4(9.3)vs79.3(4.6) 28d: 92.4(3.9)vs80.4(4.9)	NA	14d: 458.8(25.0)vs393.6(22.6) 21d: 476.1(23.9)vs388.5(15.6) 28d: 594.7(52.1)vs411.8(19.9)	A + C1
Liu et al. 2018[28]	7d: 86.59(3.48)vs68.52(3.87)	7d: 550.98(29.39)vs466.92(42.19)	NA	A + B
Roh et al. 2017[27]	3d: 81.5(11.3)vs52.9(12.5) 7d: 84.7(9.1)vs47.8(12.3)	immed: 15.21(4.81)vs7.16(4.03)	NA	A + B
Park et al. 2017[26]	5d: 95.64(3.25)vs87.32(3.67)	NA	1d: 1.53(0.01)vs1.00(0.02)	A + C2
Park et al. 2016[12]	5d: 92.17(5.35)vs80.54(10.73)	NA	C2-5d: 2.195(0.208)vs1.000(0.031) C3-5d:1.207(0.118)vs1.000(0.074)	A + C2/C3
Ghanbarzadeh et al. 2016[24]	5d: 76.0(9.6)vs44.0(21.1)	NA	NA	A
Camargo et al. 2016[23] *(a/b:2/4-month tobacco exposure)*	a-7d: 61(14)vs44(18) b-7d: 77(18)vs59(15)	NA	NA	A
Xue et al. 2015[22]	7d: 54.73(1.36)vs47.27(1.43)	NA	NA	A
Park et al. 2015[21]	5d: 72.2(11.5)vs41.2(10.2)	NA	5d: 1.32(0.13)vs1.09(0.03)	A + C2
Kim et al. 2015[25]	5d: 95.23(2.85)vs91.00(3.77)	NA	NA	A
Arnold et al. 2014[14]	7d: 83.3(6.3)vs69.6(11.9)	NA	7d: 9.8(1.3)vs239.3(139.3)	A + C2
Schweizer et al. 2013[20] *(a/b:24h before flap elevation/ after flap elevation)*	a-5d: 84(5)vs62(10) b-5d: 88(4)vs62(10)	a-5d: 468(69) vs268(105) b-5d: 502(114)vs268(105)	NA	A + B
Kim.TK et al. 2009[19]	7d: 29.8(0.51)vs21.44(0.65)	NA	NA	A
Kim.YS et al. 2009[4]	7d: 93.79(6.06)vs52.63(6.55)	7d: 61.8(11.7)vs41.2(6.5)	NA	A + B
Arnold et al. 2009[17]	4d: 74.0(3.2)vs78.0(3.3)	NA	NA	A

Format: Nd: M(SD) 'Experiment' vs M(SD) 'Control'; M: mean, SD: standard deviation,Nd: N days after surgery.
Measurement method: A(Tissue survival rate): Digital photographs + Image processing software (%); B(Blood fow): Laser Doppler flowmetry (PU).
C(VEGF expression): C1 Enzyme-Linked Immunosorbent Assay (ng/mg); C2 quantitative real time polymerase chain reaction (qRT-PCR, fold change); C3 Western Blot.

Table 3. Characteristics of other outcomes.

Other outcomes	Studies
CD31	Roh et al.2017 (+) Park et al.2015 (−)
CD34	Park et al.2017/Park et al.2016/Kim et al.2015 (+)
Number of vessels	Chen et al.2019 (+)
Microvascular density	Chen et al.2019 (+)
Vessel diameter	Park et al.2015 /Kim et al.2015/Xue et al.2015 (+)

(+): Favours[BTXA]; (−): Favours[Control].

of this systemic review after excluding each article (Figure 7), suggesting stable results of this study. Meanwhile, the publication bias of flap tissue survival was assessed using both Begg's test ($p = 0.088$, $p > 0.05$, Figure 8(a)) and Egger's test ($p = 0.033$, $p < 0.05$, Figure 8(b)). Consequently, according to trim & fill analysis with funnel plot of flap tissue survival rate, there was no significant change in the effect size when compared with the original funnel plot (Figure 9), indicating that the possible publication bias of this analysis had none obvious effect on the results [18]. Both methods above indicated the stability of the results of this systemic review. In addition, no sensitivity analysis or test of publication bias was conducted owing to the small amount of studies reported result of blood flow or VEGF expression.

Discussion

Summary of evidence

Flap reconstruction is a major technique in aesthetic and reconstructive surgery, which is widely applied in resurfacing tissue defect in the clinical setting. However, it remains to be clarified with respect to its role in increasing the survival rate of flap since the occurrence of flap vascularization is multifactorial and may lead to necrosis. Surgical delay was developed for the first time to improve blood supply of flap and achieved satisfied outcome. However, it is a double-edged sword with complicated surgical procedures that greatly restricts its application clinically. Accordingly, a variety of pharmacological agents are explored in the experimental stage to prevent the occurrence of flap ischemia, such as sympatholytics, vasodilators, calcium channel blockers, hemorheological agents, prostaglandin inhibitors and anticoagulants [17]. However, the proposed medicine may also results in systemic effects and serious side effects eventually. Therefore, current attention has been paid to search for topical application mode to meet the need of both excellent therapeutic outcomes and less side effects. In this situation, the use of BTXA becomes a hot-topic in the research of flap surgery.

An understanding on the intrinsic mechanism of BTXA is quite important to extent its application in clinical flap surgery. Besides, beneficial effects of BTXA may result from different mechanisms of action. Firstly, BTXA can improve the blood flow of flap. As for the reasons, BTXA may inhibit acetylcholine release to block the induction of muscle contraction. Under normal conditions, acetylcholine releasing can be promoted by proteins from the soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE). The heavy chain of BTXA can attach to cell-surface proteins such as E-cadherin [33] and fibroblast growth factor receptor (FGFR) [34], while the light chain cleaves SNARE proteins, synaptosomal-associated protein (SNAP)-25, thus inhibiting acetylcholine release into the neuromuscular junction. Therefore, BTXA can reduce smooth muscle contraction in vessels to increase flap blood flow. Besides, as Tai S.R et al. reported [27], BTXA can inhibit the secretion of norepinephrine *via* suppressing the activation of dopamine pathway and in turn increase the blood flow of flap as well. In this systemic review, four studies described a statistically significant increase in blood flow following intervention with BTXA [4,20,27,28]. Anyway, it remains to be further explored in terms of the mechanisms of using BTXA to increase the blood flow of flap.

Furthermore, Carmargo et al. observed increased lumen diameter, external arterial diameter, and lumen/wall thickness ratio in BTXA + flap group, which undoubtedly would contribute to the increase of blood flow and viability of flap [35]. Meanwhile, three studies claimed an increased vessel diameter in BTXA + flap

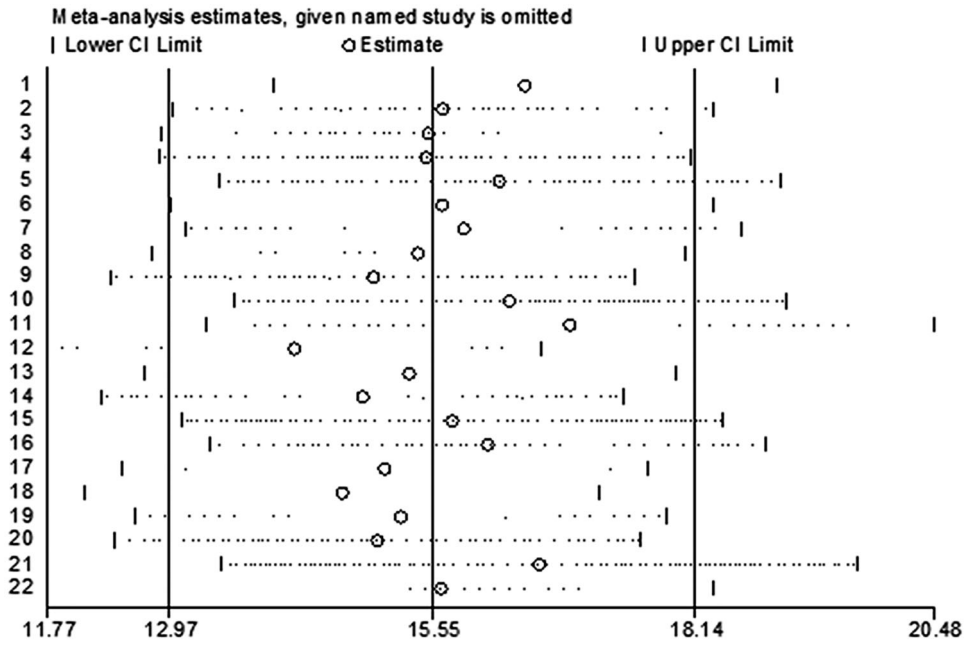

Figure 7. Sensitivity analysis of flap tissue survival rate.

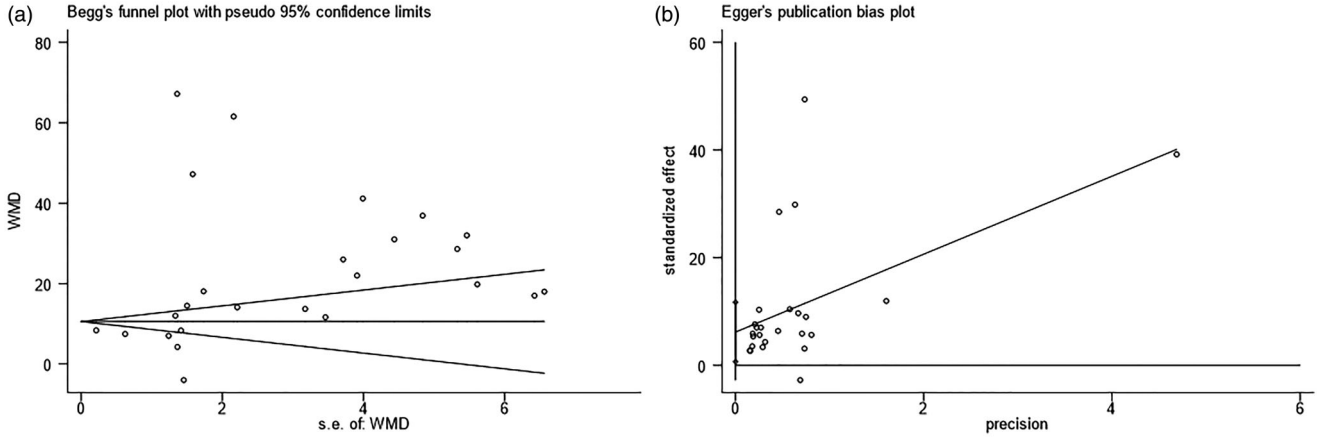

Figure 8. Begg's (a) and Egger's (b) Funnel Plot of flap tissue survival rate.

group, with statistically significant difference [21,22,25]. At molecule level, an increase of VEGF, CD31 and CD34 expression was found in BTXA + flap group when compared with saline/no treatment + flap group [14,19], which would lead to vasodilation and endothelial proliferation, and eventually the increase of blood flow. In this meta-analysis, five studies reported statistically significant increase of VEGF expression in BTXA + flap group than that in saline/no treatment + flap group based on the detection using ELISA, qRT-PCR or Western Blot [12,14,21,26,29]. It verified the positive effect of BTXA on expression of VEGF at mRNA/protein level. In addition, three studies described the same result of increased CD34 expression [12,25,26]. While only one study showed positive outcome of upregulated CD31 expression following BTXA intervention [27], while one study supported a decreased CD31 expression [21]. It may be explained by various reasons such as the difference of flap size, injection time and operator's experience. In short, vessel activity-related molecules did show an increase of expression in BTXA + flap group, which may lead to vasodilation and neo-angiogenesis, and hence increase the blood flow.

In addition to the improvement in blood flow, BTXA could also inhibit intracellular accumulation of reactive oxygen species (ROS) in vascular endothelial cells, so as to prevent flap from suffering ischemia-reperfusion injuries and thus improve the survival of flap, as suggested by Uchiyama et al. [36].

Thirdly, microcirculation may also exhibit an essential role in improving flap survival. For example, Roberto et al. [13] found that BTXA-potential diameter and assumed increase of 15% in diameter would contribute to a theoretical 75% increase in arterial blood flow over a long period. The proposed significant increase in perfusion may be beneficial to improving flap survival. Besides, Chen et al. supported the same theory on the basis of statistically significant increase of the number of vessels and microvascular density after intervention with BTXA [29].

Furthermore, BTXA could decline the secretion of inflammatory mediators such as IL-1 and TNF-α [14]. Thus, BTXA may have an effect on mediating inflammatory response and hence explain the increase of flap survival rate. Besides, Kucukkaya et al. reported a decrease in the amount of wound graft contraction in animals with BTXA injection [37]. Meanwhile, Lee et al. proposed that

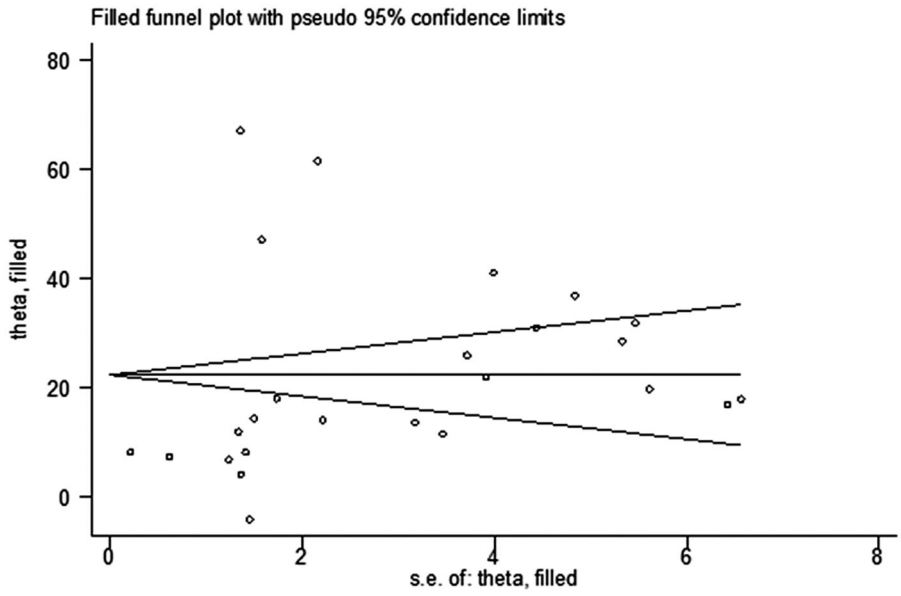

Figure 9. Trim & fill analysis of funnel plot of flap tissue survival rate.

BTXA could block TGF-β1 signaling and interrupt the differentiation of fibroblasts to myofibroblasts, thus declining the risk of hypertrophic scar to maintain the stability of flap [38]. Consequently, BTXA combined with flap surgery may improve flap survival through several mechanisms.

Recently, one study conducted by Steven et al. [39] also reported the effect of BTX on flaps, which however, focused on different outcome indicators(vessel diameter dilation and percentage of animals with thrombosis). For further outcome, a subgroup analysis was conducted in our systemic review. It revealed that the effect of BTXA in pretreatment subgroup was significantly higher than that in injection after flap elevation subgroup. It is thus assumed that the timing of administrating BTXA may be responsible for the effect of BTXA. Furthermore, Arnold et al. found a significant decrease of inflammatory mediator 2 days after BTXA injection [14], suggesting the time-delay effect of reaching its peak of effect. More interestingly, Schweizer et al. [20] compared the flap survival rate of BTXA injection after flap elevation with that before flap surgery, with the absence of statistical difference between groups. It indicated that the effect of pretreatment BTXA was inferior to its injection after flap elevation. In their study, BTXA was applied one day before flap surgery. Our authors speculated that BTXA may take a longer period of time (two days for example) to reach the peak of its effect, which may be the reason for the results reported by Schweizer et al.

Limitations

Some limitations of our review should be discussed. Firstly, we should discuss the effect of publication bias as only published articles can be included in this review and there are few published studies with negative outcomes. Funnel plots were used to evaluate the effect of publication bias. Secondly, the study had a smaller sample size owing to the limited amount of studies that could meet both the inclusion criteria and the characteristics of animal researches, which may reduce the credibility of outcomes in this study to some extent. Thirdly, studies enrolled in our review produced heterogeneity, and our meta-analysis of flap tissue survival rate also had significant heterogeneity. It may be attributed to the differences in measurement methods, measurement units, and animal species in each experiment. Besides, different research conductor, different operations with animals and different lab environment may also explain such heterogeneity. The quality of different flap surgery performed by different conductor may be the key factor resulting in high heterogeneity owing to the difference in their proficiency and operating skills. A subgroup analysis was also performed according to the timing of injection to explore the source of heterogeneity with positive result obtained.

Conclusions

In conclusion, our meta-analysis supports that BTXA combined with flap surgery may have a positive effect on improving the flap tissue survival rate, blood flow of flaps and VEGF expression. Besides, the timing of injection may be an important factor for exerting the effect of BTXA on flap surgery. However, the shortage of sufficient data of some outcomes promotes subsequent qualitative analysis. In our future exploration, we will emphasize on well-designed preclinical and clinical studies based on larger sample size to further clarify the effect and mechanisms of BTXA on flap surgery in humans.

Disclosure statement

None of the listed authors have conflicts of interest or any disclosures.

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