Electrohydraulic intracorporeal lithotripsy of salivary duct stones – in vitro and animal investigations†

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Summary. Background: The performance potential of extracorporeal lithotripsy as a method for the treatment of salivary duct stones has been demonstrated in the scope of routine clinical applications. The question thus arises whether electrohydraulic intracorporeal lithotripsy as applied in urology and gastroenterology might equally serve as a useful method for curing sialolithiasis. Materials and methods: (a) In vitro experiments: 58 salivary duct stones and 11 extirpated human submandibular glands were treated by three different electrohydraulic modalities. (b) Animal experiments: The dilated Stensen's duct and several other types of tissue (muscle, parotid gland, facial nerve) of six rabbits were exposed to electrohydraulic shockwave emission. Results: 53 out of 58 salivary duct stones (91%) were fragmented, 39 (67%) of which had a residual stone diameter of less than 1.5 mm and 14 (24%) of more than 1.5 mm. No effects were observed in five cases only. Fragmentation remained independent of the mineralogical stone composition and the type of implemented lithotriptor. The smaller the diameter of the probe and the larger the stone, the higher the number of shockwaves that was required for fragmentation. Complete fragmentation could not be achieved with the smaller probes. In human submandibular glands detectable tissue lesions could be macroscopically and histologically evidenced after application of electrohydraulic shockwaves in vitro. Immission of electrohydraulic shockwaves into the dilated Stensen's ducts of rabbits led to duct perforation after one to five single pulses. Furthermore, lesions of nerves and blood vessels were observed in the environment of the duct. These effects occurred with all the employed electrohydraulic devices, and with the entire range of applied probe diameters and intensity levels. In our opinion, the induced damage is probably the combined result of the direct effect of the plasma and of the resultant stress wave. Conclusions: Indications of the described method for the treatment of stones in narrow human salivary ducts should be clearly restricted due to the high risk of serious damage to tissue.

Keywords: salivary stones, sialolithiasis, electrohydraulic intracorporeal lithotripsy

Introduction

Electrohydraulic lithotripsy is the oldest technique used in the fields of urology and gastroenterology to treat stone diseases by intracorporeal application of shockwaves [3, 4, 13, 14, 17, 20]. Advances in the technological
performance level of electrohydraulic lithotriptors and progressive miniaturization of the implemented probes and endoscopes have improved the prospects for applying electrohydraulic-intracorporeal lithotripsy in the therapy of sialolithiasis of the large salivary glands of the head – as a method that is alternative, viz. complementary to extracorporeal lithotripsy of sialoliths \[10\]. Salivary duct stones exhibit a different composition and also a different hardness compared to biliary and renal calculi. It was therefore necessary to conduct basic investigations as to the fragmentability of sialoliths by means of intracorporeally applied shockwaves.

Earlier studies on the application of electrohydraulic shockwaves to tissue in animal experiments or on the treatment of the urinary and biliary tracts have reported cases of bladder and ureter perforation as well as damage to tissue of the ductus choledochus. Therefore, the tissue-destructive potential in the region of the narrow salivary ducts and of the adjacent anatomic structures had to be elucidated by in vitro and in vivo investigations.

**Materials and methods**

**Electrohydraulic lithotriptors**

The following three commercial lithotripter modalities were used for shockwave applications:

1. Lithotron EL 23 (Walz Elektronik GmbH, Rohrdorf, Germany);
2. Riwalith 2280 (Richard Wolf GmbH, Knittlingen, Germany);
3. Calcutript (Karl Storz GmbH, Tuttingen, Germany) (Figure 1).

The available probe diameters covered a range from 1.6 French (F) to 3.0 F. The basic physical principles of operation are identical in all three cases: a high-voltage that is built up by a series of condensers in the generator is discharged within a fraction of a second; at the tip of the probe a spark discharge between the anode and cathode takes place, with a consecutive thermal expansion of the generated spark-discharge plasma (local temperatures as high as 10,000 K) and the ambient liquid medium. This leads to the propagation of a spherical shockwave. The intensity of the shockwave pulse can be preselected in all the modalities. The intensity levels applied in our investigations are listed in Table 1.

In vitro investigations

(1) **Salivary duct stones**: Fifty-eight salivary duct stones were included in the investigations. The average weight of the calculi was 170.3 mg (range: 10–991 mg), and the largest median longitudinal diameter was 6.7 mm (range: 3.8–20.0 mm).

The concretions assigned to the different lithotriptors, the probe dimensions as well as intensity values are given in Table 1.

All 58 stones were subjected to mineralogical analysis after lithotripsy had been carried out. These investigations included sieve analysis to determine residual fragment size and X-ray diffraction measurements (diffractometer by Phillips Co.) for analysis of the chemical composition of the stones.

(1.1) **Stone fragmentation**. Before exposure to shockwaves, the salivary duct stones were immersed in physiological saline in a plastic cone with 1.5 mm perforations at its base which were permeable to stone fragments of this size. The electrohydraulic probes could thus be brought in direct contact with the concretions and exposed to shockwaves using different probe sizes, modalities and pulse intensities (Figure 2). The salivary duct stones were divided up into three groups according to size: Size category 1, 2–5 mm; Size category 2, 5–10 mm; Size category 3, >10 mm.

(1.2) **Experimental set-up**

(1.2.1) **Investigation of basic fragmentability**. Thirty stones (six groups of five stones each) were exposed to shockwave treatment with the EL (Walz Co., Rohrdorf, Germany) at intensity settings 1 and 2 in order to elucidate the basic question of fragmentability of salivary duct stones, viz. the dependence of fragmentability on pulse intensity, stone size and mineralogical stone composition. In these experiments the probe size was kept at a constant 3.0 F. The median number of shockwaves required to achieve “therapeutically adequate” fragmentation, i.e., a residual stone size smaller than 1.5 mm (Table 2), was determined for each group of stones. On the basis of anatomic studies fragments of this magnitude appear adequate for achieving spontaneous stone clearance \[11\].
Table 1. Overview of the different types of applied electrohydraulic lithotriptors, intensity settings and probe sizes, with the respectively treated collection of stones and reference to the therapeutically fragmented (complete fragmentation), incompletely fragmented (partial fragmentation) and non-fragmented

<table>
<thead>
<tr>
<th>Generator/intensity setting/probe diameter (F)</th>
<th>Number of stones</th>
<th>Complete fragmentation</th>
<th>Partial fragmentation</th>
<th>Non-fragmented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithotron 1/3</td>
<td>15</td>
<td>15</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lithotron 2/3</td>
<td>15</td>
<td>15</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lithotron 1/2</td>
<td>7</td>
<td>—</td>
<td>7</td>
<td>—</td>
</tr>
<tr>
<td>Calcutrot 1/3</td>
<td>7</td>
<td>7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Calcutrot 1.2/3/1.6</td>
<td>7</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Riwoth/5/2.4</td>
<td>7</td>
<td>—</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

(1.2.2) Investigation of the dependence of fragmentation on the type of lithotriptor and the size of the probe.

Groups of stones comprising seven concrements were treated with various probe sizes, intensities and different modalities (Table 1). The primary point of interest of these investigations was the dependence of stone disintegration behaviour on the type of lithotriptor and the probe size. The maximum number of shockwaves was restricted to 900 individual pulses due to considerations of clinical therapy (firstly, preliminary investigations had shown that no further improvement of the achievable degree of fragmentation could be expected in going beyond this number of shockwave pulses, and, secondly, that a clinically acceptable duration of treatment sessions could no longer be maintained) and the fraction of 'therapeutically adequate' fragmented stones, viz. of partially disintegrated stones (>1.5 mm) was recorded (Table 1).

(2) In vitro investigations of human tissue. Eleven submandibular glands that had been carefully exposed together with the efferent duct and extirpated in the course of therapeutically indicated neck dissections were preserved in 0.9% saline. After a maximum 1 h following excision of the gland the lumen of the duct could be explored by a venous cannula under microscopic control.

After the lithotriptor probe had been inserted in the efferent duct, single pulses were applied under simultaneous irrigation with saline. In these experiments the site of shockwave application was located in the mid-section of the duct (in one case) (Figure 3) and in the transition region between the efferent duct and the glandular parenchyma. In the latter case the application site was surrounded by glandular parenchyma. The position of the probe was always kept parallel to the surface of the epithelium of the salivary duct.

Animal experiments

(1) Animals. Six rabbits belonging to the breed 'Deutsches Riesenlangohr' with an average body weight of 3970 g (2465–4635 g) were used for the investigations. The animals were anaesthetized by intragluteal injection of a mixture of ketamine (Ketanest", 20 mg/kg body weight) and xylazine (Rompun", 4 mg/kg body weight). Three hours after completion of the shockwave treatment 5 ml of T61" (a mixture of embutramide, mebezoniumiodide and tetracainhydrochloride) was administered intravenously

Table 2. Median number of single pulses necessary to achieve therapeutically adequate disintegration in dependence of the stone group and the intensity setting (3 French probe of the Lithotron modality, six groups of five stones each)

<table>
<thead>
<tr>
<th>Group size</th>
<th>Intensity setting 2–5 mm</th>
<th>5–10 mm</th>
<th>larger than 10 mm</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>2000</td>
<td>4600</td>
<td>8150</td>
</tr>
<tr>
<td>2</td>
<td>1100</td>
<td>1400</td>
<td>2900</td>
</tr>
</tbody>
</table>

Figure 2. Application of shockwaves to a salivary duct stone (S) (fixed with tweezers) in vitro within a saline water bath. The electrohydraulic probe (P) is in contact with the stone surface. A big fragment (F) is seen at the bottom of the funnel used for the in vitro experiments.
to sacrifice the animals. Subsequently, the treated organs were excised and passed on for acroscopic and histological examination.

(2) Experimental set-up

(2.1) Direct shockwave application to the masseter muscle, facial nerve and the parotid gland. The facial nerve, the masseter muscle and the parotid gland of three rabbits were chosen to determine the effects of exposure to electrohydraulic shockwaves in vital tissue.

After the parotid region of the test animals had been prepared, the different probe sizes of the individual lithotripsy modalities were tested under varying intensity conditions during continuous irrigation with saline (in analogy to the in vitro investigations):

- probe positioned at a distance of 0, 2, 4 and 6 mm from the tissue, electrohydraulic shockwaves applied parallel to the surface of the tissue;
- probe positioned at a 45° angle to tissue and perpendicular pulse application at distances of 0, 2, 4 and 6 mm from the tissue.

(2.2) Direct shockwave application into the obstructed efferent duct of the parotid gland of the rabbit

(2.2.1) Ligature of Stensen's duct. A sufficiently broad lumen of the duct was an essential prerequisite for the intraductal insertion of the electrohydraulic probe. As a first step, a ligature of the efferent duct of the salivary gland of the rabbit was performed according to the method described by Wallenborn in 1964 [22]. For this purpose two overlapping single-button sutures (2.0 silk fibre) were applied after a vertical incision was made in the same plane, reaching from the posterior lid angle to the angulus mandibulae (Figure 4a). In this way a duct congestion with a consecutive duct dilation of approx. 2 mm was attained (Figure 4b).

(2.2.2) Experimental procedure. Three weeks later, the obstructed efferent duct was inspected, its condition

Figure 3. Application of shockwaves to the efferent duct of a surgically extirpated human submandibular gland. The onset of perforation during treatment can be recognized in the mid-section of the duct. SG, Surgically extracted submandibular gland; arrow, onset of perforation in the duct system; EP, electrohydraulic probe with irrigation system inserted into the lumen of the duct.

Figure 4. (a) Placement of the ligatures in order to obstruct and congest the efferent duct of a rabbit. (b) Status 14 days later: obstructed and congested salivary duct (the arrows outline the efferent duct of the parotid gland (PG) of the rabbit). (c) Application of electrohydraulic shockwaves to the obstructed duct with consecutive duct perforation (arrow, tip of the probe that has perforated the efferent duct while shockwaves were applied during a treatment session).
assessed and the respective lithotripter probe was introduced together with a cannula to achieve the required irrigation. This was achieved by simple removal of skin tissue and subcutaneous tissue, without having to excise the efferent duct from the ambient tissue.

Shockwaves could thus be applied intraductally to three rabbits, i.e. five efferent ducts, without any problems, while one untreated glandular duct served as a control. The first site of shockwave application was located at the hilus of the gland. Here the efferent duct was already surrounded by glandular parenchyma and the position of the electrohydraulic probe was ‘intraglandular’. The second application site was located halfway to the orifice leading to the vestibulum oris. The probes were always kept positioned in the centre of the duct and strictly parallel to the epithelium surface without direct contact to tissue.

In all three lithotripsy systems single pulses with lowest intensities were administered intraductally using different probe sizes (1.6–3.0 F). If a macroscopic lesion became visible, the experiment was terminated. The prescribable maximum number of applied pulses was the same as that determined by the in vitro stone disintegration experiments.

Results

In vitro investigations

(1) Lithotripsy of salivary duct stones. Fifty-three out of 58 salivary duct stones treated in vitro (i.e. 91%) were successfully fragmented. Thirty-nine stones (67%) had a residual fragment size smaller than 1.5 mm, and 14 stones had a residual fragment size larger than 1.5 mm. Five sialoliths (9%) were refractory to fragmentation (Table 1). The number of pulses necessary to achieve complete disintegration (residual fragment size ≤1.5 mm) decreased with increasing probe diameter, increasing intensity of the electrohydraulic shockwaves and decreasing stone size (Table 2).

Fourteen concrements that could be fragmented only partially (residual fragment size >1.5 mm) and the five stones that withstood shockwave application had all been treated with probes smaller than 3 F in diameter.

The mineralogical stone composition and the different types of lithotripsy systems had no influence on the basic fragmentability of the sialoliths.

(2) In vitro tissue reactions. In all the in vitro sonication experiments tissue ruptures and duct perforations became observable after the first two to five applied single pulses (Figure 3). Tissue lesions were noted even when the tip of the probe was placed strictly parallel to the glandular parenchyma. In experiments with a parallel probe position in the glandular duct, microscopic examination revealed a region showing a depletion of duct epithelium and a transition to duct perforation surrounded by cellular detritus consisting of pyknotic cell nuclei and denatured cell components. Such tissue destruction was observed when the probe was positioned at half-distance from the ostium of the duct and also when the tip of the probe was surrounded by glandular parenchyma in the hilar region.

Animal experiments

(1) Shockwave application to the masseter muscle, the facial nerve and the parotid gland. When a strictly parallel alignment of the probe and a distance of more than 2 mm were maintained during shockwave application, neither macroscopic nor microscopic tissue lesions could be detected. When the distance of the probe tip from the exposed tissue was less than that distance, exposure to shockwaves regularly led to bleeding and haematomas for all application angles, independent of the probe size, number of pulses, intensity, device employed and type of exposed tissue. On a microscopic level, necroses were detected in the muscular tissue with a plaque-shaped decomposition pattern and granulocytic demarcations as a sign of intravital tissue lesions. Haematomas with granulocytic and haemorrhagic extravasation were observed at the perineurium of the facial nerve and in the surrounding connective tissue. At higher pulse intensities and reduced distances from the exposed tissue nerve fibres with enhanced eosinophilia, mutilated cell nuclei and distinct intrafascicular haemorrhages occurred.

Typical caverns of destruction with bleeding, fibrinous coatings and granulocytic infiltrations became visible in the parotid gland as well, in association with ruptures of larger efferent ducts that were accidentally struck by shockwaves and that exhibited a strongly eroded ductal epithelium.

(2) Shockwave application into the obstructed efferent duct of the parotid gland of rabbits. Despite the strictly parallel application of shockwaves and sufficient irrigation of the obstructed efferent ducts with saline, macroscopic perforations appeared already after one to three applied single pulses, even when the smallest available probe diameters and the lowest intensity settings were used (Figure 4c). In addition, the shockwaves also damaged tissue immediately adjacent to the duct: in three instances erosion of a larger vein occurred with massive bleeding.

The histological features exhibited by the glandular parenchyma which had been fibrosed and atrophied by the preceding obstruction included the following:
intraductal bleeding, with epithelial defects of the efferent ducts, slightly inflamed demarcations and haematomas. The macroscopically visible duct perforation could also be assessed microscopically.

The described effects occurred independent of the probe size and shockwave modality, i.e. also when the smallest available probe size (1.6 F) and the lowest shockwave intensity level were used.

Discussion

Intracorporeal electrohydraulic lithotripsy was introduced approximately 30 years ago for the treatment of bladder concrements [4–6]. This method is still employed today in the treatment of bladder calculi under direct endoscopic control. In analogy to urolithiasis, this technique was successfully applied for the first time in 1977 to lithotripsy of choledochus concrements [13, 14]. Whenever large concrements cause acute complications and cannot be removed by papillotomy and basket-catheter extraction in the scope of ERCP (endoscopic retrograde cholangio-pancreatography), electrohydraulic lithotripsy of gall bladder stones may be indicated; however, only if the following precautionary measures are taken: stone grasped securely by the dormia basket, balloon catheter or other dilation devices serving to position the probe securely in the centre of the biliary duct placed at a sufficient distance from the duct wall in all planes. These safety measures must also be observed when applying lithotripsy to the ureter [14, 17, 19, 20].

In 1989 Cook et al. reported for the first time – in a case study devoted to a single patient – on electrohydraulic intracorporeal lithotripsy of salivary duct stones of a human patient under anaesthesia [1]. Königberger et al. had also applied this method to human patients under endoscopic control and had reported no serious adverse effects [15].

In view of the topographic anatomy of the human efferent salivary ducts that are located in close proximity to the nerve (facial nerve, lingual nerve, hypoglossal nerve) and vascular structures (lingual artery, retromandibular vein) and in consideration of the complications described in urological and gastroenterological applications [2, 8, 12, 18, 21], thorough in vitro and animal investigations were mandatory before proceeding with clinical applications.

The present investigation proves that salivary duct stones – independent of their mineralogical parameters and the employed generator – can be reliably disintegrated by electrohydraulic-intracorporeal lithotripsy. Therapeutically adequate fragmentation was achieved for 39 of the 58 salivary duct stones (67%), i.e. the residual fragments were smaller than 1.5 mm. All of the remaining concrements that were partially fragmented or refractory to fragmentation were treated with probe diameters smaller than 3 F and at low intensity levels. The lower the selected intensity level for the same probe diameter, the higher the number of single pulses required to achieve fragmentation.

These findings point towards the existence of a lowest energy threshold for electrohydraulic-intracorporeal fragmentation of salivary duct stones, because the smaller the probe diameter and the preselected intensity level (=input), the higher is the emitted energy available for stone fragmentation at the tip of the probe (=output). This means that the probe diameter and the intensity can only be lowered down to a defined threshold level, if fragmentation of a salivary gland stone is still to be achieved. This was demonstrated, for example, by the finding that only two out of seven stones could be completely disintegrated when the smallest implemented probe diameter (1.6 F) was used. At intensity settings below this energy threshold, complete fragmentation is no longer possible or only over an unduly long period of time. Hence, there exists a limit to a further miniaturization of the probe dimensions used in intracorporeal electrohydraulic lithotripsy.

Rabbits served as representative experimental models for animal studies: on one hand the anatomic relations between the facial nerve and the parotid gland comparable to those in humans, and on the other hand we found ligation and congestion of the glandular duct – based on the publication of Wallenborn et al. [22] – to be a very practical method of dilating the efferent duct of the gland so as to allow the electrohydraulic probes to be inserted into the lumen of the duct without problems.

The obstructed Stensen's duct of a rabbit then corresponds in size to the human salivary ducts (1.5–2 mm) [11]. As the median diameter of a salivary gland concrement is 6.7 mm, it did not appear possible to place sialoliths into the efferent duct system of the parotid gland of a rabbit without the risk of causing epithelial defects which could later not be distinguished from authentic treatment-induced damage to tissue. Therefore, valid conclusions as to the primary effects of electrohydraulic shockwave application could have been derived only in a limited measure.

Within the framework of our investigations macroscopically and histologically discernible haematomas, tissue defects and erosion craters were clearly recognized when shockwaves impinged on nerve and muscle tissue and salivary gland tissue – as long as the tip of the probe was kept at a distance of less than 2 mm from the tissue surface. The type of implemented modality and the size of the probe had no influence on the extent of the detectable cellular damage. No tissue destruction
whatsoever was observed in all planes of sonication after shockwave exposure from a distance of more than 2 mm.

When electrohydraulic shockwaves were applied into Stensen’s duct of a rabbit, duct perforation with distinctly recognizable damage to the adjacent tissue occurred already after one to three single pulses – independent of the probe sizes pulse intensities and the type of system – even if the tip of the probe was aligned exactly parallel to the surface of the epithelium. In this context it must be emphasized once more that duct perforation was regularly found when shockwaves were administered to surgically excised human efferent salivary gland ducts already after two to five single pulses, independent of the site of shockwave exposure. However, stone disintegration required a minimum of 400 single pulses.

Two effects that arise during intracorporeal application of electrohydraulic shockwaves can be held responsible for the occurrence of perforations at an early stage. When the shockwave is generated, a spark discharge between the cathode and anode at the tip of the probe occurs, and a plasma with local temperatures of up to 10,000 K is formed. In the literature [2, 8, 15, 18, 19] this plasma effect, which is caused by too small a distance between tissue and the tip of the probe, is considered to be the cause of cellular damage, next to the direct effect of shockwave impingement. It is ultimately too difficult to judge to what extent the tissue-destructive potential is increased by shockwave amplification caused by wave reflection at the tissue–air interface in the case of the exposed efferent ducts. This is nevertheless feasible on the basis of physical considerations [9]. Investigations by Bhatta et al., Harrison and Lear showed that substantial damage to tissue of the ductus choleductus and the bladder [2, 8, 16] also occurs in the case of a reduction or absence of leaps in selected impedance values (in situ and waterbath applications).

In our in vitro and in vivo investigations we chose different application sites (mid-section of the efferent duct and the transition zone between the glandular parenchyma and the efferent duct), and detected no differences in tissue reactions for different tissue layer thicknesses, varying geometries and thus modified shockwave reflection behaviour. The same effects were observed, when the efferent duct of the parotis of the rabbit remained in the tissue domain.

Moreover, a substantial part of the efferent duct system of the human glandula submandibularis is also located in close proximity to the air–mucosa interface, so that our experimental arrangement reflects the given anatomic conditions in human patients.

In view of the narrow efferent salivary gland ducts (max. diam. 1.8 mm) – and on the basis of our investigations and the clinical experiences gained by Harrison and Tanaka [8, 21] – the required safety distance of 1–2 mm from the surrounding tissue cannot be maintained when probe sizes which provide efficient performance are to be implemented. Therefore, the clinical indication of electrohydraulic-intracorporeal lithotripsy in the scope of human sialolithiasis appears questionable – in view of the danger of duct perforation as well as lesions of ambient nerve and vasal structures that result from the described plasma and shockwave effects. Even if a large proportion of the energy dissipated in the treatment of human patients is transferred to the concerment in case of direct contact with the stone, misapplied shockwave transmission in the narrow efferent ducts of the salivary glands with the above-mentioned consequences cannot be excluded. In this context, it must be pointed out once again that tissue lesions can be detected already after one to three single pulses, whereas a minimum of 400 pulses are required to achieve therapeutically adequate fragmentation.

**Summary assessment**

The following conclusions can be derived from the present investigations:

1. Electrohydraulic-intracorporeal lithotripsy allows fragmentation of salivary duct stones to be performed reliably.

2. There exists a minimum fragmentation threshold below which reliable stone disintegration can no longer be achieved. Therefore, miniaturization of the ultrasound probes cannot go below a certain geometrically defined limit. Already with a probe size of 1.6 F as used in our experiments, therapeutically adequate fragmentation (< 1.5 mm) could be achieved with only two out of seven sialoliths.

3. Clinical application of the described method to sialolithiasis in human patients should be approached with great caution, because there is a high risk of perforation of the efferent duct system and of surrounding anatomic structures already after administration of one single pulse, as demonstrated by in vitro animal experiments using the lowest intensity settings and smallest probe diameters. This holds in particular in view of the fact that other minimally invasive methods for treating stone disorders of the salivary gland, such as extracorporeal shockwave lithotripsy and intracorporeal laser lithotripsy, associated with a minimum of adverse effects are available and offer a high level of reliability [6, 7].
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